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19TH CONGRESS OF THE EUROPEAN HEMATOLOGY ASSOCIATION

MILAN, ITALY
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On behalf of the EHA Board and the Scientific Program Committee we are pleased to present the Abstract Book of the 19th Congress of EHA.

The Scientific Program Committee has compiled an exciting and topical program of Simultaneous and Poster Sessions from over 2,300 submitted abstracts. Join our expert moderators for a walk along the (e)posters in your field of interest on Friday and Saturday and attend one of the 40 Simultaneous Sessions on Saturday and Sunday. The six Best Abstracts have been selected for presentation during the Presidential Symposium on Saturday afternoon.

In addition, please find at the end of the book also the late breaking abstracts that were submitted, reviewed and selected in April. The Scientific Program Committee is very excited about the quality of the studies represented in these abstracts and feels that they are a nice addition to the program. The eight selected oral presentations will be presented on Saturday in two Late Breaking Simultaneous Sessions. Furthermore, eleven posters are available for viewing in the Poster Area and will be presented on Saturday as well.

On behalf of the EHA Board, the committees and all the people involved in this years' EHA congress, we thank you for coming to Milan and wish you a good meeting.

Pieter Sonneveld
Chair Scientific Program Committee

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Late breaking abstracts

Official Organ of the European Hematology Association

haematologica
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The origin of a name that reflects Europe's cultural roots.

Ancient Greek

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

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

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

Modern English

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Haematologica/The Hematology Journal, as the official organ of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identity.

POSTER SESSION I

Acute lymphoblastic leukemia - Biology 1

P100

NOVEL STAT5B MUTATIONS AS DRIVERS OF T-CELL ACUTE LYMPHO BLASTIC LEUKEMIA

M Kontro[1,*] H Kuusanmäki[2], S Eldfors[2], T Burmeister[3], EI Andersson[1] TH Brümmendorf[4], Ø Bruserud[5,6], H Edgren[2], BT Gjertsen[5,6], M Itälä-Remes[7] S Lagström[2], O Lohi[8], JM López Martí[2], T Lundán[9], MM Majumder[2], A Parsons[2], T Pemovska[2], H Rajala[1], K Vettenranta[10], O Kallioniemi[2], S Mustjoki[1], K Porkka[1], C Heckman[2]
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Background: T-cell acute lymphoblastic leukemia (T-ALL) is caused by the cooperation of multiple oncogenic lesions. Recent evidence supports that IL-7 and its receptor IL-7R contribute to T-ALL development (Zenatti *et al.*, 2011). The two main pathways induced by IL-7R are JAK/STAT5 and PI3K/AKT/MTOR. Activating mutations to *IL7R*, *JAK1, JAK2* or *JAK3* are estimated to occur in 20-30% of all T-ALL patients (Cools 2013). STAT5 plays an important role in many hematologic malignancies but constitutive STAT5 activation often is a secondary event. Mutations in *STAT5B* (N642H) were recently described in LGL-leukemia in patients with an unusually aggressive and fatal form of the disease (Rajala *et al.*, 2013). Here we report novel activating *STAT5B* mutations as drivers of T-ALL.
Aims: The aim for the study is to determine the prevalence and functional effects of *STAT5B* mutations in T-ALL.
Results: Sequencing of a relapsed T-ALL index patient revealed 3 different somatic missense mutations in *STAT5B* (T648S, N642H, I704L). To investigate the prevalence of these mutations in T-ALL we used targeted next generation sequencing (MiSeq, Illumina) to sequence the SH2 dimerization and the transactivation domains of *STAT5B* from 68 T-ALL patients. In this validation cohort we detected *STAT5B* mutations in 5 additional patients, all of which occurred in the SH2 domain. One patient had a Y665F mutation whereas 4 patients had N642H mutation. Altogether 6 of 68 patients in the cohort had *STAT5B* mutations. To investigate the effect on transcriptional activity and phosphorylation of *STAT5B* mutations identified in the index patient (*i.e.* N642H, T648S, I704L), the mutant and wild type (WT) *STAT5B* constructs were transiently transfected into HeLa cells together with a STAT5 specific luciferase reporter plasmid. Western blot analysis showed that the N642H induced strong constitutive phosphorylation of STAT5B, while the I704L mutation induced phosphorylation to a lesser extent. Compared to WT *STAT5B* the N642H and I704L mutants increased transcriptional activity by 26- and 17-fold, respectively, however the T648S mutation had no effect. Using *ex vivo* drug testing the *STAT5B* mutated blasts were resistant (EC50 >=1 uM) to inhibitors of PI3K (*e.g.* idelalisib, XL147), dual inhibitors of PI3K/MTOR (PF-04691502, dactolisib) and MTOR inhibitors (temsirolimus, everolimus). Furthermore the blasts showed no response to AKT1 inhibitors (MK-2220) or JAK inhibitors (ruxolitinb, tofacitinib). In contrast, the cells were most sensitive to the pan-BCL-2 inhibitor navitoclax (EC50 82 nM). To assess the expression of BCL-2 family members (BCL-2, BCL-XL, BCL-XS and MCL1) in *STAT5B* mutated blasts from the index patient qRT-PCR was performed. RNA from the peripheral blood CD3-positive fraction of a healthy donor and the mononuclear BM fraction from two T-ALL patients with no *STAT5B* mutations served as controls. While BCL-2 and MCL1 expression were similar across all samples, BCL-XL expression was higher in the *STAT5B* mutated blasts compared to controls by 12- and 4-fold in the diagnostic and relapse sample, respectively.
Summary and Conclusions: *STAT5B* mutations are recurrent (8.8%) in T-ALL and their occurrence underlines the significance of the IL7R-JAK-STAT5 pathway in the pathogenesis of T-ALL. These mutations are activating and thus likely oncogenic. Our results suggest that BCL-XL inhibition is cytotoxic to *STAT5B* mutated T-ALL blasts, and therefore therapies targeting specifically BCL-2 may be ineffective.

P101

PHF6 LOSS DRIVES IL7R ONCOGENE ADDICTION IN T-ALL

K Durinck[1,*] P Rondou[1], I Van de Walle[2], C De Bock[3,4], N Van Roy[1], Y Benoît[5], B Poppe[1], T Taghon[2], J Soulier[6], J Cools[3,4], P Van Vlierberghe[1], F Speleman[1]
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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a genetically heterogeneous disease. Genetic subgroups are marked by overexpression of particular driver oncogenes that disrupt normal thymocyte differentiation, while other lesions occur across different genetic subtypes and impact on proliferation, self-renewal and survival. In this latter group, the *PHF6* gene is frequently targeted by loss-of-function mutations or deletions, with the highest prevalence in *TLX1* or *TLX3* rearranged T-ALLs. Further insights into the tumor suppressor role of PHF6 will contribute to the deciphering of the complex rewiring of the cellular program of normal thymocytes during malignant transformation and will serve as a prelude to a rational design for targeted therapy.
Aims: Gain insight in the role of PHF6 through transcriptome analysis following modulation of *PHF6* expression in precursor T-cells and T-ALL lymphoblasts and functionally validate relevant candidate PHF6 target genes both *in vitro* and *in vivo*.
Methods: Transcriptome wide perturbation effects were measured following PHF6 knock down in the *PHF6* wild-type T-ALL cell lines Jurkat, ALL-SIL and MOHITO (mouse) and will be evaluated upon PHF6 reconstitution in *PHF6* deficient T-ALL cell lines HPB-ALL and DND-41. In parallel, gene expression profiles were established from cord blood $CD34^+$ progenitor T-cells, cultured for short-term on an OP9-DL1 feeder layer, with stable knockdown of PHF6. Murine bone marrow transplant assays were performed to assess the effects of PHF6 deficiency *in vivo*. In addition, a large primary cohort of T-ALL patients was screened for the association between *PHF6* mutations and other genetic defects.
Results: Modulation of *PHF6* expression induced robust transcriptional regulatory effects of PHF6 on *IL7R* expression. *IL7R* encodes a cytokine receptor critically involved in normal T-cell development and acts as a bona fide oncogene in subsets of T-ALLs. Using the IL7-dependent MOHITO mouse T-ALL cell line, we show that IL7 stimulation induced prolonged and enhanced induction of the IL7R downstream mediators (pJAK1, pJAK3 and pSTAT5) in *PHF6* knock down cells as compared to *PHF6* expressing controls, suggesting a functional PHF6-IL7R-JAK-STAT pathway in murine T-ALL. Furthermore, genomic analysis of an extend series of primary T-ALL samples unraveled a significant association between *PHF6* and *JAK3* mutations in T-ALL patients. To further explore the molecular basis for TLX1-PHF6 interaction, we looked into the TLX1 driven transcriptome and observed suppression of *IL7R* expression. In view of these findings, we hypothesize that loss of PHF6 rescues immature TLX1 overexpressing thymocytes from reduced IL7R-dependent survival/proliferative signals, thus providing an essential cooperative event in TLX1 driven leukemogenesis. Given the almost exclusive occurrence of *PHF6* mutations in T-ALL, we assumed a specific function for PHF6 in normal T-cell development. Indeed, stable PHF6 knock down in immature human thymocytes demonstrated strong effects on normal differentiation in the OP9 culture system in keeping with a context dependent regulatory role for PHF6 in T-cell development.
Summary and Conclusions: Our data provide fundamental novel insights into the role of PHF6 in T-cell development and transformation. We demonstrate a crucial role for PHF6-controlled IL7R expression, the latter which is down-regulated in TLX1 overexpressing T-ALLs, thus suggesting an essential cooperative role for PHF6 during TLX1 driven T-ALL development. These novel observations mark the IL7R-JAK-STAT signaling pathway as an important entry point for further therapeutic intervention in TLX1 driven leukemias.

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LEAFING THROUGH THE GENOME OF B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA BY NEXT-GENERATION SEQUENCING TECHNOLOGIES

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Background: The molecular characterization of B-lineage acute lymphoblastic leukemia ALL (B-ALL) currently relies on the identification of recurrent structural rearrangements (*ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1*, *MLL* rearrangements) - with a diverse distribution and impact on outcome between children and adults. However, many of these alterations alone do not induce leukemia in experimental models. Furthermore, a considerable proportion of patients lack recurrent chromosomal alterations, thus suggesting that additional submicroscopic lesions could have a role in leukemogenesis.
Aims: 1) To describe the molecular landscape of "genetically unclassified" B-ALL patients; 2) to evaluate the association between specific alterations, affecting one gene or gene category, and age-cohorts; 3) to assess the "targettability" of the mutated genes and the suitability of genetically-driven therapies.
Methods: The genome of 15 B-ALL patients (and their paired normal DNA, Discovery panel) without any major chromosomal abnormality and belonging to different age cohorts, namely children (2-15 years), adolescents/young adults (AYA, 16-40 years) and adults (>40 years) was analyzed by whole-exome sequencing (WES, Agilent SureSelect Human All Exon 50Mb, Illumina HiSeq 2000). WES was then broadened to 72 additional B-ALL cases (Screening panel 1) and we focused on the genes resulted from the Discovery panel and on known driver genes. We then selected the genes recurrently mutated for a further screening by conventional Sanger sequencing (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems), or targeted amplicon sequencing (Genome Sequencer Junior, 454 Life Sciences, Roche) of the Screening panel 2 (N=66). Overall, 29 children, 72 AYA and 52 adults were included in the study.
Results: WES of the Discovery panel highlighted 139 non-silent mutations (9.3/sample) affecting 135 genes mainly involved in RAS signal transduction, transcription and chromatin remodeling, regulation of cell proliferation, as well as genes encoding for tyrosine kinases. Indeed, Screening 1 and 2 revealed that the RAS pathway is frequently altered in this B-ALL subgroup, with 25% of cases being affected. *FLT3* was mutated in 9% of cases with the mutations being mainly represented by internal tandem duplications (36%) and affecting the tyrosine kinase domain in 28% of cases. More importantly, *FLT3* mutations clustered in adults (13%) and AYA (8%), while only 3% of children displayed *FLT3* mutations. *KRAS* and *NRAS* were mutated in 8.5 and 9% of cases, respectively, with the mutations targeting almost exclusively the G12/G13 hotspot. Moreover, we found a trend to accumulate in childhood. The screening of the genes associated to a high-risk phenotype showed that *JAK2* and *IL7R* are mutated in 4% of cases. Notably, among the B-lineage specific genes, we detected *PAX5* mutations in 13% of patients with an incidence increasing with age. Interestingly, besides the well-known P80R we observed other 2 hotspots: G30 and S55.
Summary and Conclusions: By next-generation sequencing, we have shed light on the genome of "genetically unclassified" B-ALL. This approach showed that this subgroup is characterized by 9 mutations/sample and a considerable heterogeneity of mutations distribution across different age cohorts. This pattern of lesions might sustain a diverse outcome between children and adults, and prompt the development of therapeutic strategies targeted according to the differential recurrence of the above reported alterations.

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IMPACT OF VIRUSES ON THE PATHOGENESIS OF SPORADIC CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: INTEGRATION AND PRESENCE OF 25.525 VIRAL GENOMES TESTED BY NEXT GENERATION SEQUENCING

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Background: The incidence of childhood acute lymphoblastic leukemia (ALL) is rising steadily. Accumulating epidemiologic evidence suggests that viral infections early in life account for this increase. The putative infectious agent is still unknown. Theoretically, transforming viruses may integrate into the genome of precursor B-cells, disturbing differentiation and proliferation control. Alternatively, common pathogens may act indirectly, eliciting an abnormal immunological response resulting in autonomous precursor B-cell proliferation.
Aims: Previous attempts to identify a candidate virus by low-throughput techniques were unsuccessful due to detection limits: 95% probability to detect viral genomes >9 kb present in at least one copy per cell was achieved. But the smallest virus genomes are about 3 kb, and many oncogenic viruses are smaller than 9 kb. We aimed at developing a next generation sequencing based, bioinformatics pipeline for the detection of known and novel viruses in childhood ALL.
Methods: Whole-genome sequencing of leukemic and remission bone marrow from 15 children with precursor B cell ALL was performed. Data sets of five healthy probands derived from the 1000 genomes database (www.1000genomes.org) were used as controls. Paired-end sequences were aligned against the human reference genome (GRCh37.55) employing BWA. Aligment was repeated with all unmapped reads with higher sensitivity and mapping concordance ≥85% using Bowtie2. Remaining unmapped reads were aligned against 25,525 viral reference genomes (Genome Information Broker for Viruses database, GIB) allowing multiple matches. Repeat sequences and regions of high homology with the human reference were filtered. Paired-end reads of both human and viral sequence were considered for breakpoint detection of viral integration. Viruses with at least two single-end alignments or one spanning read were chosen for manual inspection and validation.
Results: *In silico* simulations showed that a typical virus genome (≥2 kb) and viral integration sites are detected with >99.9% probability within ≥100 million sequencing reads by the developed bioinformatics pipeline. 442 million sequencing reads were generated per sample on average. About 10% could not be mapped to the human reference and thus potentially encoded viral sequences. Few viruses were detected in healthy controls: Herpesviridae (EBV, n=5, HHV-7, n=3) and Adenoviridae (ADV, n=2). In the patient samples, viral DNA corresponding to common human pathogens (Anelloviridae, Herpesviridae, and Parvoviridae) was detected in 11 cases. No evidence was found for the presence of other human or non-human DNA viruses.
Likely due to contaminated blood transfusions the incidence of the Anelloviridae TTV and TTMDV increased from two cases at diagnosis to six in remission. At diagnosis six patients were positive for at least one other virus. EBV (n=3), VZV (n=1), and HHV-7 (n=3) were detected. In four relapse samples EBV (n=3), HHV-7 (n=3), and HHV-6 (n=1) were identified. None of the patients of whom both diagnosis and relapse samples were available (n=8) showed persistence of a virus.
Most viruses detected at remission were not found in leukemic samples of the same patient: HSV (1/1), VZV (1/1), CMV (2/2), EBV (0/1), HHV-6 (2/2), HHV-7 (2/6), Parvovirus B19 (1/1), TTV (4/5), TTMDV (1/2). EBV was detected in leukemic cells of six out of 15 patients, but only in one corresponding remission sample.
Summary and Conclusions: Our analyses suggest that the sought-after virus(es) could be very common ones provoking an exceptional immunological response of the B-cell compartment in genetically and immunologically susceptible children.

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THE FIRST LNCRNA LANDSCAPE OF MAJOR GENETIC T-ALL SUBSETS AND GUILT-BY-ASSOCIATION ANALYSIS FOR ETP-ALL SPECIFIC LNCRNAS

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a hematologic cancer caused by uncontrolled proliferation of immature thymocytes that arrested during differentiation. T-ALL can be classified into molecular genetic subgroups that are defined by overexpression of transcription factor oncogenes including *LYL1*, *TLX1*, *TLX3*, *TAL1*/*LMO2* or *HOXA*. The *LYL1*$^+$ T-ALL subtype characteristically shows an immature immunophenotype and is closely related to the recently identified poor prognostic T-ALL subtypes including early T-cell precursor ALL (ETP-ALL) or early immature T-ALL.
Aims: Long noncoding RNAs are emerging as important players in cancer development. In order to assess the possible role of lncRNAs in T-ALL, we aimed to perform lncRNA expression profiling in primary T-ALL samples, T-ALL cell lines and normal immature thymocytes to define whether a specific lncRNA expression landscape is linked to particular T-ALL subgroups and driver genes.
Methods: We analyzed a clinically and genetically annotated cohort of 64 T-ALL samples (including 15 immature, 17 TLX and 25 TAL-rearranged T-ALL patients), 7 T-ALL cell lines and sorted subsets of immature thymocytes from four healthy donors using an in house developed Agilent platform covering all protein coding genes and 12,000 previously annotated lncRNAs. This dataset was further expanded by RNA-sequencing data from an independent cohort of 31 T-ALLs (Atak *et al.*, PLoS Genetics, 2013).
Results: First, we validated our datasets by demonstrating similar clustering of major genetic subsets based on the expression pattern of protein coding genes. Next, we searched for subgroup specific lncRNA expression signatures in T-ALL. Each of the genetic subgroups was characterized by a distinct subset of differentially expressed lncRNAs, including 461 TLX1-specific, 1494 TAL1-specific and 1448 immature-specific lncRNAs ($p < 0.05$, corrected for

multiple testing). Using the top 50 most differentially expressed lncRNAs for each subgroup, individual patients could be assigned to their appropriate molecular genetic T-ALL subtype (Pearson clustering). Given their proposed association with poor clinical outcome, we further focused on the early immature T-ALL subtype and looked for putative functionally relevant lncRNAs in this genetic subgroup. To this end, a guilt-by-association was performed for the top 10 most differentially expressed known lncRNAs derived from the Agilent platform and an additional top 10 expressed previously unannotated lncRNAs identified through RNA-seq analysis. Of further interest, several immature T-ALL specific lncRNAs were located in the immediate vicinity of important protein coding genes implicated in early haematopoiesis and leukemia such as *MYB*, *RUNX2* and *MEF2C*. Further analyses of selected immature T-ALL associated lncRNAs also showed strong differential expression between $CD34^+CD4^-CD8^-$ *versus* $CD4^+CD8^+$ double positive thymocytes, pointing at important roles for these lncRNAs in normal differentiation. Finally, we selected several top candidate ETP-ALL specific lncRNAs for further functional analyses in order to unravel their exact role in early haematopoiesis and malignant T cell transformation as a prelude for possible lncRNA oriented molecular therapy in immature T-ALL.
Summary and Conclusions: This is the first comprehensive analysis of lncRNA expression in primary T-ALL samples and their normal thymic counterparts. Our finding that lncRNA expression patterns follow the previously established genetic classification is of importance as it marks functional relevance for lncRNAs in T-ALL development.

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PTEN MICRO-DELETIONS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA ARE CAUSED BY ILLEGITIMATE RAG-MEDIATED RECOMBINATION EVENTS

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is characterized by distinct chromosomal abnormalities and mutations. The Phosphatidylinositol 3-kinase (PI3K) signal transduction pathway is often aberrantly activated in various cancers. The main negative regulator of PI3K activity is the phosphatase and tensin homolog (PTEN) tumor suppressor gene. In T-ALL, *PTEN* is inactivated by point-, frameshift insertion/deletion mutations and entire locus deletions. For some seemingly *PTEN* wild-type or monoallelic mutated T-ALL patients that lack total PTEN protein, the mutational mechanisms remain unclear.
Aims: Our goal is therefore to investigate copy-number variations among *PTEN* exons and to detect potential additional *PTEN* deletions.
Results: Here, we show that *PTEN* can be inactivated by micro-deletions spanning introns 1-3 or introns 3-5 in 8% of pediatric T-ALL patients. These micro-deletions were clonal in 5 out of 146 patients, whereas in 8 patients they were present at the sub-clonal level and only detected through PCR techniques. Specific sequences flanking these deletions together with insertion of random nucleotides between the breakpoints pointed to illegitimate RAG-mediated activity. The cryptic RAG recombination signal sequences (cRSS) that flanked the breakpoints drive RAG-dependent recombination in an *in vitro* reporter system as efficiently as established RSSs from TCR gene segments. Similar to other PTEN-inactivating events, *PTEN* micro-deletions are strongly associated with the TALLMO T-ALL cluster, characterized by TAL1 or LMO2 chromosomal rearrangements. As these leukemias frequently display rearranged αβ T-cell receptors at a maturation stage with ongoing RAG activity, our results imply that the TALLMO subgroup has an increased chance of acquiring *PTEN* micro-deletions. Notably, primary and secondary xenotransplants of human *TAL1*-rearranged T-ALL cells in NSG-mice displayed sub-clonal *PTEN* micro-deletions, and we identified equivalent micro-deletions in thymocytes of healthy individuals.
Summary and Conclusions: We propose that *PTEN* micro-deletions result from ongoing RAG activity that is perpetuated during the leukemogenic process, thereby contributing to clonal diversification and disease progression.

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CMYC-TRANSLOCATIONS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: *CMYC* is a transcription factor which regulates critical cell functions such as metabolism, proliferation, and survival. In T-ALL high *CMYC* expression is mainly caused by activation of *NOTCH1* and post-transcriptional mechanisms mediated by *PTEN*.
Aims: To delineate the genetic profile and clinical-hematological features of human T-ALL with *CMYC*-translocations.
Methods: We investigated 62 adults and 132 children belonging to the AIEOP, GIMEMA, and UK clinical trials. *CMYC* was studied by LSI MYC Dual Color, Break Apart Rearrangement Probe (Vysis-Abbott) and homegrown G248P8135G5, RP11-367L7 and RP11-26E5 clones. Affymetrix HU133 Plus 2.0 arrays were used for whole transcriptome profiling (GEP) and Cytoscan HD Platform for SNPa. CI-FISH was performed as described (La Starza R, Leuk Res 2013). *NOTCH1*, *FBW7*, and *PTEN* were analysed by DHPLC (Transgenomic) and sequencing (AB3500 Genetic Analyzer).
Results: CI-FISH and/or predictive analysis by microarray classified 155 cases into 5 distinct molecular subtypes, *i.e. TAL/LMO* (56), *HOXA* (48), *TLX3* (31), *TLX1* (15), and *NKX2-1* (5). *CMYC* reciprocal translocations were detected in 12 cases and involved *TCR* loci in 6 cases while remained undetermined in the other 6. FISH showed that the 8q24 breakpoints clustered at the telomeric region of *CMYC* in all cases with *TCR* translocations, while in 3/6 non-*TC*R translocations breakpoints fell upstream (1 case) or within (2 cases) fosmid G248P8135G5. As type B abnormalities, *CMYC*-translocations occurred in all cases in association with other changes (range: 1-10 additional abnormalities). Association with the TAL/LMO subgroup was significant (Pearson Chi-square, P=0,018). Frequent concurrent rearrangements were *CDKN2A/B* deletions (58,3%) and *PTEN* deletion and/or mutation (41,6%). *NOTCH1* and *FBXW7* mutations were found in a single case. In accordance with their secondary nature, *CMYC*-translocations were found in variable sized subclones (range: 8-62%) in 6 cases, suggesting a contribution to disease progression rather than to disease initiation. Notably, *CMYC*-positive clones appeared to be treatment-resistant. In one case, paired diagnosis/relapse samples showed an increase in size of the CMYC clone from 8% to 100%, whereas other abnormalities $ETV6^{del}$, $BCL1B^{del}$, and $WT1^{del}$ disappeared. In a second case, backtracking to initial diagnosis did not show the *CMYC*-translocation that at relapse was present in 60% of cells. Within the *TAL/LMO* subgroup, 7 translocated *CMYC* specimens with available material for GEP showed high *CMYC* expression in the fourth quartile. Interestingly, in the same quartile, *CMYC* translocated cases showed significantly upregulated *CD44* expression and absence of activated *NOTCH1* signaling compared to cases without these translocations.
Summary and Conclusions: *CMYC*-translocations occurred in about 6% of *NOTCH1* independent T-ALL with marked leukocytosis (>50.000/mmc in 90%; >100.000/mmc in 60% of cases) and a cortical/mature differentiation arrest (60% of cases). *CMYC*-translocations clustered in *TAL/LMO* positive T-ALL with co-occurrence of poor prognostic markers, such as high *CD44* expression and *PTEN* inactivation. Our findings recapitulate murine models in which c-Myc had a crucial role on maintenance and self-renewal of leukemia-initiating cells resulting into resistance to chemotherapy and disease relapse. Early identification (and eradication) of small *CMYC*-positive subclones not only at diagnosis but also during treatment might be helpful to prevent disease progression.

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REVEALING EXPRESSION, POST-TRANSLATIONAL MODIFICATIONS AND PROTEOLYSIS IN CHILDHOOD ACUTE LEUKEMIA USING A NOVEL FLOW CYTOMETRY-BASED METHOD OF AFFINITY PROTEOMICS

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Background: Acute leukemia (AL) is the most common childhood malignancy. It is driven by a number of aberrations detectable at the DNA and mRNA level, but the functional consequences of these alterations at the cellular level are not fully understood. Proteins are the entities that form connection between gene expression and cellular response. Therefore, more effective and sensitive approaches to detect changes in proteome are needed. In the present study we develop and validate new affinity proteomics based tool for analysis of clinical samples.
Aims: Using Size-exclusion Chromatography - Microsphere-based Affinity Proteomics (SEC-MAP) we are able to resolve expression and activation (*e.g.* phosphorylation) of proteins in AL cells.
Methods: SEC-MAP array is a set of 1728 populations of fluorescently-labeled latex microbeads each carrying an antibody against a human protein. We isolate the cellular proteins from membranes, nuclei and cytoplasm using detergents, label them with biotin and separate them using gel chromatography into 24 fractions. These fractions are incubated with SEC-MAP microbeads and the

antibody-protein binding is detected using fluorescently-labeled streptavidin by flow cytometry.
Results: We have compared the data collected by SEC-MAP array in leukemic cell lines (n=11) and healthy peripheral blood B-cells, T-cells and monocytes with classical flow cytometry-based immunophenotyping. Thirty-four markers for leukemia classification correlated qualitatively within both methods. Next, we evaluated leukemia classification markers in bone marrow or peripheral blood from fifty-seven patients with leukemia at diagnosis. We were able to correctly classify all patients' samples to myelo-, B- and T-cell origin using SEC-MAP technology. We have further examined the expression of 499 proteins in 69 diagnostic samples of AL. The analysis was performed using in-house automatic software created in R-project. For the normalization of protein expression we have used loess normalization commonly used in mRNA profiling studies. Due to ability of SEC-MAP to separate proteins according to their size we have not only quantified the expression of proteins but also evaluated proteins' size that could serve as a sign of proteolysis. We have detected signs of proteolysis in twelve samples (based on *e.g.* cleaved PARP1, BLNK and BAD). These samples were therefore excluded from the final analyses. Moreover we have evaluated the sensitivity to proteolysis of four commonly used house-keeping proteins (β-actin, β2-microglobulin, AKT1 and ABL1). ABL1 and AKT1 were cleaved while β-actin and β2-microglobulin were not detected in their cleaved forms in the proteolytically degraded diagnostic samples. Therefore we propose to use ABL1 and AKT1 as house-keeping proteins whenever proteolysis has to be excluded. So far we have identified 44 proteins (*e.g.* SH2D1A, TCF7, GLUD1, TCF3, CD72) differentially expressed in different subtypes of AL and validated their expression with different methods (*e.g.* flow cytometry, western blot, real-time quantitative PCR).
Summary and Conclusions: In summary, SEC-MAP is a high-content method of functional proteomics that combines the capacity of DNA microarray and high-throughput evaluation by flow cytometry. It can detect changes in expression, post-translational modification and subcellular localization of hundreds of proteins in relatively small-size sample (only 10 million cells needed).
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RNA-SEQ ANALYSIS TO DISSECT THE BIOLOGY OF EARLY RESPONSE TO TREATMENT IN HIGH RISK *VS.* STANDARD RISK CHILDHOOD BCP-ALL PATIENTS

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Background: Acute Lymphoblastic Leukemia (ALL) is the most frequent type of childhood leukemia. It is a multi-step complex process, characterized by the expansion of a pre-leukemic clone after a variable latency time, accumulating cooperative genetic events required for the full transformation and clinical manifestation. Recently, the technological advances for genome-wide profiling have allowed both a better understanding of the molecular basis and heterogeneity of pediatric ALL, and a more precise definition of risk factors. However, incidence and cure rates differ among children, reflecting a diverse response to drug treatment and defining low-risk and high-risk patients. The comprehension of the leukemia biology, as well as the risk prediction, could be enhanced by investigations that address the individual differences.
Aims: The aim is to apply a whole-transcriptome sequencing approach (RNA-Seq) to characterize and compare low- *versus* high-risk patients to identify new genetic explanations for their different early response to therapy, as reflected by the minimal residual disease (MRD) monitoring; moreover, we focused on the identification of novel pre-leukemic and leukemogenic events.
Methods: Total RNA was extracted from primary leukemic blast samples of 10 pediatric ALL patients, included in the Italian AIEOP-BFM ALL2000 protocol. Genome-wide DNA profiling was performed by Affymetrix whole-genome 2.7M arrays. RNA-Seq experiments were carried out by using an Illumina GAIIx platform. Different strategies, such as RT-PCR and/or FISH analyses, were employed for validation.
Results: We analyzed the transcriptome of 10 childhood ALL cases (4 low- and 6 high-risk patients, according to MRD classification), not carrying any other clinical or genetic risk factor. For each case, we identified the already known and novel transcripts, single-nucleotide variants, alternative splicing events and related expression levels. Priority was given to putative fusion transcripts, which could originate from intra- or inter-chromosomal structural rearrangements. We identified 67 fusion events. Strikingly 60 out of 67 events were identified as intra-chromosomal fusions, and 59/60 were involving two contiguous genes or whose gene loci were even overlapping (the so-called "con-joined genes", CGs). These new fusion transcripts are being validated, in the same patient cohort. Furthermore, among the intra-chromosomal fusions, the NUP214-ABL1 fusion was identified in one high-risk patient. This event is noteworthy, since it was previously known as involved mainly in T-ALL and to be responsive to kinase inhibitors, and was found here in a high risk B-cell phenotype leukemia. Moreover, among the seven putative inter-chromosomal fusions, the novel PAX5-POM121C fusion was identified in one low-risk patient (and confirmed by RT-PCR and Sanger sequencing on an independent primary sample).
Summary and Conclusions: RNA-Seq represents one of the most suitable and comprehensive approaches to identify the genetic alterations harbored by leukemia clones. Our analyses detected highly recurrent novel fusion genes, originated either by classical inter-chromosomal or by intra-chromosomal rearrangements (recently defined as con-joined genes). Further evaluations will address SNPs, mutations, changes in expression profiles and alternative splicing events that could be related to a different associated risk of relapse and the feasibility of a screening of the candidates on a large population of consecutive cases.

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DARATUMUMAB TREATMENT ALONE OR IN COMBINATION WITH VINCRISTINE RESULTS IN THE INHIBITION OF TUMOR GROWTH AND LONG TERM SURVIVAL IN PRECLINICAL MODELS OF ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Daratumumab is a human antibody that binds to CD38 on the cell surface and induces cell killing by multiple mechanisms including complement mediated cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP) and apoptosis.
Aims: Efficacy of daratumumab as an anti-tumor therapy was evaluated in 9 ALL tumor cell lines *in vitro* and in *in vivo* tumor models.
Methods: The expression of CD38, CD 46, CD55 and CD59 on tumor cell lines was determined using flow cytometry. ADCC assays were carried out using tumor cell lines as target cells and human PBMC as effector cells in a ratio of 50:1. CDC assays were carried out using human serum at 10%. For *in vivo* model, NALM-6 ALL cells were injected intravenously via the tail vein in SCID mice and the animals were treated with daratumumab alone (10 mg/kg QWx5), vincristine (0.5 mg/kg QWx5) alone or in combination. For patient-derived ALL models, tumor cells were injected intravenously and the dosing was initiated when tumor burden in the peripheral blood was no more than 10%. The animals were treated with daratumumab alone (10 mg/kg QWx3) or a non-specific control antibody. Tumor growth in the blood was monitored as circulating CD45+ cells by flow cytometry.
Results: Evaluation of the expression of CD38 in 9 ALL cell lines suggested that CD38 expression varied among different cell lines but the majority (88%, n=8/9) had >1000 CD38 receptors per cell. Treatment with vincristine did not modulate CD38 expression. Daratumumab induced apoptosis in 5 out of 9 (55%) cell lines in the presence of a cross-linking agent and <20% apoptosis was observed in 4 cell lines with or without cross-linker. Interestingly, in tumor cell killing assays, daratumumab induced minimal ADCC (5-20%) and low levels of (2-5%) CDC mediated cell killing. In addition, no direct correlation was observed between CD38 expression and the extent of ADCC and CDC. The levels of complement inhibitory proteins (CIP) (CD46, CD55 and CD59) were evaluated to determine if these proteins affected CDC in response to daratumumab but no direct correlation was observed between CDC and CIP expression. In patient-derived $CD38^+$/$BCR\text{-}ABL^+$ B-ALL-7015 model, daratumumab treatment resulted in significant tumor growth inhibition $p<0.001$) by day 22 compared to the control antibody. In $CD38^+$/$BCR\text{-}ABL^-$ T-ALL-7473 model, treatment with daratumumab resulted in significant tumor growth inhibition by day 7 ($p<0.05$) but the animals developed disease by day 14 and were sacrificed on day 21. In NALM-6 cell line-based model, treatment with daratumumab either alone or in combination with vincristine showed significant prolongation of survival. The animals in the control group died by day 22 and the animals in vincristine group died by day 43, however, 80-100% of the animals in daratumumab alone or in combination with vincristine survived beyond day 88.
Summary and Conclusions: These data suggest that daratumumab inhibits growth of ALL tumors that express high levels of CD38 and may offer therapeutic benefit in the clinical setting of ALL.

P110

EVALUATION OF TP53 MUTATIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): RESULTS FROM IRON (INTERLABORATORY ROBUSTNESS OF NEXT-GENERATION SEQUENCING) II STUDY

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Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous disease that affects children and adults, with a different incidence and outcome. While there is a great knowledge of recurrent molecular aberrations (*i.e. BCR/ABL1*, *MLL* rearrangements, *E2A/PBX1*, *ETV6/RUNX1* in B-lineage ALL, *NOTCH1* and *FWB71* in T-lineage ALL), *TP53* mutations have not been widely investigated until recently; even at present, its evaluation was carried out in relatively small cohorts of patients, and in children at recurrence of disease.
Aims: Within the IRON (Interlaboratory RObustness of Next-generation sequencing) II study, 949 ALL samples were evaluated for *TP53* mutations to establish their incidence at diagnosis and relapse in both adults and children, and to correlate their presence with molecular and immunophenotypic subtypes.
Methods: Ten centers worldwide participated to the study. To identify *TP53* mutations, the 454 Life Sciences, a Roche company GS Junior system was used. A custom assay plate was designed to screen exons 4 to 11. This 96-well plate allowed studying eleven samples simultaneously, through the use of Multiplex Identifier (MID) sequences that identify each sample. For analysis, Quality Control (QC) analysis excluded samples with a coverage less than 80 reads in forward and reverse directions. The variant analysis was performed centrally, using Amplicon Variant Analyzer (AVA-Roche 454) and Sequence Pilot (JSI Medical Systems) software. A cut-off ³ 2% was applied to define variants. The *TP53* at ENSG00000141510 was used as reference sequence.
Results: A total of 877 samples were evaluable for variant analysis: 307 were pediatric and 558 adult; for 12 cases age was not available. The pediatric cohort was composed of 44 T-ALL and 263 B-ALL: B-ALL cases included 16 *BCR/ABL1+*, 13 *MLL* rearranged, 13 *E2A/PBX1+*, 43 *ETV6/RUNX1+* and 175 negative for the above aberrations. The adult cohort included 117 T-ALL and 441 B-ALL, the latter group thus subdivided: 126 *BCR/ABL1+*, 43 *MLL* rearranged, 9 *E2A/PBX1+*, 244 negative for recurrent fusion genes. Molecular characterization was not available for a total of 22 cases.
In 95 samples variants were identified, of which 64 were deleterious mutations: 40 missense (42.1%), 3 nonsense and 3 splice variants -all known IARC mutations- and 11 indels plus 7 frameshift (18.9%), not reported in any database; mutations affected mostly exons 5 (26.6%) and 8 (29.7%).
Overall, 664 patients were exclusively analyzed at diagnosis and 144 at relapse; 69 matched copies at diagnosis and relapses were analyzed. *TP53* mutations were observed at similar rate in B- and T-ALL 4.23% and 4.7% respectively.
Focusing on B-ALL subgroups evaluated exclusively at diagnosis, mutations were detected in 0.9% *BCR/ABL1+*, 7.5% *MLL* rearranged, 5.5% *E2A/PBX1+,* 3.3% *ETV6/RUNX1+*, 4.5% in molecularly negative cases; this latter subgroup was further evaluated for hypodiploidy and mutations were detected in 20% of cases. In B-ALL, comparison between diagnosis and relapse showed an increase of *TP53* mutation rate in both children (2.3% *vs* 8.3%, p=0.031) and adults (5.1% *vs* 11.5%, p=0.059). A similar trend was observed in T-ALL.
Summary and Conclusions: *TP53* mutations affect B- and T-ALL and their incidence does not significantly differ between children and adults. Within B-ALL molecular subsets, they were more recurrent in *MLL* rearranged, *E2A/PBX1+* and molecularly negative cases. An association with hypodiploidy is confirmed. Finally, a significantly increased incidence of *TP53* mutations at relapse is observed for both cohorts. Correlation with outcome is warranted.

P111

INVESTIGATION OF PH-LIKE ALL BY GENE SET ENRICHMENT ANALYSIS AND IDENTIFICATION OF THEIR SPECIFIC EXPRESSION GENES

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Background: Recently, "Ph-like" or "*BCR-ABL1*-like" acute lymphoblastic leukemia (ALL) has been proposed as a subset of B-cell precursor (BCP-) ALL with unfavorable outcome. Although Ph-like ALL lacks *BCR-ABL1* as well as other well-known fusion transcripts, they exhibit a gene expression profile of *BCR-ABL1*-positive ALL-like signature. Since some of Ph-like ALL possess tyrosine kinase (TK)-related fusion transcript and have sensitivity for tyrosine kinase inhibitor, it is important to distinguish Ph-like ALL from other fusion gene-negative BCP-ALLs. The diagnosis of Ph-like ALL is essentially performed by clustering analysis based on gene expression data of a group of patients using Affymetrix GeneChip®, diagnostic method applicable to individual patient using other microarray system is strongly desired. Therefore, we have developed methods to diagnose Ph-like ALL using Gene Set Enrichment Analysis (GSEA). As we presented previously, GSEA and clustering analysis identified distinct subsets of BCP-ALL with approximately 40% overlapping, whereas GSEA can also detect a group with poor prognosis including tyrosine kinase-related fusion transcript-positive cases (ASH 2013).
Aims: In this study, we intended to Ph-like ALL by GSEA based on gene expression data using different microarray system. Furthermore, we also investigated Ph-like ALL specific expression genes to develop alternative diagnostic method for Ph-like ALL.
Methods: Gene expression of 152 BCP-ALL cases enrolled on Tokyo Children Cancer Study Group (TCCSG) trials, including TCCSG L07-1602 and L09-1603, were analyzed by microarray Agilent Whole Human Genome DNA Microarray 4x44K v2, respectively. Based on these expression data, Ph-like ALL were investigated using GSEA, a computational method that ascertains whether a given gene set is significantly enriched in a list of genes ranked by their correlation with a phenotype of interest. The specific expression genes of Ph-like ALL, as well as Ph1 ALL, were investigated by fold-change analysis using GeneSpring GX software (Agilent).
Results: 10 cases (6.6%) of Ph-like ALL were identified in 152 cases of BCP-ALL by GSEA. They included the cases with Ph-like-related fusion transcripts, such as *P2RY8-CRLF2*, *IGH@-CRLF2*, *SNX2-ABL1*, *EBF1-PDGFRB*, as well as *ATF7IP-PDGFRB*, a novel fusion transcript involving *PDGFRB*. Some of them revealed early relapses. Next, genes specifically expressed in Ph-like and Ph1 ALL were explored. In similar to Ph1-ALL, high expression of *GPR110*, *MUC4*, *SEMA6A*, *GREM1*, *IL2RA* and *BAALC*, and low expression of *RICS*, *SOX11*, *RGS1*, *RGS13*, *BCL2L11*, *NR4A1*, *NR4A3* and *BTG3* were observed in Ph-like ALL. Interestingly, high-expression of *CRLF2* and *LDB3*, and low expression of *ABCC4* and *CCNA1* were observed only in Ph-like ALL cases.
Summary and Conclusions: We presented that GSEA can identify Ph-like ALL cases expressing uncommon TK-related fusion transcripts with poor prognosis. Since GSEA is applicable to identification of Ph-like ALL from individual patient based on the gene expression data using other microarray system than GeneChip®, it should be more practical for clinical use. We also identified specific genes expressed in Ph-like ALL cases and the development of method to distinguish Ph-like ALL from other BCP-ALL based on expression of these genes is now underway.

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ROLE OF THE HYSTONE DEACETYLASE INHIBITOR GIVINOSTAT (ITF2357) IN TREATMENT OF CRLF2 REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Novel genomic abnormalities of the *CRLF2* and *JAK2* genes have been recently reported in a subset of childhood ALL patients without known chromosomal aberrations, leading to the deregulation of the CRLF2 cytokine receptor pathway and associated to a poor prognosis. Inhibition of CRLF2/JAK2 signalling has the potential to become a therapeutic intervention for this subgroup of patients. In addition to the use of JAK2 inhibitors, numerous reports indicate that a broader antitumor activity is necessary to effectively treat tumor cells with aberrant JAK2 signalling. Alterations of epigenetic programmes is a common feature of tumor cells and novel therapies involving HDAC inhibition have been employed to treat different kind of neoplasms due to the key involvement of acetylation in the regulation of transcription and a number of signal transduction pathways. Previous studies have shown that the HDAC inhibitor Givinostat/ITF2357 has potent anti-tumor activity against hematological malignancies, including myeloproliferative neoplasms (MPN) carrying the JAK2V617F mutation. Indeed, the molecule is currently being evaluated in a phase1/2 study in patients with polycytemia vera.
Aims: In 10% of pediatric high-risk ALL and about 20% in Down Syndrome ALL patients, *JAK2* mutations are characterized by a specific and frequent R683G mutation. Interestingly R683 and V617, commonly mutated in MPN, are located in the same JAK2 pseudokinase domain. This opens the possibility that givinostat could have an inhibitory activity also on ALL blasts positive for JAK2 mutations and on ALL with disregulation of JAK/STAT pathway independently of JAK2 mutations.
Results: Here we demonstrate that givinostat at low concentrations inhibits proliferation and induces apoptosis in human B cell precursor leukaemia MHH-

CALL4 and MUTZ 5 cell lines positive for JAK2 mutations (R683G and I682F respectively) and *CRLF2* rearrangements. Givinostat inhibited proliferation with IC50 between 0.08-0.17 uM and induced apoptosis with IC50 between 0.17-0.25 uM. We investigated by RQ-PCR the expression of some target genes of the JAK-STAT pathway and B cells development. At 100-200 nM, Givinostat downregulated of the expression of *JAK2*, STAT5A and its target cMyc together with CRLF2 and IL7 receptor genes. Key factors of B cell development (EBF1, PAX5, IKZF1, E2A) were also downmodulated. The phosphorilation of target proteins downstream to CRLF2-mediated JAK-STAT pathway were assayed by phosphoflow analysis. Givinostat inhibited the phoshorilation of Stat5 both at a basal level and after stimulation with the CRLF2 ligand TSLP. These analysis were also performed on blasts from different BCP-ALL patients. Thus, cells from secondary xenograft with wt Jak2 and overexpressing CRLF2 due to P2RY8-CRLF2 fusion, were used. A low concentration (200 nM) of Givinostat inhibited proliferation and induced cell death of blasts at a three-fold greater rate than vehicle. Moreover, Givinostat inhibited Stat5 phosphorylation induced by TSLP stimulation. Downmodulation of basal level of genes of the JAK/STAT pathway was confirmed in these cells.

Summary and Conclusions: Altogheter, our data indicate that Givinostat inhibits the proliferation and induces apoptosis of ALL CRLF2 positive cells bearing or not JAK2 mutations through a specific downmodulation of the genes involved in JAK/STAT pathway and inhibition of their downstream effector molecules. Studies on xenograft models of BCP-ALL leukemia in NOD/SCID mouse are in progress.

P113

TELOMERE DNA CONTENT AND TP53 STATE PROVIDE AN INSIGHT INTO GENOMIC INSTABILITY IN OLDER ADULT PATIENTS WITH ALL

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Background: Genetic abnormalities in acute lymphoblastic leukemia (ALL) are a major contributing factor to age-related inferior outcomes. Unfavorable genetic anomalies - especially low hypodiploid/near triploid and complex karyotypes - rise with increasing age. Telomere shortening is a hallmark of aging. Telomere attrition results from critical telomere shortening, which -in the absence of TP53 - generates end-to-end chromosome fusion, causing genomic instability. Previous studies assessing cancer genome telomere DNA content in epithelial tumors revealed a significant association between cancer genome telomere gain (relative to matched non tumor sample)and frequency of structural variations/mutations characteristic of genomic instability. We hypothesized that older adult patients with ALL would exhibit leukemic telomere changes which could confer genomic instability and may account for poor risk genetics.

Aims: To evaluate the genetic landscape of patients aged ≥60 with *de novo* ALL by assessing cytogenetics, TP53 status and copy number alterations (CNA) of key ALL genes IKZF1, CDKN2A/B, PAX5, EBF1, ETV6, BTG1, RB1 and the PAR1 region (CSF2RA, IL3RA, CRLF2 and P2RY8) at diagnosis. To assess the relationship between genetics and leukemic telomere DNA content.

Methods: Genomic DNA from diagnostic samples of 52 patients aged ≥60 enrolled in the prospective UKALL14, UKALL60+ and MRD feasibility trials was assessed for CNA using Multiplex Ligation Probe Amplification (MLPA) p335-B1 kit. Relative telomere length was quantified using monochrome multiplex quantitative PCR (qPCR) (Cawthon 2009) in diagnostic and matched remission DNA (N=22). Relative Telomere/Single copy gene (β globin) ratio generated for each leukemic DNA sample was compared to that generated for matched remission DNA and expressed as a log2 ratio. Telomere gain was defined as log2 ratio >0 and loss as <0. TP53 was assessed by fluorescent *in situ* hybridization for deletions and direct sequencing of hot spots exons 5-8 for mutations (N=34). Minimal residual disease (MRD) was assessed by immunoglobulin gene/T cell receptor rearrangement or BCR-ABL qPCR.

Results: Patient characteristics are shown in the Table 1. Fourteen of 52 patients (27%) and 12 of 33 (36%) Philadelphia negative patients had ≥4 genetic anomalies by MLPA, significantly higher than the 3% seen in a younger cohort from the UKALLXII/ECOG2993 trial. The median number of CNA was 2 (range 0-7). Ten had TP53 deletions and/or mutations (del±mut). Eight of 22 patients (36%) had telomere gain. Those with telomere gain had significantly more CNA (p<0.01); median 4 (range 2-7) compared to 1 (range 0-2) in those with telomere loss (p<0.01). Of the 22 patients where telomere state was evaluated, cytogenetic data was available in 20. Patients with telomere gain were more likely to have low hypodiploid/near triploid or complex karyotype (5/7) and those with telomere loss were more likely to have t(4;11), t(9;22) or standard risk cytogenetics (12/13); p<0.01. Six of 8 patients with telomere gain had TP53 del±mut *versus* 2 of 12 with loss (p=0.01). There was no association between MRD state post induction and CNA frequency or telomere state. Paired diagnostic/relapse DNA samples were available in two patients. In both cases, relapse DNA showed telomere gain relative to the diagnostic DNA and had new CNA and/or cytogenetic anomalies.

Table 1. Patient characteristics.

Summary and Conclusions: The prevalence of genetic anomalies in our older cohort was strikingly higher than that of a published younger cohort. Of note, the pattern of telomere DNA alteration in the older adult ALL was similar to that reported in solid tumors. There was a significant association between telomere gain and both CNA frequency and nonreciprocal translocations. The prevalence of TP53 del±mut in those with telomere gain supports previous observations that unchecked telomere dysfunction plays a role in genomic instability. This could account for the poor risk genetic abnormalities characteristic of ALL in older adult patients. However leukemic telomere gain could be a response to rather than the cause of a genetic crisis caused by constitutional telomere attrition. A potential causal relationship will be explored in future mechanistic studies.

P114

FUNCTIONAL STUDIES OF IK6 EXPRESSION SHED LIGHT ON THE COOPERATING MECHANISMS UNDERLYING THE GAIN OF MALIGNANCY IN B-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Alterations of the transcription factor *IKZF1* gene occur in approximately 70% of *BCR-ABL1* and 29% of *BCR-ABL1*-negative ALLs. Deletion of exons 4-7 is the most common alteration, resulting in the expression of dominant negative cytoplasmic isoform, Ik6. We found *IKZF1*Δ4-7 in 45% of *BCR-ABL1*-negative ALL by a sensitive (10^{-4}) PCR method in 161 consecutive 25-59 year olds enrolled on UKALL14; approximately double the reported incidence. *IKZF1*Δ4-7 was typically subclonal with <0.01% of cells bearing *IKZF1*Δ 4-7 in the subclonal cases, compared to in >90% of cells in cases where *IKZF1* was part of the major clone. Analysis of paired relapse samples from 14 diagnostic cases with *IKZF*1Δ4-7 showed patterns discrepant in both directions, as well as concordant suggesting that the gain of malignancy conferred by *IKZF1* lesions must be context dependent.

Aims: We aimed to study the context and mechanisms by which *IK6* expression contributes to enhanced leukaemogenesis and to determine cooperating lesions.

Methods: Three (two *BCR-ABL1* neg, NALM-6 and REH and one *BCR-ABL1* pos, SD-1) *IKZF1* wild-type pre-B ALL cell lines were evaluated by MLPA (P335-100R). We over-expressed dominant negative isoform, *IK6*, by retroviral transduction with MSCV-IK6-IRES-mRFP (kindly provided by Charles Mullighan). Cells were flow-sorted to >95% purity by monomer-red fluorescent protein expression. mRFP alone vector-transduced cells were the control. We evaluated drug sensitivity to vincristine, daunorubicin and cyatarabine, cell prolifer-

ation kinetics (MTS assay) and DNA double strand break (DSB) repair following exposure to γ irradiation by confocal microscopy for antiphosphorylated H2AX. Data are from 3 independent experiments.
Results: MLPA confirmed the *IKZF1* wild type status of all cell lines prior to manipulation. SD-1 showed no other genetic abnormalities. Nalm-6 (near diploid karyotype) and REH (ETV6-RUNX1 positive) both showed homozygous deletion of *CDKN2A* and *CDKN2B* and duplication of *PAX-5* exon 10. REH also showed deletion of *ETV-6* and *BTG-1*. The doubling time of SD-1/IK6 was significantly greater than that of SD-1/mRFP, 14.5 *vs* 28.3 hrs (p=0.042), whereas there was no significant difference in doubling times in either of the *BCR/ABL1* neg cell line pairs. SD-1/IK6 cells were significantly less sensitive than SD-1/mRFP to all the drugs tested, at all the 3 concentrations tested (p values<0.05). By contrast, there was no difference in drug sensitivity between Nalm-6/IK6 and REH/IK6 and their control counterparts. The Figure 1 shows sensitivity to daunorubicin as an example. SD-1/IK6 showed significantly less DSB repair after γ irradiation than SD-1/mRFP at each of the time-points tested (20min (p=0.031), 3hrs (p=0.043), 5hrs (p=0.042), and 24hrs (p=0.014)). By contrast there was no difference in DSB repair in either of the *BCRABL-1* negative cell line pairs.

Figure 1.

Summary and Conclusions: Our data show that overexpression of *IK6* in a B-precursor ALL cell lines conferred a higher growth rate, drug resistance and compromised DNA DSB repair when *BCRABL1* was present. None of these changes occurred in the two *BCR-ABL1* negative cell lines studied, despite the presence of other potential cooperating lesions. Our data suggest a starting point and a model for further exploration of the link between *IKZF1* mutations and *BCR-ABL1* pos ALL and work is ongoing. By contrast, the poor risk phenotype conferred by IK6 expression in *BCR-ABL1* neg ALL cannot be modeled or elucidated further by this dataset suggesting that alternative co-operations to those revealed by MLPA analysis are in operation.

P115

ETV6-RUNX1 DE-REGULATES THE CYTOSKELETON AND MIGRATION PROPERTIES OF B CELL PROGENITOR CELLS

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Background: Although ETV6-RUNX1 fusion is a frequent initiating event in childhood leukemia, its role in leukemogenesis is still unknown. The main impact of the fusion itself is to generate and sustain a clone of clinically silent pre-leukemic B cell progenitor (BCP) cells. Second hits, occurring even several years later, are required for overt disease. The understanding of the features and interactions of ETV6-RUNX1 positive cells during this "latency" period might help explaining how they can persist, and whether they could be prone to additional genetic changes. In particular, the site of localization and interaction with the microenvironment is crucial to sustain the hematopoietic stem cells in quiescence and the survival of both normal and pre-leukemic cells.
Aims: The aim of this work was to investigate whether the ETV6-RUNX1 pre-leukemic clone showed alterations in its adhesive and migratory properties that could provide a rationale for its persistence and proliferation.
Methods: We have employed two different model systems to study the ETV6-RUNX1 pre-leukemic phase. Indeed, this type of study is not feasible in clinical samples at diagnosis of leukemia, where the analysis of the fusion function is confounded by the additional genetic abnormalities. We have therefore used a murine progenitor cell line (Ba/F3), with hormone inducible ETV6-RUNX1 expression and we confirmed our results in pre-BI cells, primary cells derived from a wild-type mouse fetal liver.
Results: We observed that the expression of ETV6-RUNX1 in Ba/F3 cell line resulted in changes in the cellular morphology and phenotype: several molecules involved in cell adhesion were deregulated in expression and we observed an increase in the adhesion of ETV6-RUNX1 positive cells to murine endothelial cell lines. We showed that the expression of the fusion in Ba/F3 cell line caused alteration in the expression of genes regulating cell shape, formation of pseudopodia, cell migration, actin and microtubule organization. In particular, among the most over-expressed genes in ETV6-RUNX1 positive cells, we identified two negative regulators of CDC42. Consistently, we observed a reduction of CDC42 at the transcription and protein level. CDC42 not only has a pivotal role in cell cycle progression, but also in cytoskeleton rearrangement during directional migration. In parallel, we investigated the migration abilities of ETV6-RUNX1 inducible Ba/F3 cells. Interestingly, we found that the fusion gene significantly impaired the chemotactic response to CXCL12, although the cell-surface expression of the receptor CXCR4 was unaffected. Indeed, the CXCL12 chemotaxis defect was not due to a general impairment of movement of ETV6-RUNX1 positive cells, as their spontaneous motility and migration towards a general stimulus was instead increased. We then excluded a possible role in this migration defect of several players of CXCL12/CXCR4 pathway, including CXCR7, FLT3L receptor, CCL2/CCR2 and EGF/EGFR axes. We were able to demonstrate that ETV6-RUNX1 impairs the calcium flux, a very proximal CXCR4 signaling event, and the phosphorylation of ERK kinase, as a downstream event. Importantly, the results observed in the Ba/F3 ETV6-RUNX1 inducible expression system were reproducible in primary pre-BI cells.
Summary and Conclusions: The abnormalities described here could alter the interaction of ETV6-RUNX1 pre-leukemic BCP cells with the microenvironment and contribute to the pathogenesis of the disease, with potential implications to develop strategies for effective eradication of leukemia.

P116

ECTOPIC EXPRESSION OF THE HEDGEHOG PATHWAY LIGANDS SHH AND IHH IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

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Background: The hedgehog (HH) pathway plays an important role in the development of normal tissues and mutations in HH pathway genes have been identified in various solid tumors. In addition, ectopic activation of the HH pathway has been reported in hematological malignancies and leads to increased growth, stem cell survival, resistance to therapy and relapse.
Aims: Based on the importance of the HH pathway in solid cancers, hematologic malignancies and in normal T-cell development, we investigated its potential role as an oncogenic factor and a potential target for therapy in T-ALL.
Methods: We analyzed gene expression profiles of human T-ALL samples, and determined the effects of HH ligands IHH or SHH stimulation on T-ALL cell lines. SMO, GLI1, SUFU or control siRNA were transfected by electroporation in the same cell lines. For bone marrow (BM) transplantation assays, retroviral Shh, Ihh, JAK3(M511I) and JAK3(L857Q) constructs, were used to transduce primary BM cells and transfected cells were injected in irradiated recipient mice.
Results: Analysis of gene expression profiles of primary T-ALL samples identified a subset of T-ALL cases with elevated expression of the SHH and IHH ligands, with a clear correlation with increased levels of the main activators of the pathway, SMO and GLI1. In addition, a strong correlation between hedgehog ectopic expression and certain T-ALL genes was observed. Inhibition of the HH pathway caused a decrease of proliferation of various T-ALL cell lines, which was associated with downregulation of GLI1 and BCL2, two well-established HH pathway target genes. Furthermore, knock down of negative regulators of the pathway led to increased cell proliferation. These data indicate that a subset of T-ALL cases have ectopic expression of HH ligands that may activate an autocrine loop. One of the genes that was found to be co-expressed with components of the HH pathway in T-ALL patients was the JAK3 kinase. For this reason we took advantage of a JAK3 mutation driven mouse leukemia model in order to decipher the role of the ectopic HH pathway activation in a leukemic background. Expression of JAK3 M511I or JAK3 L857Q activating mutants in the BM cells of mice led to the development of a fatal transplantable T-ALL disease with a latency of >150 days. The immunophenotype of the leukemic clones were variable and clonal populations could arise at the CD4CD8 double negative, CD4CD8 double positive or CD8 single positive stages. Ectopic expression of the HH ligands in the JAK3 mouse leukemia model did not lead to an acceleration of the disease, but caused a consistent block in differentiation of the leukemia cells at the CD4CD8 double positive and CD8 single positive stage in mice expressing the Ihh and Shh ligand respectively. Also in wild type animals, ectopic expression of Ihh or Shh caused a block of differentiation of CD4CD8 double positive and CD4CD8 double negative thymocytes. As a consequence, the percentage of mature T cells in the blood and spleen was significant reduced. Moreover, all mice expressing the HH ligands showed enlarged lymph nodes with a major lymphocytic infiltration.
Summary and Conclusions: In this report we demonstrate that the HH pathway is activated in a subset of T-ALL patients by ectopic expression of the ligands by the leukemia cells. In addition, HH activation affects T-ALL cells growth *in vitro* and causes a block of differentiation in a JAK3 mutant driven mouse T-ALL model. In conclusion, our data indicate that HH pathway can play a role at least in a fraction of T-ALL patients and therefore considered as a new target for therapy in T-ALL.

Acute lymphoblastic leukemia - Clinical 1

P117

NON-INTENSIVE, BUT NO-INTERRUPTIONS PROTOCOL PROVIDES COMPARABLE RESULTS IN AYA AND OLDER ADULTS WITH PH-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA: THE RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA (RALL) STUDY GR

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Background: It is now broadly discussed that so called "pediatric approach" applied in adult ALL is more reasonable and effective especially in adolescence and younger adults (AYA), and pediatric schedules are considered to be more aggressive comparing to adults protocols.
Aims: RALL has initiated a pediatric oriented not in intensity but in prolonged non-stop cytostatic drugs application protocol and in 2009 has started a prospective multicenter trial for adult Ph-negative ALL "ALL-2009" based on the following criteria: 1) less intensity but no-interruptions during induction/consolidation/maintenance; 2) prolonged up to the end of treatment L-asparaginase (Σ=590.000 IU) if tolerable; 3) autologous stem cell transplantation with BEAM conditioning for T-cell ALL followed by maintenance. Allo-BMT was an option for high risk patients with sibling donors. The study is registered on the ClinicalTrials.gov public site; NCT01193933.
Methods: From Jan, 2000, till Jan, 2014, 30 centers enrolled 231 pts: median age 28 years (15-55 years), 104f/127m; in 2,6% phenotype was unknown (n=6), B-lineage ALL - 62,8% (n=145), T-lineage ALL - 34,2% (n=79), biphenotypic - 0,4% (n=1). Cytogenetics was available in 50% of patients (n=116) and 47,4% of them (n=55) had normal karyotype (NK). 27,8% of patients (n=55) were in the standard risk (SR) group (WBC <30 for B-lineage, <100 for T-lineage; EGIL BII-III, T-III; LDH <2N; no late CR; t(4;11)-negative), 72,2% (n=143) - in the high risk (HR) group (WBC ≥30 for B-lineage, ≥100 for T-lineage; EGIL BI, T-I-II-IV; LDH >2N; late CR; t(4;11)-positive), 18 patients were not qualified by risk group. The analysis was performed in Jan, 2014.
Results: Blast cell count ≥25% at +8 day leaded to the substitution of prednisolone by dexamethazone in 60% of patients. The portion of non-responders to PRD was 51% in SR and 64% - in HR groups (p=0,06). CR rate in AYA was=97 (93,2%); in older adults ALL=75 (82,4%) with 4 (3,8%) and 13 (14,3%) induction deaths and 3 (2,9%) and 3 (3,3%) resistant leukemia from 104 and 91, respectively. L-asparaginase was stopped due to toxicity in 19% of patients, but it did not influence long-term results. 20 of 65 (31%) CR T-ALL patients underwent autologous BMT after BEAM conditioning and proceeded to the maintenance. No relapses were registered in this group. Allogeneic BMT was performed in 9 patients on our protocol. At 48 mo OS for the whole group constituted - 65,6%, DFS - 69,3%. OS was substantially better (p=0,01) in AYA (72%) than in older adults (57,3%), but DFS did not differ much (p=0,3): 71% *vs* 66,6%, demonstrating high efficacy of the treatment approach in both groups. OS and DFS differed in B-ALL patients with NK in comparison with abnormal karyotype: 80% *vs* 57%,(p=0,01) and 81% *vs* 61%, respectively (p=0,02), but not in T-ALL patients.
Summary and Conclusions: Our data demonstrate that the applied treatment is rather effective in both age cohorts though OS is somewhat worse in older adults. DFS approached to 66,6% in older adults and exceeded 70% in the AYA. Abnormal karyotype for B-ALL is a steady risk factor adversely influencing OS and DFS.

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SIGNIFICANCE OF MRD BY BCR-ABL QUANTITATIVE PCR DURINGCOMBINATION TREATMENT WITH VARIOUS TKIS PLUS CHEMOTHERAPY FOLLOWED BY ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR PH-POSITIVE ADULT ALL

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Background: Recently, next-generation tyrosine kinase inhibitors (TKI) that inhibit the BCR-ABL tyrosine kinase more potently than imatinib have been used in the treatment of Ph+ ALL.
Aims: This study evaluated the clinical significance of MRD from the analysis of two sets of prospective data on the treatment outcomes of imatinib and nilotinib plus multiagent combination chemotherapy for newly diagnosed adult Ph+ ALL.
Methods: Patients with newly diagnosed Ph+ ALL and either 15 or 18 years old or over were eligible for each clinical trial when they met the inclusion criteria and gave informed consent. All patients received induction treatment composed of vincristine/prednisolone/daunorubicin (mVPD) plus concomitant imatinib or nilotinib. After achieving hematologic complete remission (HCR), either five cycles of consolidation therapy with concomitant TKI followed by 2 years of TKI maintenance (non-alloHCT) or allogeneic hematopoietic cell transplantation (alloHCT) was performed as post-remission therapy. MRD was evaluated in terms of the ratio between the amount of *BCR-ABL*/*G6PDH* RNA of peripheral blood, and was monitored from the time of HCR and every 3 months thereafter (for alloHCT recipients, just before alloHCT and every 3 months afterward) for 2 years. MRD was monitored with a LightCycler-T® (9;22) Quantification Kit (Roche Diagnotics [Schweiz] AG, Switzerland) with a sensitivity of 1×10^{-6}. In terms of molecular response, MR3 was defined as the achievement of $\leq1\times10^{-3}$, and molecular complete remission (MCR, MR6) was defined as the achievement of $\leq1\times10^{-6}$.
Results: Eighty-seven patients on an imatinib-based protocol (cohort I) and 90 patients on a nilotinib-based protocol (cohort N) were sufficient for MRD analysis. The median age of patients in cohort N was significantly higher than that of cohort I (47 *vs.* 41 years old; p=0.035). The HCR rate of all patients in both cohorts was 92.7% and the MCR rate at the time of HCR was 52.4%, and the cumulative rate of MCR was 93.9% among 164 patients who achieved HCR, which were not significantly different between cohort I and N. When treatment outcomes were compared according to the MRD level at four time points, the proportion of patients achieving MR6 in cohort N was slightly higher than that of cohort I with near statistical significance at 3 time points. After 35.0 months of follow-up for surviving patients, the 3-year hematologic relapse-free survival (HRFS) rate was 59.9%, which was significantly higher in cohort N than in cohort I (73.5% *vs.* 48.5%; p=0.001). The 5-year overall survival (OS) rate was 35.0%, which was higher in cohort N than in cohort I, with marginal statistical significance (57.0% *vs.* 39.2%; p=0.053). The achievement of MR3 at the time of HCR was an important prognostic factor (5-year OS rate, MR3 *vs.* no MR3=33.9% *vs.* 14.3%; p=0.001) and showed a significant difference in outcomes, especially in the non-alloHCT cohort (5-year OS rate, MR3 *vs.* no MR3=32.3% *vs.* 50.0%, p=0.877 in the alloHCT cohort and 25.3% *vs.* 6.9%, p<0.001 in the non-alloHCT cohort). The achievement of MR6 at 3 months after alloHCT was an important prognostic factor in both cohort I and N.
Summary and Conclusions: Combination therapy with nilotinib is superior to that of imatinib in terms of the achievement of deep molecular response, the prevention of hematologic relapse, and the OS. MRD monitoring based on the *BCR-ABL/G6PDH* was a meaningful method for the prediction of outcome, and the achievement of MR3 at 3 months after HCR and MR6 at 3 months after alloHCT were important prognostic indices for predicting OS.

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THE POOR PROGNOSTIC VALUE OF IKZF1-DELETION IN CHINESE ADULT COMMON B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND THE SUPERIORITY OF STEM CELL TRANSPLANTATION OVER CHEMOTHERAPY FOR IKZF1 DELETED PATIENTS

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Background: Early studies suggest that IKZF1 deletions are associated with an adverse prognosis, especially in pediatric B-cell acute lymphoblastic leukemia (B-ALL) patients. Nevertheless, the prognostic role of IKZF1 deletions alone in BCR-ABL-positive or BCR-ABL-negative patients in adult common B cell (Com-B) ALL is not well defined. It remains unclear whether HSCT (hematopoietic stem cell transplantation) is superior to chemotherapy alone as postremission treatment for IKZF1 deleted patients.
Aims: The objectives of the study were designed to evaluate the prognostic role of the deletions of IKZF1 in adult Com-B ALL and to compare the efficacy of HSCT with that of chemotherapy alone as postremission treatment for IKZF1 deleted adult ALL.
Methods: Untreated 190 adult ALL patients were recruited from 2007-2012. Patients received 1-2 cycles of induction therapy and, if in remission, were allowed to select ongoing systemic chemotherapy or HSCT. BCR-ABL positive patients were treated with chemotherapy or HSCT plus tyrosine kinase inhibitors (TKIs). Deletions in the exons 4 to 7, exons 4 to 8, exons 2 to 7, exons 2 to 8 of the IKZF1 gene (types △4-7, △4-8, △2-7, and △2-8) were detected using multiplex RQ-PCR, multiplex fluorescent PCR and sequence analysis. The study's end points of disease-free survival (DFS) and overall survival (OS) were analyzed using the Kaplan-Meier method.
Results: The detection rate of IKZF1-deletion in untreated 190 ALL patients was 33.7%, including 32 (66.7%) with BCR-ABL1-positive ALL, and 32(23.0%) with BCR-ABL1-negative ALL. The 64 positive cases included 4 (18.2%) cases with type progenitor B ALL, 6 (22.2%) cases with precursor B ALL, 54 (45.0%) cases with Com-B ALL, and no one case with T ALL. In the 120 Com-B ALL, patients with IKZF1 wide type (n=66) had a significantly better prognosis than those with IKZF1-deletion (n=54) (OS time: 63.2±2.0 *vs* 34.7±2.3 months, *P*=0.035; DFS time: 40.9±3.5 *vs* 19.8±2.4 months, *P*=0.007; Cumulative incidence of relapse (CIR): 47.8% *versus* 29.7%, *P*=0.027). Patients with IKZF1-deletion but not BCR-ABL rearrangement (n=27) experienced inferior outcome than patients with BCR-ABL rearrangement but not IKZF1-deletion (n=13) (DFS time: 14.4±2.2 *vs* 43.0±5.8 months, *P*=0.005, Figure 1 A; CIR: 52.5% *versus* 15.4%, *P*=0.033) and patients without IKZF1-deletion or BCR-ABL rearrangement (DFS time: 14.4±2.2 *vs* 39.2±4.0 months, *P*=0.004; CIR: 52.5% *versus* 27.5%, *P*=0.037). In Com-B ALL-HSCT group, IKZF1-deleted patients (n=25) had an unfavorable outcome compared to IKZF1 wild-type patients (n=41) (DFS time: 19.8±2.4 *vs* 40.9±3.5 months, *P*=0.017) while in chemotherapy-alone group there was no difference between patients with IKZF1-deletion (n=22) and patients with IKZF1 wild-type (n=23). In IKZF1-deleted group, patients received HSCT acquired longer OS time (39.2±1.8 *vs* 27.7±5.1 months, *P*=0.004, Figure 1 B) and DFS time (23.4±2.8 *vs* 12.7±3.0 months, *P*=0.014, Figure 1 C) than those received chemotherapy only. Independent negative prognosis value of IKZF1 deletions and positive prognosis value of HSCT were confirmed by multivariate analysis.

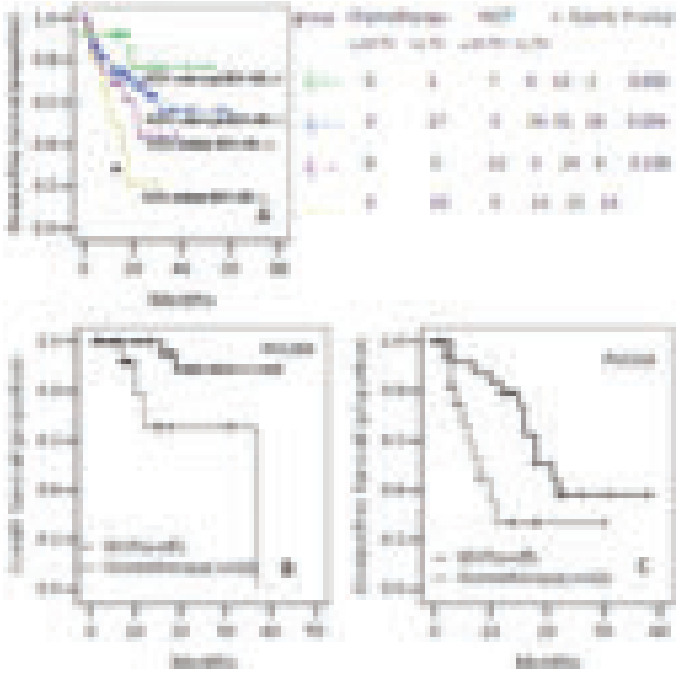

Figure 1. Survival curves of patients with IKZF1-deletion in the Com B ALL. Results from DFS ratios (Figure 1A) indicate that patients with IKZF1-deletion but not BCR-ABL rearrangement experience a poor prognostic outcome. Each subgroup is compared with IKZF1 deleted and BCR-ABL negative subgroup (see asterisk), respectively. Results from both OS (Figure 1B) and DFS (Figure 1C) ratios exhibit that HSCT is superior to chemotherapy alone as postremission treatment for patients with IKZF1-deletion.

Summary and Conclusions: IKZF1 deletions are associated with poor prognosis which is even more obvious than BCR-ABL rearrangement due to the use of TKIs in Chinese adult Com-B ALL. HSCT is superior to chemotherapy alone as postremission treatment for patients with IKZF1-deletion.

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PROGNOSTIC IMPACT OF IKZF1 DELETIONS IN PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA TREATED ACCORDING TO NOPHO PROTOCOLS – THE SWEDISH EXPERIENCE

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Background: Recently, *IKZF1* deletions have been shown to be associated not only with the leukemogenic process but also to confer a poor prognosis in all risk groups of B-cell precursor ALL. In a previous study, we used Multiplex Ligation-dependent Probe Amplification (MLPA) to investigate the presence of *IKZF1* deletions in bone marrow DNA from 116 children diagnosed with BCP ALL in a single center and treated according to NOPHO protocols. Deletions were detected in 16% of cases; both event free survival and overall survival were significantly reduced in the *IKZF1*-deleted group compared to the group with intact *IKZF1* (Öfverholm *et al.* Leukemia 2013).
Aims: The aim of our study is to further validate the prognostic impact of *IKZF1* deletions, and to ascertain if they are an independent risk factor when minimal residual disease (MRD) data are taken into account.
Methods: We investigated 362 pediatric BCP ALL cases accrued between 1992 and 2013 and uniformly treated according to the NOPHO-ALL 1992, 2000, and 2008 protocols in Sweden. The *IKZF1* deletion status, based on either SNP array (n=246) or MLPA (n=116) analyses, was known in all cases.
Results: Preliminary analyses indicate that the prognostic significance of *IKZF1* deletions is retained in the larger cohort, with the highest impact on survival being observed in the group lacking risk-stratifying aberrations at diagnosis. Updated survival analyses and the clinical implications of *IKZF1* aberrations will be presented at the conference.
Summary and Conclusions: We conclude that our data support the relevance of *IKZF1* deletion as a prognostic factor for childhood BCP ALL treated according to NOPHO protocols.

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COMPARISON OF BONE MARROW AND PERIPHERAL BLOOD SAMPLES FOR MINIMAL RESIDUAL DISEASE MONITORING IN INFANTS WITH MLL-REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA TREATED BY MLL-BABY PROTOCOL

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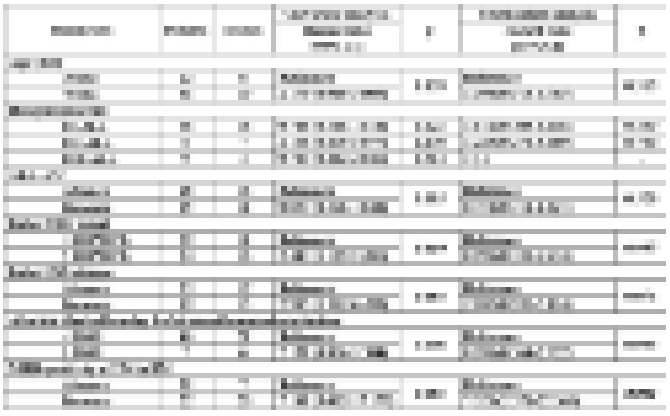
Background: Minimal residual disease (MRD) is powerful tool for prediction of treatment outcome in leukemia patients of various age groups, including infants with acute lymphoblastic leukemia (ALL). In the vast majority of cases only bone marrow (BM) samples are used for MRD detection.
Aims: To estimate prognostic significance of MRD in BM and peripheral blood (PB) by qualitative detection of different *MLL* fusion gene transcripts (FGt) in infants with ALL treated by MLL-Baby protocol.
Methods: Fifty three infants (20 boys and 33 girls) and with defined *MLL* rearrangements were included in the current study. Median age was 5.3 months (range 0.03-11.80). MRD detection was performed from BM and PB samples by real-time quantitative PCR and nested RT-PCR with sensitivity non-less than 1E-04. MRD-negativity was defined as absence of FGt in the both assays. Median of follow-up period in the observed group was 5.2 years. TPs for MRD assessment were as follows: day 15 of remission induction (time point (TP) 1), at the end of remission induction (TP2), after each course of all-trans retinoic acid (ATRA) administration (TP3-TP9). Informed consent was obtained in all cases.
Results: We estimated 142 paired BM/PB samples. 79 samples were double positive, 41 were double negative Thus concordance between MRD results in BM and PB samples achieved 84.5%. Concordance varied between different TPs of MLL-Baby protocol from 79.0% to 100%. The highest concordance rate was at TP4 and TP7 (92.3% and 100% respectively). All discrepant results (22 samples 15.49%) were BM-positive/PB-negative. MRD-positivity at TP4 in BM led to unfavorable outcome. Event-free survival was significantly lower in MRD-positive group in comparison to MRD-negative one (9.9±6.1 *vs* 75.9±8.0, p=0.001). MRD-

positivity at this TP in BM was the only significant factor in the diagnostic model where initial risk factors (age at diagnosis, initial WBC count, immunophenotype, CNS disease, presence of *MLL-AF4*) were combined to response criteria (number of blast cells at day 8 of dexamethasone prophase) (Table 1). We could not find any TP when MRD data obtained from PB samples had prognostic values.

Table 1.



Summary and Conclusions: Despite high qualitative concordance rate between BM and PB samples we could not show prognostic significance of MRD monitoring by FGt detection in PB. Univariate and multivariate analysis revealed that MRD-positivity at TP4 in BM was significant and independent prognostic factor of unfavorable outcome

P122

***IN VIVO* RESPONSE TO REMISSION INDUCTION POLY-CHEMOTHERAPY IN NOD/SCID/HUALL REFLECTS PATIENT RISK AND OUTCOME**

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Background: Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in children and adolescents. Continuous improvement of therapy regimens increased the 5-year event free survival rate to 80%. Therapy response to initial remission induction treatment, which typically combines vincristine, corticoids, and asparaginase, is an important factor for patient risk stratification. Nevertheless, overall 20% of patients diagnosed with ALL encounter relapse. Thus, novel therapeutics are urgently needed and will most likely be combined with already established treatment regimens. For this reason, we took advantage of our previously established NOD/SCID/huALL xenograft model, which resembles human disease and is a suitable model for evaluating novel substances in a preclinical setting.
Aims: In this study, we aimed to establish a poly-chemotherapy treatment regimen in our NOD/SCID/huALL xenograft model in order to test the therapy effectivity by combining vincristine (V), dexamethasone (D), and asparaginase (A) on different patient-derived xenograft (pdx) ALL samples, which further can be combined with the evaluation of novel compounds.
Methods: Pdx B-cell precursor ALL samples (n=6) were established in the NOD/SCID/huALL model and engraftment efficiency was monitored weekly by the detection of human cells in the recipients' peripheral blood. Multi-agent chemotherapy combining VDA or vehicle-treatment was started upon appearance of 5% human cells and was administered for 2 weeks. Time to leukemia reoccurrence (TTR) was calculated as the time from treatment start to onset of leukemia-related morbidity of mice, which then were sacrificed confirming full-blown leukemia by the detection of high percentages of human cells in spleen, bone marrow, and peripheral blood.
Results: In each mouse receiving VDA of all 6 individual pdx ALL samples, a significant delay of post-treatment leukemia manifestation was observed compared to vehicle-treated recipients (Paired T-test, p=.0021). Interestingly, leukemia manifestation after VDA treatment in one pdx ALL sample did not reoccur within an observation time of 30 weeks suggesting induction of successful long-term remission. Previously, we showed that a short time to leukemia engraftment (TTL^{short}) of diagnostic patient ALL cells in NOD/SCID mice is strongly associated with an early patient relapse, while long time to leukemia engraftment (TTL^{long}) of diagnostic patient ALL cells in our model was shown to have favourable patient outcome. According to TTL^{short}/TTL^{long} phenotypes of pdx ALL samples we also observed clear differences of post-treatment leukemia reoccurrence after VDA remission induction treatment. Interestingly, we observed longer TTR of VDA treated TTL^{long} xenografts compared to significantly shorter TTR of VDA treated TTL^{short} xenografts, which resembles favourable outcome and higher relapse risk, respectively, suggesting that post-treatment leukemia reoccurrence is an intrinsic factor of ALL cells.
Summary and Conclusions: In summary, we successfully established an *in vivo* preclinical setting in our NOD/SCID/huALL xenograft model for analysing novel substances for the treatment of ALL in combination with already applied multi-agent chemotherapy modalities. Most importantly, we observe that our model shows leukemia characteristics of human disease, which is reflected by combination chemotherapy in association of patient relapse probability and time to leukemia reoccurrence after VDA treatment in pdx ALL samples.

P123

USE OF BCR/ABL GENOMIC BREAKPOINT FOR MRD MONITORING IN CHILDHOOD ALL AND ITS COMPARISON WITH OTHER STANDARD METHODS

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Background: Among childhood ALL subtypes, patients harbouring BCR/ABL fusion belong to those with the worst prognosis. The role of minimal residual disease (MRD) in this subgroup is unclear; however, MRD is used in HSCT indication according to the worldwide protocol for the BCR/ABL+ ALL - EsPhALL. In general, the standard method for MRD monitoring in ALL is quantification of Ig/TCR rearrangements. Quantification of the fusion transcript is easier/cheaper technique; however, as we showed previously, the correlation between these two methods in some BCR/ABL+ patients is not satisfactory. We demonstrated presence of BCR/ABL within the non-lymphoid (Ig/TCR-negative) population and worse prognosis of such patients (Zaliova, 2009). Moreover, mRNA quantification might not always correspond to the number of positive cells (possible changes of expression under particular conditions or in various cell types).
Aims: We aimed to establish MRD monitoring based on the detection of the genomic (intronic) BCR/ABL fusion and the BCR/ABL DNA quantification.
Methods: We performed multiplex long distance DNA PCR to find genomic BCR/ABL breakpoint in 15 patients with minor BCR/ABL fusion. The patient-specific fusion sequence was used for MRD quantification by qPCR. Using this approach we measured 149 bone marrow (BM) and 64 peripheral blood (PB) samples from 12 patients. The results were compared with data from Ig/TCR and BCR/ABL transcript quantification. For correlation analysis double negative results were excluded.
Results: We found genomic BCR/ABL breakpoint in 14/15 (93%) patients. Analysis of MRD data in BM samples confirmed poor correlation between Ig/TCR and BCR/ABL mRNA quantification (R^2=0.72; n=89). Moreover, we saw similar difference also when comparing the two DNA methods (Ig/TCR *vs.* BCR/ABL DNA, R^2=0.68; n=92). The correlation between BCR/ABL mRNA and BCR/ABL DNA approach was more satisfactory (R^2=0.83; n=98). Correlation in PB samples was higher (R^2=0.79, 0.80 and 0.89, respectively).
Summary and Conclusions: We believe that the BCR/ABL quantification at genomic level brings the most precise picture of leukaemic burden. Our analysis confirmed poor correlation between BCR/ABL and Ig/TCR data. As we showed previously, at least in some patients this discrepancy is caused by the presence of BCR/ABL in non-lymphoid cells. In our cohort, correlation of the two BCR/ABL based methods was in general good. However, in some patients (4/12) we observed higher genomic BCR/ABL MRD compared to the transcript levels in consecutive samples suggesting either that treatment (possibly by imatinib) can in some patients influence the BCR/ABL expression or that BCR/ABL+ cells might differ in their responsiveness to treatment (cells with low BCR/ABL expression being more resistant). This might be important from the clinical point of view as these data suggest that not only Ig/TCR monitoring, but also BCR/ABL transcript quantification might underestimate the real MRD load. We found better correlation of all methods in PB reflecting probably more uniform cell population released from BM. However, in significant number of paired samples the MRD levels were more than 1 log lower in PB compared to BM (48%, 38% and 27% using BCR/ABL mRNA, Ig/TCR and BCR/ABL DNA quantification, respectively) questioning the use of PB for standard MRD monitoring. The most reliable method for useful and reasonable MRD detection in BCR/ABL+ ALL needs to be discussed within large therapeutic protocol and, importantly, with respect to clinical data.
Support: MH CZ–DRO Univ. Hospital Motol 00064203; UNCE 204012.

P124

NEGATIVE OUTCOME IN PTEN MUTATED ACUTE LYMPHOBLASTIC LEUKEMIA PEDIATRIC PATIENTS COULD BE MODULATED BY THE PRESENCE OF NOTCH1/FBXW7 MUTATIONS

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Background: Recent advances in the knowledge of biology of acute lymphoblas-

tic leukemia (ALL) and the use of risk-directed therapy have led to a significant improvement in survival of pediatric patients. However, T-cell ALL patients have poorer prognosis than B-cell precursor ALL cases and biomarkers to stratify patients in different risk groups are needed. NOTCH1 activating mutations have been described to confer a better prognosis in several studies, but their effect on outcome is controversial. Recently, a role of PTEN and/or RAS mutations on prognosis of pediatric and adult ALL patients has been suggested.
Aims: To analyze the prognostic effect of clinical and biological variables, including mutations of NOTCH1/FBXW7 and PTEN in a series of homogeneously treated pediatric ALL patients diagnosed in a single center.
Methods: Newly diagnosed ALL patients aged 0-18 years, enrolled in the consecutive SHOP (Spanish Hemato-Oncology Pediatric Society) protocols ALL-SHOP-99 and ALL-SHOP-2005 between 2003 and 2013 were included. Mutations of NOTCH1 (exons 26, 27 & 34), FBXW7 (exons 8-12) and PTEN (exon 7) were screened by PCR and direct sequencing.
Results: A total of 216 patients diagnosed with ALL (B-cell precursor ALL, n=189; T-cell ALL, n=24) were included. T-cell patients (67% males) had a median age of 7.6 years (range 2.2-15.4) and a median WBC count of 44.5 $x10^9$/L (range 1.1-588). Four patients had CNS involvement. Seventeen cases (71%) presented a T-cortical phenotype and 2 patients were diagnosed with early-T cell precursor leukemia. Point mutations in NOTCH1 and/or FBXW7 genes were found in 9 cases (9/15 analyzed, 60%) and indels PTEN mutations were present in 4 patients (4/15, 27%). Minimal residual disease (MRD) at day +14 by flow cytometry was negative (<0.01%) in 7 cases and 11 patients (46%) had MRD levels >0.1%. All cases achieved a morphological complete remission (CR) after induction with negative MRD in 16 patients (67%). After a median follow up of 3.5 years (0.02-9 years), 6 patients had died (5 of disease progression after relapse, 1 toxic death after hematopoietic stem cell transplant). Overall survival (OS) at 5 years of T-cell patients was inferior to B-cell precursor cases (67% *vs* 83%, p=0.06). In the univariate analysis, male patients showed a trend for a worse OS (60% *vs* 100%, p=0.08) and WBC count >200 $x10^9$/L associated significantly with a lower OS (40% *vs* 81%, p=0.035). PTEN mutations also conferred a significant worse prognosis (OS of 33% *vs* 90%, p=0.01). By contrast, NOTCH1/FBXW7 mutations showed a trend for a better outcome, but the low number of cases precluded the finding of statistical significance. When NOTCH1/FBXW7 and PTEN mutations were combined, we observed significant differences in survival, with the worst survival corresponding to PTEN mutated & NOTCH1/FBXW7 wild type cases. Noticeably, the only case with double PTEN& NOTCH1/FBXW7 mutation is still alive in first CR after 2 years of follow-up. Similar results were obtained for event free survival. Further analysis to complete the study in the whole series of patients is currently undergoing.
Summary and Conclusions: We have reproduced the value of clinical variables (gender and hyperleukocytosis >200 $x10^9$/L) as prognostic factors in pediatric T-ALL, and demonstrated the adverse effect of PTEN mutations in our patients. Our observations, in line with previous reports (Trinquand *et al.*, 2013), suggest a modulating effect of NOTCH1/FBXW7 mutations on the worse outcome of PTEN mutated cases, although a larger number of patients is needed to confirm our results.

P125

CORRELATIONS OF EXPOSURE AND RESPONSE FOR INOTUZUMAB OZOGAMICIN IN PATIENTS WITH RELAPSED OR REFRACTORY, CD22-POSITIVE ADULT ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Inotuzumab ozogamicin (InO) is an antibody targeting CD22, conjugated with a cytotoxic antitumor antibiotic (calicheamicin), in development for the treatment of acute lymphocytic leukemia (ALL).
Aims: As a follow-on to recent findings[1] we report here interim results of exposure-response analysis for this open-label, phase 1 study of InO in patients with relapsed or refractory CD22-positive ALL.
Methods: InO was administered intravenously on Days 1, 8, and 15 over each 28-day cycle for up to 6 cycles until progressive disease, patient refusal, or intolerable toxicity.
Results: A linear, one-compartment, population-based model described InO concentrations in serum for N=31 patients, yielding clearance of 0.0413 L/hr (SE=0.00463), and central volume of distribution of 5.02 L (SE=0.524); inter-individual variability were 72.1% and 34.0%, respectively. Cumulative area under the serum concentration curve (cAUC; sum over all treatment cycles) and average minimum concentration (Cmin; average across all treatment cycles) were graphically correlated against each patient's response outcome (N=27 subjects). The median cAUC in patients who experienced a complete response (CR; 249.2 hrxµg/mL) was higher than for patients who failed treatment (non-CR; 68.2 hrxµg/mL). Similarly, the median Cmin in patients with CR (40.6 ng/mL) was higher than for patients with non-CR (4.42 ng/mL). The median pre-treatment blast count was lower in patients with CR (0/uL) than in patients with non-CR (553/uL). cAUC across all patients appeared to be negatively trended with higher blast cell count.
Summary and Conclusions: In summary, InO exposure is an important driver of InO response in the treatment of ALL, and this exposure appears to be contemporaneously influenced by the presence of pre-treatment circulating blasts.

Reference

1. DeAngelo D, *et al.* Weekly inotuzumab ozogamicin in adult patients with acute lymphoblastic leukemia. Dec 7-10, 2013, American Society of Hematology Meeting, New Orleans, LO. Abstract No. 3806.

P126

REDUCED PENETRANCE OF INHERITED SUSCEPTIBILITY TO PRE B-ALL CAUSED BY GERMLINE TRANSMISSION OF PAX5 C.547G>A

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Background: Although the overall survival rate for children affected with precursor B cell leukemia (pre B-ALL), which is one of the most common childhood malignancies exceeds 80% the overall survival of relapsed ALL is much less and needs improvement.
Aims: Since the development of pre B-ALL is multifactorial and so far the relevance of hereditary alterations is insufficiently understood, the identification of potential susceptibility loci that predispose to leukemia is extremely important in order to develop individual disease based treatments.
Methods: We describe a family from Ashkenazi Jewish origin with three cases of pre B-ALL. We performed SNP array analysis using the Illumina Human660W-Quad-v1 BeadChip and whole exome sequencing on a HiSeq 2000 (Illumina) of germline and leukemic samples.
Results: Applying cytogenetic and SNP array analysis we identified the homozygous loss of a chromosome 9p region involving the CDKN2A/B encoding region in the leukemic samples. Additionally we identified a heterozygous *PAX5* c.547G>A SNP (p.Gly183Ser) located on chromosome 9p downstream of the CDKN2A/B region in the germline of the affected children, as described previously in two families from Puerto Rican and African American ancestries (Shah *et al.*, 2013). The acquired chr. 9p aberration in the leukemic cells subsequently leads to loss of the wt *PAX5* allele and hence to reduced Pax5 activity, due to retention of the *PAX5* allele encoding p.Gly183Ser. In this pedigree, the *PAX5* c.547G>A variant is transmitted from the grandfather to the fathers and subsequently to five out of seven children, three of whom developed pre B-ALL. Hence the inherited predisposition to pre B-ALL follows an autosomal dominant mendelian transmission with reduced penetrance.
Summary and Conclusions: Thus we confirm and extend the recently reported genetic susceptibility to pre B-ALL in families carrying a *PAX5* c.547G>A germline mutation in combination with structural chr, 9p aberrations and subsequent loss of heterozygosity of the wt *PAX5* allele. With respect to the reduced penetrance of pre B-ALL development the selection of additional mutations, which cooperate with inherited *PAX5* c.547G>A, are likely and currently under investigation.

P127

EXPRESSION OF THE IMMUNOGLOBULIN SUPERFAMILY CELL MEMBRANE ADHESION MOLECULE CD146 IN ACUTE LEUKEMIA

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Background: The expression of the immunoglobulin superfamily cell membrane adhesion molecule CD146 has been observed on several human cell types including a subset of T- lymphocytes as well as in a number of solid tumors.
Aims: Aim of this study was to investigate CD146 expression in Acute Leukemia (AL) by a multiparametric flow cytometric approach.
Methods: We studied 162 patients diagnosed with AL (121 AML, 38 B-ALL, 3 T-ALL) and 10 healthy controls admitted to 3 italian centers between 2010 and 2012. The purity of CD146+ leukemic blasts was assessed by flow cytometry and CD146 positivity was considered for an antigen expression of at least 30%. Conventional cytogenetic analysis of Bone Marrow or Peripheral Blood samples was performed in all patients. Fluorescence *In Situ* Hybridization (FISH) was performed using the following probes: 11q23/*MLL*, 14q32/*IGH*, 8q24/*MYC*, *RUNX1-CBFA2*, *CBFB/MYH11*, *ETV6/RUNX1*, *TCF3/PBX1*, *BCR/ABL*. The

cut-off level for break-a-part and translocation probes was set at respectively at 3% and 1.5%. Statistical analysis used Fisher's exact test and Mann-Whitney test for categorical and numerical variables respectively. A ROC analysis on 38 B-ALL was performed to illustrate the overall discriminator potential of CD146 expression for Ph+ B-ALL.

Results: In our series median age was 57 yrs (range 2-87) and Male:Female ratio was 1.1. A CD146 expression >30% was detected in 4/121 cases of AML (3.3%, median age 69, range 28-87 yrs) and 11/41 ALL cases (26.8%, median age 37.5, range 2-80 yrs) (p<0.001). No associations were found for CD146 expression with sex and age. In 10 healthy controls CD146 expression ranged between the 0.5-1% of the total number of the lymphocytes. In CD146+ AML cases the mean percentage of CD146+ blasts on the total number of cells analysed was 15% which was significantly higher than that observed in healthy controls (p<0.01). The four CD146+ AML cases were classified as secondary AML not otherwise specified. No associations were found between karyotype and CD146 expression. Nine out of 38 B-ALL cases were CD146+ (mean value 67.1%, 44 –98%). When comparing CD146+ to CD146- blasts no significant differences were observed in the expression of common diagnostic markers (TdT, CD19, CD22, HLA-DR) while CD10 and CD20 expression resulted higher in the CD146+ cases though without statistical significance (p=0.21). Of 38 B-ALL cases analysed: 42.1% had normal karyotype, 18.4% a t(9;22) translocation, 10.5% a t(12:21), while t(1;19) and t(4;11) were present in 5.3% and 2.6% of cases respectively. The remaining cases presented hyperdiploid karyotype (7.9%) or other aberrations (13.1%). Of the 9 CD146+ B-ALL cases: 7 had a t(9;22) translocation, one had a t(1;19) and the latter hyperdiploid karyotype. CD146 expression strongly associated with the presence of Ph+ abnormality (p<0.001). A ROC analysis was performed to illustrate the discriminatory potential of CD146 expression for Ph+ B-ALL cases with an area under curve (AUC) of 0.98 (95% CI: 0.95, 1,00) and a CD146 cutpoint of 44% (sensitivity 100%, specificity 93.6%: positive likelihood-ratio 15.5, negative likelihood-ratio 0). Three cases of T-lineage ALL cases were investigated also due to their rarity as compared to B-ALL: in 2/3 cases the mean percentage of CD146+ blasts was 95% of the total number of blasts.

Summary and Conclusions: In conclusion, our results showed a higher expression of CD146 in ALL compared to AML. Moreover a strong association between the presence of the t(9;22)/*BCR-ABL* and CD146 positivity in B-ALL was also observed suggesting that CD146 may be considered in the setting of clinical trials as additional marker especially in Acute Lymphoblastic Leukemia patients.

P128

MLL REARRANGEMENT CONFERS THE POOREST PROGNOSIS AS THE SECOND MOST FREQUENT CHROMOSOME ABERRATION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA, DATA FROM A SINGLE INSTITUTION IN CHINA

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Background: Chromosomal rearrangements of mixed-lineage leukemia (MLL) gene are associated with poor prognosis in infant and children acute lymphoblastic leukemia (ALL), but it was relatively marginally addressed in adult ALL. In fact, the prognostic significance of MLL rearrangement might raise up as tyrosine kinase inhibitors (TKIs) have bridged the gap between Ph-positive subset and Ph-negative group.

Aims: Herein we presented 24 MLL-rearranged cases by a single institution-based retrospective analysis among 330 cases of adult ALL to shed light to clinico-pathological features and outcome of this subset among the whole cohort.

Methods: A retrospective study was undertaken to describe the clinical, pathological, immunological features and outcome of MLL-rearranged population among adult ALL from JAN 2006 to JAN 2012 in Nanfang Hospital.

Results: Twenty-four MLL-rearranged patients were identified among 330 adult ALL, being the second (n=24, 7%) most frequent chromosome aberration as following Philadelphia chromosome (n=66, 20%). The MLL-rearranged population included 22 primary patients and 2 cases secondary to breast cancer, a median age of 30-years old (range 16-73). MLL subset had significantly higher white blood count (median=123 G/L) and higher frequency central nervous system involvement (29%) than Ph-positive and Ph/MLL double-negative (DN) group. ProB was the predominant immunophenotype (n=14, 58%) in MLL subset, followed by comB (n=5) and early T-cell precursor (ETP, n=4), while common-B was dominant in both Ph (n=49, 74%) and DN (n=123, 51%) group. Furthermore, proB and ETP subset of the whole cohort were strongly associated with the poorer outcome of 20% and 15% of 5-year overall-survival (OS), respectively, comparing with of 40% in comB (proB *Vs* comB: p=0.001; ETP *Vs* comB: p<0.001) and 45% in non-ETP-T ALL (NET) (proB *Vs* NET: p=0.051; ETP *Vs* NET: p=0.023) group. The 5-year OS of MLL subset was only 11%, significantly lower than 27% of MLL-negative group (p=0.002), respectively. Pediatric-inspired regimen (n=41, 12%) produced significantly superior 5-year OS than adult protocol (60% *versus* 40%, p<0.001). Similarly, allogeneic hematopoietic stem cell transplantation (allo-HSCT, n=129, 39%) yielded 40% of 5-year OS, comparing to 20% in non-HSCT group (p<0.001). Multivariate survival analysis confirmed MLL rearrangement (p=0.019) and immunophenotype (p=0.022) were independent prognostic factors (Figure 1).

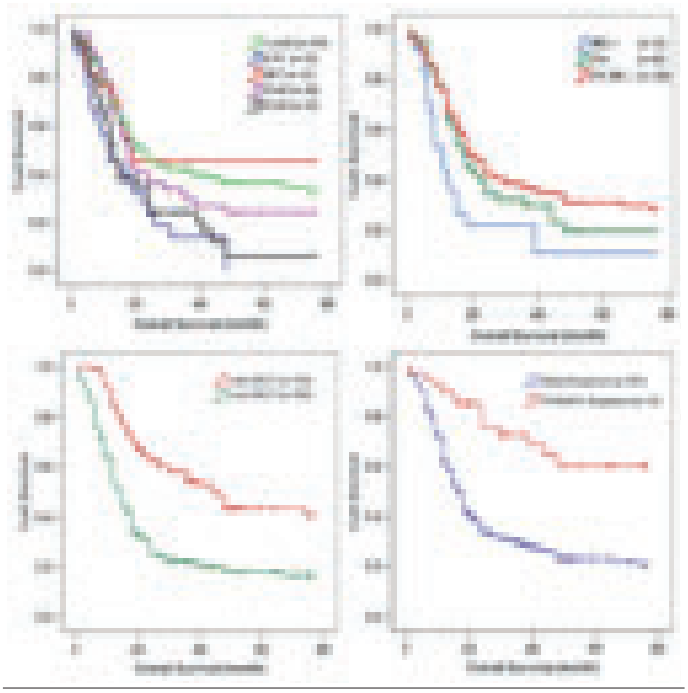

Figure 1.

Summary and Conclusions: Our data showed MLL rearrangement confers the poorest prognosis as the second most frequent chromosome aberration in adult ALL and it deserves more research efforts.

P129

COMBINED TRG AND IGH CLONALITY TESTING ON THE PGM USING LYMPHOTRACK™ REAGENTS & BIOINFORMATICS

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Background: Assays that identify clonal lymphocyte populations by detecting over represented V-J gene rearrangements within the antigen receptor genes of clinical specimens are used on a routine basis to assist in the detection of lymphoproliferative disease. Sensitive detection of tumour-specific DNA sequences, identified in initial samples, can also assist in determining therapeutic efficacy and often provide the first indication of clinical relapse. PCR-based methods run on capillary electrophoresis instruments are the current gold standard for clonality testing. However, these methods often do not provide sufficient sensitivity and, absent separate sequencing steps, are incapable of identifying the specific V-J DNA sequence data required to track clones in follow up testing. The emergence of massively parallel sequencing (MPS) platforms has facilitated development of powerful new approaches for clonality detection and monitoring. Here we report performance of an MPS assay and associated bioinformatics developed for the PGM platform to detect and monitor *IGH* and *TRG* clonality.

Aims: To develop a rapid, easy to use multiplex PCR NGS assay for the combined analysis of TCRG and IGH for clonality detection on the PGM NGS sequencing platform.

Methods: Optimized V and J consensus primers targeted all *IGH* and *TRG* variable (V) and joining (J) region gene segments that are rearranged in lymphoid cells. Separate multiplex PCR master mixes amplified *IGH* and *TRG* gene rearrangements while incorporating sequence indices into products, which allowed simultaneous sequencing and tracking of as many as 12 individual samples for each marker, plus controls, in each run. Amplicons were purified, quantified and pooled for library formation. The harmonized, quantified libraries were loaded onto the OneTouch ES. The enriched emulsion PCR libraries were sequenced using the Ion 316 Chip Kit v2 and Ion PGM Sequencing 400 Kits. Data were analyzed using Invivoscribe proprietary bioinformatics software run on standard Windows platforms.

Results: The PGM platform provided sustainable, rapid turn around times, frequently less than 36 hours from processing of primary sample to result. This assay and bioinformatics package reproducibly identified clonality and DNA sequences for *TRG* and *IGH* V-J gene rearrangements. Automated data out-

puts include frequency distributions and V-J gene usage, as well as complete DNA sequences for up to 1 million sequence reads. DNAs from cell lines serially diluted into polyclonal tonsil DNA, confirmed the linearity and low run-to-run variance of the assay. Good linearity (R^2>0.95) and reproducibility were achieved. Genomic DNA from peripheral blood (PB), tonsil, and bone marrow aspirates were tested for *TRG* and *IGH* gene rearrangements yielding excellent results.

Summary and Conclusions: We have previously developed *IGH* and *TRG* NGS assays for the Illumina MiSeq and HiSeq platforms. Now we demonstrate an adapted workflow for the IonTorrent PGM that is quicker and less costly for laboratories handling small numbers of samples. These assays run on the PGM platform can simultaneously identify both clonal *IGH* and *TRG* V-J rearrangements, associated specimen-specific V-J region DNA sequences, provide frequency distribution of V region and J region segment utilization, and can be used to monitor their presence in subsequent samples. When coupled with the bioinformatics and visualization software this assay provides robust detection and enhanced data-rich outputs.

Acute myeloid leukemia - Biology 1

P130

AML WITH EVI1 REARRANGEMENTS ARE CHARACTERIZED BY FREQUENT SF3B1 AND IKZF1 MUTATIONS

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Background: Acute myeloid leukemias with *EVI1* translocations (*EVI1*-t AML) are a rare subtype of AML accounting for less than 3% of patients. The most frequent rearrangement, inv(3)(q21q26.2)/t(3;3)(q21;q26.2);*RPN1-EVI1*, is a distinct genetic entity in the WHO 2008 classification. It is characterized by multilineage dysplasia, atypical megakaryocytes with normal or high platelet counts and a poor outcome.

Aims: Co-occurring gene mutations are unknown in *EVI1*-t AML. We performed a comprehensive genome-wide analysis of mutations in *EVI1*-t leukemias using next-generation sequencing (NGS) to identify recurrent mutations that may provide key insights into the biology of this disease.

Methods: We performed transcriptome analysis of 9 adult *EVI1*-t leukemias, including 5 samples with *RPN1-EVI1* fusion and 4 with other rearrangements. Results were compared to our cohort of 143 sequenced AML with various other cytogenetic anomalies. Libraries were prepared with standard TruSeq protocols and sequencing was performed using HiSeq2000 (Illumina). Non tumoral DNA isolated from buccal swabs or saliva was used to confirm the somatic status of the identified mutations.

Results: Within a set of 35 genes frequently mutated in AML and other myeloid malignancies, *RAS* pathway mutations were the most frequent type in *EVI1*-t AML (6/9), followed by mutations in genes involved in RNA splicing (5/9 samples: 4 *SF3B1* and 1 *U2AF1*). *SF3B1* mutations were exclusive to the *RPN1-EVI1* subgroup and rarely detected (2/143) in non *EVI1*-t leukemias (*EVI1*-t *vs* non *EVI1*-t, p<0.0001). All other mutations identified in *EVI1*-t AML are shown in Figure 1. To identify novel mutations, we analyzed all genes with variants called in ≥2 *EVI1*-t specimens. After removing polymorphisms (dbSNP v.137), 20 genes were selected. Targeted sequencing of non-tumoral DNA revealed non-annotated inherited polymorphisms in 19/20 genes. One gene, *IKZF1*, contained 4 somatic mutations in 3/9 samples which were confirmed by Sanger sequencing of tumor cDNA. No *IKZF1* mutation was found in non *EVI1*-t AML (3/9 *vs* 0/143, p=0.0001). To our knowledge, recurrent *IKZF1* mutations have not been described in AML. In acute lymphoblastic leukemia, *IKZF1* alterations can result in haploinsufficiency or in the expression of a dominant negative isoform. Two samples shared an *IKZF1* N159S mutation. This mutant, located in one of the N-terminal zinc finger (DNA-binding) domain, is expected to have a dominant negative effect similar to the G158S variant previously described. The two additional mutations identified, R213X and p.N270KfsX6, are predicted to result in truncated proteins. *IKZF1* is located on chromosome 7p12.2 and monosomy 7 is the most frequent cytogenetic anomaly associated with *EVI1*-t leukemias. Two samples had a monosomy 7 and *IKZF1* expression was lower in these samples than in those without monosomy 7 (average RPKM: 14 *vs* 36, p=0.06), suggesting that monosomy 7 might result in *IKZF1* haploinsufficiency in *EVI1*-t AML. In null mice, the loss of Ikaros is associated with abnormal red cells, increased megakaryocytes and elevated platelet counts (Lopez *et al.,* PNAS, 2002), a phenotype similar to that described in *EVI1*-t leukemias.

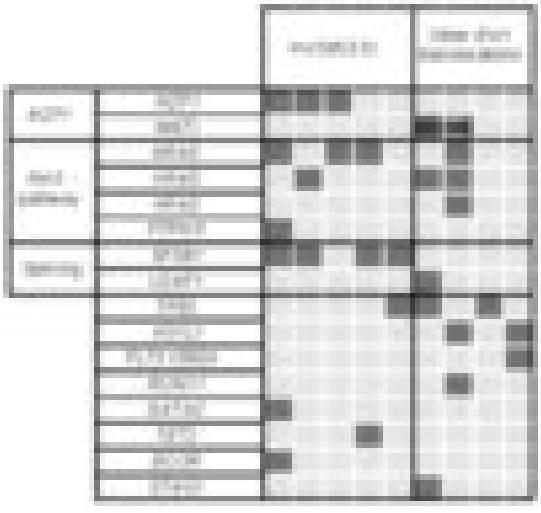

Figure 1. Mutations in *EVI1*-t AML.

Summary and Conclusions: We provide the first genomic characterization of AML with *EVI1* rearrangements by NGS. This study reveals frequent *SF3B1* and novel recurrent *IKZF1* genetic lesions. Although these results need to be confirmed in a larger cohort, we propose that *IKZF1* mutations and deletions might contribute to the distinct phenotype of these leukemias, especially in the *RPN1-EVI1* AML subgroup.

P131

SEQUENTIAL CARBOXY-TERMINAL PHOSPHORYLATION OF EVI1 UPON DNA DAMAGE

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Background: The *EVI1* proto-oncogene is overexpressed in 10% of acute myeloid leukaemia (AML) and is associated with very poor prognosis. *EVI1* overexpression is also a key event in leukaemic transformation in Fanconi Anaemia (FA), an inherited defect in the DNA damage response with extreme predisposition to malignancy. The *EVI1* gene encodes a DNA binding protein with roles in gene expression and epigenetic regulation. Detailed understanding of EVI1 protein regulation is incomplete, however an important role for post-translational modification is emerging. We hypothesised that protein phosphorylation might be govern EVI1 function.
Aims: Identify and characterise EVI1 phosphorylation events plus their downstream effects.
Methods: Endogenous EVI1 from EVI1-overexpressing FA-derived SB1690CB AML cells was analysed after enrichment using mass spectrometry. EVI1 protein was analysed from untreated and irradiated (X-rays; 10 Gy) cells and specific phosphorylation events identified. Phospho-specific antibodies were generated and used to confirm EVI1 phosphorylation on a specific serine residue, assess phosphorylation dynamics and characterise the effect of kinase inhibitors. Phosphorylation site-mutated EVI1 was expressed in bone marrow progenitor cells and the impact on myeloid transformation determined.
Results: In untreated cells we detected an EVI1 phosphorylation site on serine-860 (Ser860). However, after DNA damage EVI1 was phosphorylated on both Ser860 and Ser858. Induction of the doubly phosphorylated form of EVI1 was seen within 15 min of DNA damage and peaked after one hour. In contrast, the cellular pool of EVI1 phosphorylated on S860 alone was reduced, suggesting that Ser858 and 860 phosphorylation is dynamically phosphorylated in response to DNA damage. The Ser858 phosphorylation site matches the ATM kinase motif. Treatment with the ATM inhibitor KU-55933 abrogated the DNA damage-dependent phosphorylation on Ser858. Increase in the proportion of blast cells was detected in serial replating assays with phosphomimetic EVI1 mutants compared with a non-phosphorylatable EVI1, indicating that S858 and S860 phosphorylation modulates EVI1 function.
Summary and Conclusions: DNA damage modulates EVI1 phosphorylation, which may regulate its function in myeloid transformation and self-renewal.

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SETBP1 MUTATIONS IN 106 PATIENTS WITH THERAPY-RELATED MYELOID NEOPLASMS

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Background: Therapy-related myeloid neoplasms (t-MN) are myeloid disorders developing in patients treated with radiotherapy and/or chemotherapy for cancer or autoimmune diseases. t-MNs are characterized by high incidence of complex karyotypes and frequent monosomies and/or deletions of chromosomes 7 and/or 5, whereas the recently identified mutations of epigenetic regulators and of the spliceosome machinery are rare, with the exception of SRSF2. Recently, new sequencing technologies have enabled large screening of somatic mutations in myeloid malignancies, leading to the discovery of new hot spot mutations in genes candidate as leukemic transformation drivers. Among these, SET binding protein 1 (SETBP1) mutations, previously associated to Schinzel-Giedion syndrome, were reported in several myeloid neoplasms, in particular in patients with chromosome 7 alterations and SRSF2 mutations.
Aims: Object of our study was to determine the frequency of SETBP1 mutations in a cohort of 106 t-MN patients.
Methods: The study population included 106 patients affected by t-MN. According to the proportion of blasts, there were 53 t-MDS and 53 t-AML. Karyotype was abnormal in 52 of 81 (64.19%) patients in whom the karyotype was available. Chromosome 7 alterations were present in 16/81 (19.75%).
Mononuclear cells (MNCs) were separated from the BM at the time of diagnosis by Ficoll gradient centrifugation. DNA was extracted using a QIAamp DNA Mini Kit (Qiagen). Detection of SETBP1 mutations was performed by Sanger sequencing (Life technologies). t-MN patients were also tested for mutations in DNMT3A, IDH1, IDH2, SRSF2, U2AF1 and SF3B1. Paired T-test was performed to test the association between SETBP1 mutations and other screened genes and patient's karyotype.
Results: We identified three point mutations in the SKI-homologous domain of SETBP1 in our patient cohort (3/106; 2.83%). Two patients had a G870S (COSM1234973) and one a S869R mutation. Two of three SETBP1 mutated patients (both carriers of G870S mutation) also had a SRSF2 mutation at position P95 (P95H and P95R with contextually P96 insertion). No other associations between SETBP1 mutations and spliceosome machinery or epigenetic regulators somatic mutations were found. All three SETBP1-mutated patients had a different primary tumor (Non-Hodgkin lymphoma, breast and thyroid cancer), but interestingly, all patients had developed a t-MDS (one RAEB1 and two RAEB2). None of the t-AML patients resulted mutated. Looking at karyotype associations, the two patients carriers of a G870S mutation had a complex karyotype without chromosome 7 aberration, whereas the S869R mutation carrier had a chromosome 7 deletion. The low number of SETBP1-mutated t-MN patients precluded survival analysis in this cohort.
Summary and Conclusions: We found a low incidence of mutations in the SKI-homologous domain of SETBP1. In our t-MN patients SETBP1 and SRSF2 mutations were frequently associated, whereas there was no association between SETBP1 mutation and chromosome 7 alteration, suggesting a limited role of these mutations in t-MN pathogenesis.

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FANCONI ANEMIA AND DNA-REPAIR GENE VARIANTS IN THERAPY-RELATED MYELOID NEOPLASMS

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Background: Therapy-related myeloid neoplasms (t-MN) include myelodysplastic syndromes and acute myeloid leukemias occurring as a late effect of chemotherapy and/or radiotherapy for a primary malignancy or for autoimmune diseases. The incidence of this complication has been raising in the last years due to the prolonged survival and the higher number of treated patients. Still, less than 5% of patients exposed to cytotoxic drugs and radiotherapy develop a t-MN, suggesting underlying individual susceptibility. The association of breast and other cancers to myeloid neoplasms is frequent in Fanconi Anemia (FA), a syndrome characterized by chromosomal instability, developmental abnormalities, aplastic anemia and predisposition to cancer. So far, mutations in FANC genes have been rarely described in hematological malignancies outside the syndromic picture of FA.
Aims: We were interested in the prevalence of FANC and other DNA-repair gene variants in t-MN following cytotoxic treatment for breast cancer and lymphoproliferative diseases.
Methods: The test- patient cohort included 37 patients with a t-MN. According to the proportion of blasts, there were 19 t-MDS and 18 t-AML. The primary malignancy was Hodgkin lymphoma (HL) in 7 patients, non-HL in 12 patients and breast cancer in 18 patients. DNA isolated from BM-MNCs at t-MN diagnosis was analyzed using Agilent HaloPlex system and validated with Sanger sequencing and Pyrosequencing. We selected 41 genes, including 14 FANC pathway genes and 27 further DNA repair genes. For the ATM SNV analysis, we included BM samples from further 60 t-MN patients, adding to a total of 97 t-MN patients (48 t-MDS and 49 t-AML). Control samples were obtained from 129 Caucasians with a negative history for previous malignancies and normal PB cell counts. Differences in the distribution of ATM SNV between patients and controls were evaluated using the Fisher's Exact test.
Results: DNA-repair and FANC gene variants were frequent in our t-MN patients, with 21 of 37 patients (57%) carriers of at least one genomic variant. There were no differences in latency from the primary cytotoxic treatment and t-MN in mutated *vs* unmutated patients and the median overall survival after t-MN diagnosis was similar for the two groups. The gene with the highest frequency of variant sequences was TP53 (15 variants in 10 patients). Looking at FANC genes, we found 7 heterozygous variants in 6 patients (16%), including two FANCA (L6F and S90T), three FANCD2 (T1376A, P256S and M1023V), one FANCJ (I364V) and one FANCC (L36F). Six variants were novel, according to the NCBI and Cosmic databases, whereas the FANCA L6F had been previously described (*www.1000genomes.org*). Control tissues confirmed that the variants were germ-line in 5 patients with available material (Table 1). The frequency of FANC variants in our t-MN cohort was higher than that reported in 200 *de novo* AML patients by the Cancer Genome Atlas Research Network.
In the extended study population, the ATM P1054R variant was significantly more frequent in t-MN compared to controls (10.3% *vs* 2.3%, p=0.018). This translates into a 4.8-fold increased t-MN risk.

Table 1. Characteristics of patients with FANC and DNA-repair variants.

Summary and Conclusions: The high incidence of FANC germline variants in our t-MN cohort may be the underlying cause for increased susceptibility to primary cancers and may induce secondary leukemogenesis.
In the same line, the higher prevalence of the ATM P1054R SNV in t-MN may increase sensitivity of hematopoietic progenitor cells to the DNA damaging effect of chemo- and radio-therapy leading to secondary leukemia.

P134

SELECTION OF A PRE-EXISTING TP53 MUTATED CLONE IN THERAPY-RELATED ACUTE MYELOID LEUKEMIA

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Background: Therapy-related myeloid neoplasms (t-MNs) are thought to arise due to mutational events in hematopoietic stem and precursor cells induced by cytotoxic treatments for a primary disorder. However, no consistent biomarker has been identified yet that unambiguously classifies a particular neoplasm as "therapy-related". This raises the possibility that other mechanisms may also be operational in their pathogenesis.
Aims: Our aim was to demonstrate that leukemia-specific mutations were pre-existing in some of these patients and we, therefore, focused on the *TP53* gene which is frequently mutated in t-MNs.
Methods: We used Sanger sequencing of paired samples - t-MN and constitutional material - for initial mutation identification and rearrangement-specific PCR for the detection of a *TP53* duplication in pre-leukemic specimens, respectively. Quantitative PCR was performed to semi-quantify the mutated clone and Ion torrent deep sequencing (Life Technologies, Carlsbad, CA) to search for co-operating mutations.
Results: We screened 53 t-MN specimens for *TP53* mutations and identified one patient with a somatic heterozygous 64-base pair duplication (NM_000546.5:c.276_339dup:p.Leu114Profs*31) who developed therapy-related acute myeloid leukemia (t-AML) with complex karyotype (46-50,XY,del(5)(q12q33),?r(7)(p22q11)[cp20]) 13 years after combined modality treatment for Hodgkin's lymphoma. This duplication was particularly amenable for detection by a highly sensitive PCR assay enabling the detection of 0.01% rearranged cells. We could not only unambiguously detect the presence of *TP53* mutated cells in the patient´s pre-treatment bone marrow but also in a lymphadenitis sample obtained seven years before lymphoma diagnosis. Quantitative PCR estimated the amount of affected bone marrow cells as <1% as compared to the t-AML specimen. Analysis of the *FLT3*, *NPM1*, *ASXL*, *TET2*, *IDH1/2*, *HRAS*, *KRAS*, *RUNX1*, *MLL*, *JAK2*, *WT1*, *PTEN* and *PHF6* genes by deep sequencing using bone marrow and leukemia specimens did not show evidence of co-operating mutations.
Summary and Conclusions: The fact that a leukemia-specific *TP53* mutation is already present before any chemo- or radiotherapy has been administered suggests a different mode of therapy-related leukemogenesis than currently assumed. Instead of inducing a leukemia-specific mutation, cytotoxic treatments have facilitated the expansion of a pre-leukemic clone in this patient.

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GENOMIC ANALYSIS OF THE CLONAL ORIGIN AND EVOLUTION OF ACUTE PROMYELOCYTIC LEUKEMIA IN A UNIQUE PATIENT WITH A VERY LATE (17 YEARS) RELAPSE

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Background: Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by a balanced reciprocal translocation t(15;17)(q22;q12-21). The introduction of all-*trans*-retinoic acid (ATRA) has considerably improved complete remission (CR) rate and survival of APL patients. APL is now a highly curable disease and late relapses beyond 7 years are very rare. Here we describe a female patient with manifestation of APL 17 years after the initial APL diagnosis (Figure 1A).
Aims: To elucidate whether the more recent manifestation of the disease is a very late relapse or a *de novo* APL in this unique patient by genomic analyses.
Methods: Whole genome sequencing (WGS) and array-comparative genomic hybridization (CGH) were performed on genomic DNA from the patient's bone marrow specimens at initial (1994) and second diagnosis (2011), and blood samples during second CR.
Results: The patient was initially treated with ATRA and chemotherapy in 1994 while ATRA and chemotherapy plus arsenic were given in 2011 (Figure 1A). WGS revealed two different *PML/RAR*α gene fusions (Chr17: 38489469-Chr15:74316176 and Chr15:74316160-Chr17:38489139) in APL cells from both samples, with the first fusion being predominant in both (Figure 1B). The fusion genes were further verified by Sanger sequencing (Figure 1B). Although the fusion genes/breakpoints were identical, significant differences in genetic aberrations were observed between the first and second APL samples, as revealed by WGS and array-CGH. Importantly, WGS and the fragment length analysis demonstrated FLT3ITD and FLT3-D835 point mutation in the first and second APL samples, respectively.

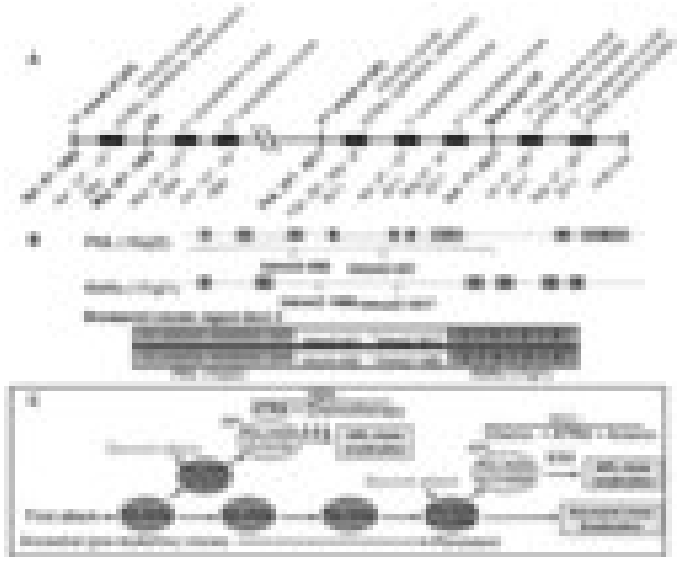

Figure 1.

Summary and Conclusions: We believe that our patient documents the longest interval between diagnosis and relapse of APL described in the literature to date. Genomic analyses show how APL clones evolved between the two manifestations of APL in this patient (Figure 1C). Likely, the patient's hematopoietic cells underwent *PML-RAR*α gene fusion following a genetic attack, leading to the generation of abnormal ancestral or pre-leukemic clones, which is consistent with a recent study. These clones then transformed into APL following acquisition of *FLT3ITD* by another genetic attack. The patient obtained a CR following ATRA treatment plus chemotherapy in 1994. APL blast clones were eradicated after treatment, but the ancestral clones carrying the *PML-RAR*α fusion gene were persistent and acquired a *FLT3-D835* point mutation later. The *PML-RARα/FLT3-D835* clones then contributed to the second onset of APL in 2011. At relapse, the patient was given ATRA and chemotherapy as induction and ATRA/arsenic acid as consolidation therapy. The patient has now remained in a molecular remission for three years, hopefully, indicating that a "non-aggressive" approach was appropriate for this patient. In summary, we here describe an extremely late relapse of APL and the leukemic clonal evolution. Very late relapses in APL, as seen in this unique patient, are more likely caused by a new genetic attack on existing pre-APL clones, which differs from early relapses resulting from the re-growth of original residual APL blasts. Genomic characterization of late relapses may have therapeutic implications.

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NOVEL MUTATED GENES IN ACUTE PROMYELOCYTIC LEUKEMIA IDENTIFIED BY WHOLE-EXOME SEQUENCING

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Background: Acute Promyelocytic Leukemia (APL) is a rare hematologic neoplasm characterized by the fusion gene *PML/RARA*. However, it is known that this abnormality itself is not able to reproduce the whole leukemic phenotype. Preliminary APL whole exome studies have identified a huge number of somatic mutations affecting 135 different genes in a non-recurrent manner, except for *FLT3*, *WT1* and *KRAS*. These findings suggest that APL is a heterogeneous disease with secondary relevant changes not yet defined.
Aims: We performed whole-exome sequencing (WES) of tumor-normal matched samples to identify new gene mutations that might carry prognostic value in APL. Novel candidate-genes, together with other previously described[1,2], were resequenced in an independent cohort of APL patients. The obtained results were further studied by *in silico* analysis to enlarge our understanding of their role in the pathology and entry genetic pathways.
Methods: WES was performed on matched samples from 5 *de novo* APL patients, as our study cohort. Library preparation and exome capture were performed according to the protocol version 2.1 from Baylor College of Medicine for sequencing with SOLID 4 platform as recommended by the manufacturer. WES data were analyzed using and in-house bioinformatic pipeline. Candidate variants were confirmed by Sanger sequencing. We extended the analysis of these variants to a validation APL cohort (n=76). Furthermore, a custom panel of 97 genes (17 genes from in-house results and 80 genes reported to be mutated in at least 1 patient from APL previous studies[1,2]) was performed on a subset of the validation cohort (n=25) using SureDesign Tool (Agilent) for NGS, according to the manufacturer's instructions. Samples were provided by the Hospital La Fe Biobank.
Results: We identified 32 SN*Vs* non-synonymous coding mutations and 18 small indels, with an average of 10 mutations per sample (range 7-14). We confirmed 17 SN*Vs* and 1 indel of the candidate variants (36%) in 17 genes by Sanger sequencing. Among them, no recurrent specific variants were observed through the study and the validation cohorts, with the exception of *FLT3*. Over the 25 patients analysed by the genes panel, we detected a mean of 7,76 mutations per sample (range 1-23). We identified a total of 92 variants affecting 54 different genes, where 33 were mutated in more than one patient. When we focused our research on these genes, we found that 10 mutated genes (2 of them, listed from our WES results) had a higher frequency in our cohort than expected in the 1000g database ($P \leq 0{,}01$). Pathways analysis of the mutated genes are been analyzed and the complete results will be presented in the meeting.
Summary and Conclusions: This study shows a comprehensive analysis of APL combining WES with the frequency assessment of somatic mutations from a custom panel of targeted genes by NGS. We identified recurrent deleterious mutations in 10 genes with a strong potential to be involved in APL pathogenesis. The relevance of the newly defined mutated genes for APL pathogenesis will require functional validation studies.
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DNA METHYLATION PATTERNS AT DISEASE RELAPSE IN ACUTE MYELOID LEUKEMIA TARGET CONVERGENT ELEMENTS AND PATHWAYS DESPITE INTER-PATIENT HETEROGENEITY

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Background: Disease relapse is a fundamental clinical challenge for Acute Myeloid Leukemia (AML) since most patients have poor clinical outcomes. The biological basis of relapse in AML remains unclear, and a role for epigenetic mechanisms has not been examined in depth. Genetic evolution of the disease after induction treatment is widely believed to underlie the emergence of chemotherapy resistant clones. However, recent reports have identified only a limited number of somatic mutations and copy number abberations upon disease relapse, and in some cases, no relapse-specific events were detected. These findings suggest a role for other mechanisms in relapsed AML. We hypothesize that epigenetic plasticity and dysregulation of biological pathways contribute to the relapsed disease phenotype in AML.
Aims: In this study, we aimed to determine epigenetic patterning (DNA methylation) and its potential functional consequences in relapsed AML.
Results: DNA methylation profiling using Enhanced Reduced Representation Bisulfite Sequencing (ERRBS) of 39-paired diagnosis and relapse AML patient samples was performed. Cells analyzed were enriched for the blast population. We identified thousands of statistically significant changes in cytosine methylation patterns. All patients acquired both hyper- and hypomethylated differentially methylated CpGs (DMC) and regions (DMR) resulting in a range of cytosine methylation patterning upon disease relapse. In spite of the variety of cytosine methylation patterns, the majority of differentially methylated cytosines are located in intergenic regions in all cases. Interestingly, however, a subset of promoters were hypermethylated in almost all patients at relapse. This heterogeneity is not uniformly driven by mutations in epigenetic modifiers known to affect DNA methylation; has been confirmed in two independent patient cohorts (n=71 and n=31 paired diagnosis and relapse AML patient samples); and is reproducible in a xenograft model of relapsed AML treated with cytosine arabinoside (Ara-C). Integrated analysis with ENCODE datasets revealed enrichment for differential cytosine methylation upon disease relapse at distal regulatory elements (including enhancers and insulators) and regions affected by histone marks associated with cell cycle and transcriptional regulation. Pathway enrichment analysis of differentially expressed genes (determined from RNA-seq) and genes affected by differential methylation within their promoters upon disease relapse revealed convergence on commonly affected biological pathways between the patients. In particular, there was a strong overlap between gene promoters differentially methylated in relapsed compared to diagnostic AML in the patients and xenografted sample ($p<0.01$, hypergeometric test), including members of the Wnt signaling pathway.
Summary and Conclusions: We conclude that there are extensive and dynamic changes in DNA methylation patterns between diagnosis and relapse in AML. Preliminary analysis demonstrates convergent epigenetic targeting of specific gene pathways that may contribute to relapsed AML pathogenesis. The genomic distribution of reprogrammed methylation also suggests a role for epigenetic plasticity at distal regulatory elements. These findings suggest that mechanisms regulating cytosine methylation may be altered from *de novo* states after exposure to induction chemotherapy or facilitate survival of minor clones of disease during treatment resulting in widespread epigenetic reprogramming upon disease relapse.

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WHOLE EXOME SEQUENCING REVEALS CLONAL EVOLUTION PATTERNS AND DRIVER GENETIC ALTERATIONS OF RELAPSED PEDIATRIC ACUTE MYELOID LEUKEMIA

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Background: Pediatric acute myeloid leukemia (AML) comprises ~20% of pediatric leukemia, representing one of the major therapeutic challenges in pediatric oncology. Nearly 40% of patients still suffer from a relapse after first-line therapies and once the relapse occurs, long-term survival rates decrease, ranging from 21% to 34%. The recent development of high-throughput sequencing technologies has provided an unprecedented opportunity to investigate comprehensive genetic alterations that are involved in the tumor recurrence of various cancers including adult AML. However, little is known about the molecular mechanisms of relapsed pediatric AML.
Aims: The purpose of this study is to identify the clonal evolution patterns and the major mutational events in relapsed pediatric AML.

Methods: We performed whole exome sequencing of 6 relapsed AML cases, in which diagnostic, relapsed and complete remission samples were available. Copy number alterations and structural variants including tandem duplications were also analyzed using whole exome sequencing data. Subsequently, deep sequencing of identified mutations was performed to evaluate intra-tumor heterogeneity and the clonal origin of relapsed clones.
Results: Whole exome sequencing of 18 samples from 6 patients with different subtypes of pediatric AML were analyzed with a mean coverage of more than x100, by which 95% of the targeted sequences were analyzed at more than x20 depth on average. The mean number of nonsynonymous mutations was higher at relapsed phase than at the time of diagnosis (14.0/case *vs* 10.5/case). Mutational signature was dominated by C>T transitions at both phases. Clonality assessment using variant allele frequencies of individual mutations revealed the presence of intra-tumor heterogeneity both at the diagnostic and at therelapse phases. In all 6 patients, relapsed AML evolved from one of the subclones that were present at the diagnostic phase. In all cases, relapsed AML was accompanied by many additional mutations that were absent (relapse specific mutations) or existed only with lower allele frequencies (relapse enriched mutations) in the diagnostic samples, indicating a multistep process of leukemia recurrence. Mutations that were specific to or enriched in relapsed specimens were found in several driver genes including *ASXL1*, *NRAS* and *CREBBP*, suggesting these mutations could contribute to tumor recurrence. In some cases, AML relapse may accompany a dynamic clonal change. For example, some *bona fide* driver mutations, such as *KRAS* mutations, that were predominant at the time of diagnosis disappeared in relapsed samples.
Summary and Conclusions: Whole exome sequencing unmasked the clonal structure of primary and relapsed pediatric AML, which helped to understand the underlying mechanism of relapse in pediatric AML. Our results suggested that the intra-tumor heterogeneity was common in pediatric AML both at presentation and at relapse and subclonal mutations involving RAS pathway genes and chromatin modifiers could contribute to the AML relapse.

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IDENTIFICATION OF SOMATIC MUTATIONS OR FUSIONS BY RNA-SEQUENCING IN ACUTE MYELOID LEUKEMIA.

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Background: For patients with diagnosis of acute myeloid leukemia (AML) the presence or absence of specific genetic alterations is useful for both prognosis and treatment choice (according to AML classification). However standard techniques, like cytogenetics and PCR, are able to identify only a limited number of alterations.
Aims: We tested RNA-sequencing in AML patients in order to validate this technique as a method to identify fusions and mutations in a clinical setting and obtain information about genetic variations in AML.
Methods: We collected samples from peripheral blood or bone marrow of 13 consecutive patients with new diagnosis AML of any type. RNA-sequencing data were generated using an Illumina Genome Analyzer IIx following standard library-prep protocols. Alignment to the reference GRCh37/hg19 genome was performed using BWA. Alignment data were processed using Samtools. Single nucleotide and small indels detection was performed using in-house software and applied to a predetermined list of 45 genes involved in AML pathogenesis (CEBPA, NPM1, FLT3, RUNX1, MLL, WT1, EZH2, NF1, MECOM(EVI1), KIT, H-RAS, K-RAS, TET2, IDH1, IDH2, DNMT3A, BCOR, BCORL1, NUP98, ASXL1, ABCB5, BAALC, CEP72, DIP2C, ROBO1, KLC1, TP53, IGFBP7, SETBP1, JAK2, NRAS, NOTCH1, CDKN2A, MPL, SF3B1, BRAF, PTPN11, SRSF2, IKZF1, GATA1, MYD88, ATM, CBL, PHF6, BCL2). The presence of fusions was assessed using FusionAnalyser.
Results: We identified a total of 9 fusions and 28 single nucleotide variants with a median number of 2 single nucleotide variants per patient (range 0-6). 5 patients had fusions that were previously detected with standard techniques (1 AML-ETO, 3 PML-RARA and 1 CBFB-MYH11); for all these patients known fusions were confirmed by RNA-sequencing. In 4 patients, previously unreported fusions were detected. Two of them were already known from the literature (ZMYM2-FGFR1 and MLL-MLLT10) and two were new (ETS2-ERG and WDFY3-WAS). The ZMYM2-FGFR1 is particularly interesting, because it is potentially targetable by using Ponatinib. Interestingly, we were able to identify new potential targeted therapies for 7 patients with single-nucleotide mutations: NOTCH1 D622E using NOTCH inhibitors; KIT N511K using Dasatinib; KRAS A155T using RAS inhibitors; MLL G256E, DNMT3A C599G, TET2 L1801R and EZH2 T80S using demethylating agents. Considering both new and already known mutations and fusions, 12 out of 13 patients could have received a patient tailored treatment based on these data (Table 1).
Summary and Conclusions: Our study demonstrates that RNA-sequencing leads to rapid detection of somatic mutations and fusions in AML patients. The possibility of recognizing a subset of genetic variations with potentially therapeutic value could pave the way to a genomic-centered, "personalized" therapy.

Table 1.

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IMPROVED COPY NUMBER VARIATION ESTIMATION FROM NEXT GENERATION SEQUENCING DATA REVEALS FOCAL GENETIC LESIONS SUCH AS MLL-PTD IN ACUTE MYELOID LEUKEMIA

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Background: Different next generation sequencing (NGS) platforms generate high-quality data which allows, in addition to the detection of mutations, small deletions or insertions (indels), for the estimation of copy number variations (CNVs). CN*Vs* in combination with concomitant single nucleotide variants (SNVs) or indels, possibly in the same regions, can be utilized for further delineation of the biology of acute myeloid leukemia (AML). Remarkably, AML patients have an exceptionally low number of CNVs, with a mean of 2.38 CN*Vs* per case as previously measured by DNA mapping arrays. The increased resolution of NGS derived high-density sequence data could enable the detection of novel genetic lesions in AML, which could serve as guides towards the identification of novel cancer-related genes.
Aims: The development of an algorithm that mitigates systematic bias from the NGS data in conjunction with the robust detection of novel genetic lesions in AML.
Methods: Whole genome sequencing was performed on 3 primary AML patients, harboring the chromosomal rearrangements inv(3)(q21q26) or t(3;3)(q21;q26), and matched *in vitro* cultured T-cells using the Complete Genomics platform. Whole exome sequencing was performed on 30 primary AML patients, 30 matched relapse samples, and 30 matched *in vitro* cultured T-cells controls using the Illumina HiSeq 2500 platform. The NGS data was analysed with an in-house developed package CNVsvd. In short, we count the number of fragments in consecutive 0.5 kb windows and compare them to the counts derived from a reference set of healthy individuals. Of a selected number of AML patients the CN*Vs* were also determined using Affymetrix 500K DNA mapping arrays.
Results: The whole genome sequencing data revealed an extensive number of small genetic lesions, more than 100 CN*Vs* per AML case, initially missed with the DNA mapping array. Most of these CN*Vs* are concomitantly detected in the matched controls and are therefore deemed to be germline CNVs, which in principal could still play a vital role in leukemogenesis. Importantly, the vast majority of CN*Vs* was corroborated by structural variants present in the NGS data. Surprisingly, on average 7 somatic CN*Vs* (sCNV) were detected per AML case using WGS data in conjunction with the CNVsvd package, while on average 2 sCN*Vs* per AML case were detected with the DNA mapping arrays. Most of these novel sCN*Vs* were small genetic lesions, between 1.5 and 6 kb in size, likely to be missed because of the lower resolution of DNA mapping arrays. Similarly, on average, a substantial lower number of CN*Vs* were detected with the DNA mapping arrays compared to those derived from whole exome sequencing data (2.4 *vs.* 9.5, p=0.0345). The power and resolution of our novel statistical tool was illustrated by the detection of acquired MLL-partial tandem duplication (MLL-PTD) lesions in 7 AML patients, which resurfaced in 4 matched relapse cases, and were not detected in the matched control samples. All MLL-PTD lesions were confirmed by standard PCR methodology.
Summary and Conclusions: Our algorithm enables reliable detection of small genetic lesions from different types of NGS data. The analyses based on NGS data revealed that AML has, on average, more genetic lesions than previously thought based on DNA mapping array data. The discordance can be mainly attributed to the increased resolution of NGS data with respect to DNA mapping arrays and the robust removal of systematic noise by our algorithm. Analysis of CN*Vs* derived from whole exome sequencing data reveals a substantial number of cases with MLL-PTD within diagnostic-relapse AML pairs, previously missed with DNA mapping arrays.

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HALOPLEX TARGET DNA ENRICHMENT ALLOWS INVESTIGATION OF COPY NUMBER AND MUTATIONAL STATUS OF KEY GENES IN ACUTE MYELOID LEUKAEMIA WITH NORMAL KARYOTYPE

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Background: Acute myeloid leukaemia (AML) is a deadly haematological malignancy for which individualized treatment based on prognostic stratification is essential to maximize chances of survival. Karyotypic abnormalities carry prognostic significance, but are absent in about 50% of patients (NK-AML), and in this subgroup analysis of gene mutations can be used to stratify patients. Recent advances in AML genomics have defined the set of genes which are recurrently mutated in NK-AML, whilst the prognostic impact of many of these has been determined. Therefore, clinical-grade sequencing platforms will be increasingly useful in clinical practice in the next few years, and this highlights the need for reliable methods targeted gene re-sequencing.
Aims: HaloPlex is a novel, rapid approach for targeted DNA enrichment that requires low amounts of input DNA. We evaluated its performance in detecting abnormalities in coding sequence and copy number of genes recurrently mutated in NK-AML, with special focus on reproducibility and on the quantitative value of data.
Methods: Genomic DNA from 43 NK-AML samples from 40 patients was subjected to Haloplex target enrichment for 24 genes. Target-enriched DNA was sequenced on HiSeq2000, and reads were aligned using BWA. Substitutions and indels were called using standard algorithms and mutations called as previously described (Papaemmanuil *et al.*, Blood 2013).
Results: We sequenced 32.26 gigabases (Gb). A mean of 66.13% mapped on-target (62.94%>74.15%). The mean coverage of the target region was 3674.69x and 91.16% of bases were covered at >30X. Read depth showed significant variability, and coverage across consecutive bases could vary by several folds. This variability was expected as HaloPlex target-enrichment employs digestion of genomic DNA and specific capture of variable-length fragments with subsequent PCR amplification. Nevertheless, we found that this variability in coverage was predictable and highly consistent across samples. Consequently, using normalized data we were able to identify an interstitial deletion of BCOR and three MLL partial tandem duplications. We also report an amplification of KRAS, an event with driver potential in solid cancers but not previously described in AML. The quantitative nature if the data was retained when looking at point mutations, as demonstrated by a narrow range of allelic frequency of heterozygous SNPs. We identified likely oncogenic mutations in 38/40 samples with a median of 3 (1-5) per sample. NPM1 mutations were the most frequent (69%), followed by FLT3 (58%), DNMT3A (35%) and TET2 (32%). As described, NPM1 mutations co-occurred with FLT3 and DNMT3A. Both mono- and bi-allelic FLT3-ITDs were reliably identified. The recurrence rates were consistent with previously published data. We estimated allelic frequency of each mutation and reconstructed the phylogeny of mutations in each. In 2 cases with serial samples we studied the dynamics of tumour evolution and described variants lost and gained at relapse, implying that subclonal evolution can be inferred using Haloplex targeted data.
Summary and Conclusions: Haloplex target enrichment followed by massively parallel sequencing is a simple, rapid and robust method for high throughput screening of gene alterations in NK-AML. It may help prognostic stratification, treatment decisions and minimal residual disease assessment in clinical practice. It can reliably call copy number alterations, substitutions and indels. However, the targeting design should be checked carefully to ensure that the inherent variability in coverage will not affect the efficiency of variant calling.

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Abstract withdrawn

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THE CAMP RESPONSE ELEMENT BINDING PROTEIN (CREB) OVEREXPRESSION INDUCES MYELOID TRANSFORMATION IN ZEBRAFISH

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Background: Pediatric acute myeloid leukemia (AML), is a heterogeneous disease characterized by multiplicity of genetic or epigenetic events that can lead to transformation. Identifying specific leukemogenic aberrations may guide the development of targeted therapy approaches for selected patient groups. We previously identified and characterized one AML proto-oncogene: the cAMP response element binding protein (CREB). Its overexpression has been found in 66% of pediatric AML patients, and represents a poor prognostic marker in human AML. *In vitro* and *in vivo* studies demonstrated that its overexpression leads to abnormal cell proliferation, cell cycle progression and higher clonogenic potential, through an aberrant transcriptional regulation of its target genes. However the molecular mechanism and the transcriptional networks by which CREB triggers leukemogenesis has never been underpinned.
Aims: The aim of this study is to investigate the effects of CREB overexpression on hematopoiesis using the zebrafish *in vivo* model. Moreover, the identification of the main deregulated CREB targets will be pursued, in order to discover relevant pathways which will be considered for further therapeutic targeting.
Methods: We developed a zebrafish model that overexpressed human CREB in myeloid precursor cells, by injecting a plasmid which expressed CREB under control of *pu.1* promoter into 1-cell stage zebrafish embryos. The plasmid was constructed using the site-specific recombination-based cloning (multisite Gateway technology) Tol2kit system. Gene expression studies were conducted by RQ-PCR and whole mount *in situ* hybridization. Hematopoiesis after CREB enforced expression was investigated by flow cytometry analysis of kidney marrow cells, by immunohistochemistry. RNA extracted from kidney of 5 CREB and 5 control zebrafish were analyzed for gene expression (GeneChip Zebrafish Genome Arrays, Affymetrix, Santa Clara, CA, USA).
Results: Results showed that CREB recognized the cyclic-AMP responsive elements at genes promoter modifying gene expression. We documented the up-regulation of its targets, particularly of *bcl-2* and *jun*. Then, we controlled gene expression of genes involved in myelopoiesis and found increased levels of *pu.1*, *mpo* and *gata1* during the primitive hematopoiesis. Then, we monitored the kidney marrow of zebrafish during growth: 6-8 months old CREB zebrafish showed an impairment of myelopoiesis with a loss of cell precursors and increased myelo-monocytes by flow cytometry analysis. From 9 to 14 months, CREB overexpressing zebrafish developed a fatal myeloproliferative neoplasm (MPN) with 41% mortality rate. A clonal expansion of mature myelo-monocitic cells were present in kidney marrow with an impairment of lymphocyte production. Histological analysis revealed that myeloid cells infiltrated extramedullary organs, such as heart, liver, and abdominal organs, leading to zebrafish death. Immunohistochemistry assays displayed that disseminated blasts expressed human CREB. Microarray analysis identified 92 CREB target genes differentially expressed, belonging to proliferation/differentiation MAPK and cell cycle/DNA repair pathways.
Summary and Conclusions: This represents the first evidence that CREB overexpression triggers myeloid transformation in zebrafish. This model will serve to identify CREB mediated genes/pathways that contribute to the myeloid transformation, with the final aim of identifying novel therapeutic targets.

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POTENT COOPERATION BETWEEN THE NUP98-NSD1 FUSION AND THE FLT3-ITD MUTATION IN ACUTE MYELOID LEUKAEMIA INDUCTION

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Background: The NUP98-NSD1 fusion, resulting from an acute myeloid leukaemia (AML)-associated cytogenetically silent chromosomal translocation, is frequently found in pediatric cytogenetically normal AML and associated with aggressive disease and poor prognosis. Co-existence of a FLT3-ITD activating mutation has been reported in more than 70% of these patients but its functional significance remains unclear.
Aims: In order to address the functional synergism between the two mutations, NUP98-NSD1 and FLT3-ITD, we determined the transforming potential of retrovirally expressed NUP98-NSD1 and FLT3-ITD in murine bone marrow *in vitro* and *in vivo*.

Methods: Lineage marker-negative murine bone marrow cells were transduced with *pMSCV-NUP98-NSD1-neo* or *pMSCV-FLT3-ITD-GFP* retroviral vectors or both. Transduced cells were analyzed for their clonogenic potential by serial plating in growth-factor containing methylcellulose followed by expansion and injection into sublethally irradiated syngeneic recipients.
Results: Expression of NUP98-NSD1 provided aberrant self-renewal potential to bone marrow progenitor cells. Co-expression of FLT3-ITD increased proliferation but impaired self-renewal *in vitro*. Transplantation of cells expressing NUP98-NSD1 and FLT3-ITD into mice resulted in transplantable AML after a short latency. The leukaemic blasts expressed myeloid markers (Mac-1^+/Gr-1^+/FcγRII/III$^+$/c-kit^{lo}/CD34^{lo}). Splinkerette-PCR revealed similar patterns of potential retroviral integration sites of *in vitro* immortalized bone marrow cells before and after expansion *in vivo*. Mice that received cells expressing solely NUP98-NSD1 did not develop AML but showed signs of a myeloid hyperplasia after long latency, characterized by expansion of Mac-1^+/Gr-1^+ cells in the bone marrow and active extramedullary haematopoiesis in the spleen. Interestingly, similar to what has been observed in patients carrying NUP98-NSD1, an increased *Flt3-ITD* to wildtype *Flt3* mRNA expression ratio was found to be associated with a more aggressive disease induced by co-expression of NUP98-NSD1 and FLT3-ITD. Additionally, the shorter latency of AML induction correlated with increased activation of the FLT3-STAT5 signaling axe as shown by increased pFLT3 levels and increased basal and GM-CSF induced pSTAT5 levels, as assessed by flow cytometry. To further underline the importance of FLT3 signaling for NUP98-NSD1 induced AML we treated *ex vivo* immortalized progenitors as well as *in vivo* established leukemic blasts with a small molecule FLT3 inhibitor (PKC-412) and found that proliferation of all cells co-expressing NUP98-NSD1 and FLT3-ITD was significantly impaired by PKC-412.
Summary and Conclusions: Our data revealed a potent cooperation between NUP98-NSD1 and FLT3-ITD for the induction of AML disease *in vivo* and underlined the significance of aberrant FLT3 signaling for maintenance of NUP98-NSD1-positive AML. In addition, we provided the rationale and a mouse model that allows to functionally explore the use of small molecule FLT3 inhibitors for NUP98-NSD1 positive AML.

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AN INDUCIBLE MOUSE MODEL FOR MLL-ENL MIXED-LINEAGE LEUKEMIA
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Background: The t(11;19)(q23;p13.3) translocation leading to expression of the MLL-ENL fusion protein is one of the most prevalent MLL gene alterations, mostly associated with mixed lineage or B-cell acute lymphoblastic leukemia. The existing retroviral and knock-in models do not closely recapitulate the human disease, possibly because of a failure to activate MLL-ENL expression in the appropriate hematopoietic compartment.
Aims: To establish an inducible mouse model for MLL-ENL that recapitulates the human disease and thereby facilitates the dissection of the mechanisms underlying MLL-ENL leukemogenesis.
Results: We used the doxycycline (DOX)-induced gene expression system to establish transgenic rtTA;MLL-ENL mice. DOX-induced *ex vivo* expression of MLL-ENL provided bone marrow and hematopoietic stem and progenitor cells with a strong long-term self-renewal capacity, causing the accumulation of immature blast-like cells upon serial replating in methylcellulose cultures. In the presence of factors favoring myelopoiesis, like IL-3, DOX removal resulted in a complete differentiation towards the granulocytic-monocytic, lineages expressing high levels of Mac1/Gr-1, whereas IL-7 favored differentiation towards the B-cell lineage characterized by the expression of high levels of B220. Induction of MLL-ENL expression in newborn or adult mice resulted in 100% of the mice in a leukemic phenotype that phenocopied pre-B- and myeloid mixed lineage leukemia. Diseased mice had excessive splenomegaly, massive lymph node and multi-organ infiltration characterized by the coexistence of at least two types of blasts of various sizes and granularity that expressed intermediate levels of myeloid markers or lymphoid markers. Transplantation of B220^{high}, blasts into sub-lethally irradiated recipients rapidly induced the disease (15.6±0.5 days) whereas transplantation of B220^{low} blasts resulted in significantly longer latency (22.5±1.1 days) suggesting that the B220^{high} population is enriched for leukemia initiating cells. Expression of the fusion gene and disease induction was DOX dosage dependent and fully reversible upon DOX removal. Interestingly, low level MLL-ENL expression resulting from the inherent leakiness of the DOX-inducible system never resulted in any disease. Transplantation of long-term hematopoietic stem cells (LT-HSCs) and granulocyte-monocyte progenitors (GMPs) from naïve rtTA;MLL-ENL mice into lethally irradiated recipients revealed that the LT-HSCs induced the disease in all the recipients whereas GMPs never resulted in any disease, suggesting that MLL-ENL preferentially transforms LT-HSCs rather than committed progenitors.
Summary and Conclusions: We established a novel inducible transgenic mouse model that phenocopies the human MLL-ENL mixed-lineage leukemia. *Ex vivo* and *in vivo* transformation and disease development was always DOX dependent and fully reversible upon MLL-ENL downregulation. Conditional activation of the fusion in different cells of the hematopoietic hierarchy suggested that in contrast to other MLL fusions, MLL-ENL leukemia originates from hematopoietic stem cells or early progenitors rather than more mature lineage-committed progenitor cells.

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LATS1 IS A NOVEL PUTATIVE TUMOR SUPPRESSOR IN ACUTE HOX-INDUCED LEUKEMIA
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Background: It is estimated that 50% of human leukemias are induced by the misregulation of Hox genes and their cofactors such as Meis1. A genetic modifier screen in drosophila showed that hth/MEIS directly interacts with YKI, a transcription co-factor and component of the Hippo signaling network. The Hippo signaling network has been proposed to play a tumor suppressive role in carcinoma development but to date its function in hematopoiesis and leukemia remains unknown.
Aims: The aim of our study is to elucidate the function of the Hippo signaling pathway in Hox / Meis induced Leukemia and to characterize its role in the murine hematopoietic system.
Methods: The core components of the Hippo pathway are expressed in the Hoxa9+Meis1-induced leukemia named FLA2 in which approximately 70% of cells represent leukemia initiating cells (LIC) (Wilhelm B *et al.*, 2011). Freshly isolated FLA2 cells were retrovirally transduced with shRNA targeting Hippo signaling network core components (MST1 and 2, LATS1 and 2, YAP and TAZ) and transplanted into recipient mice. The proportions of shRNA transduced (GFP+) cells were determined at the time of transplantation (day 0), and at the time of sacrifice. We then isolated the CD150+CD48- Lin- stem/progenitor cells from murine BM, co-infected them first with Hoxa9 and Meis1 cDNA carrying retroviruses, and then knocked down Yap or Lats1. The latency of leukemia onset after transplantation was compared to the shLuc - control. To test wether normal murine hematopoiesis is affected by the loss of function of core components of the canonical Hippo pathway, HSC-enriched CD150$^+$CD48$^-$Lin$^-$, cells were infected over 5 days by co-culture with retroviral producer cells in an arrayed 96-well format, with one shRNA per well. Directly after infection, the *in vivo* reconstituting potential of ¼ of each well was evaluated through duplicate competitive repopulation assays involving the co-transplantation of 1.5x10^5 congenic BM competitor cells into irradiated recipients. The remaining cell fraction served to asses gene transfer by GFP fluorescence measurements. Blood reconstitution was evaluated at an early (4wks) and late time point (16-20wks), tracking the contribution of the donor CD45.1+ transduced (GFP+) cells to recipient hematopoiesis over time. As baseline references we used shRNA to Luciferase (no effect) and Nup98HoxA10 overexpression (stem cell expansion).
Results: Expression of the core Hippo pathway constituents in different subpopulations of primitive murine hematopoietic cells as well as in FLA2-leukemia could be detected by quantitative RT-PCR. During the time between transplantation of FLA2-leukemia and sacrifice of the mice, the proportions of shTaz (GFP+) cells to the leukemic cell populations decreased to 10-20% of the initial day 0 values. Conversely, the Lats1 knockdown leads to >50% increase over the initial proportion of the GFP+ cells ($p < 0.05$, Mann-Whitney-Test). This effect is not limited to FLA2 leukemia. AML development in mice induced by overexpression of HoxA9 and Meis is accelerated with additional Lats 1 loss of function. The Median survival of transplanted mice is 46 days (range 45-50 days) compared to 53 days of the shLuciferase control (range 52-56 days). Yap loss of function prolongs the latency of leukemia onset significantly ($p < 0.05$, Mantel Cox Test) to >70 days. Consistent with our previous observations we detected a strong impact of LATS1 loss of function on normal HSC-function, resulting in higher long - term reconstitution rates compared the shLuciferase control. Moreover, one third of transplanted mice developed acute leukemia (myeloid and lymphoid).
Summary and Conclusions: Our observations suggest that LATS1 acts as a tumor suppressor in Hox/Meis-induced leukemia and putatively in the hematopoietic system. This indicates a possibility for a specific targeting of the Hox/Meis-activated cellular pathways.

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THE MYB-GATA1 FUSION IN THE CONTEXT OF LOW GATA1 EXPRESSION FAVOURS THE BASOPHILIC DIFFERENTIATION OF HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS
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Background: Acute basophilic leukemia with t(X;6) translocations represent a rare specific hematological malignancy encountered in male infants. The t(X;6) translocation has two consequences: i) the fusion of MYB and GATA1, two transcription factors important for the regulation of hematopoiesis and ii) the destruction of the GATA1 gene, resulting in the absence of this protein in the cells of the affected male patients (Quelen *et al.*, Blood, 2011;117(21):5719-5722). We and other have previously shown that murine hematopoietic stem cells expressing MYB-GATA1 and low levels of GATA1 display an increased self renewal and a propensity to develop acute myeloblastic leukemia. However, no evidence of a basophilic phenotype has been observed in this model thus far.
Aims: Since this genetic abnormality correlates with a characteristic basophilic leukemia, we explored whether the expression of MYB-GATA1 in cells expressing a low level of GATA1 would result in a basophilic differentiation.
Methods: Lentiviral vectors encoding MYB-GATA1 along with a TdTomato marker or a shGATA1 with a GFP marker were generated, as well as control vectors (empty vector withTdTomato or irrelevant shRNA with GFP). Normal human CD34+ stem / progenitor cells were isolated from placental blood by immunoselection. They were infected with control or relevant vectors after a 24 hour culture in medium containing IL-3, SCF and TPO. 100 000 cells were then engrafted in immunodeficient (NOD-SCID-γ) mice after myeloablation by busulfan. Twelve weeks later, hematopoiesis was examined for basophilic differentiation by analyzing the expression of CD117, CD203c, FcεRI and CD123 by flow cytometry.
Results: No mouse developed signs of leukemia. There were no anomalies of the various organs studied (spleen, liver, lungs). CD34+ cells were efficiently infected by control vectors (45-65% expressing both the TdTomato and GFP markers) and the MYB-GATA1 and shGATA1 expressing vectors (6-17% double transfectants). Efficiencies of engraftments, appreciated by measuring cells expressing the human CD45 antigen, were similar for control and MYB-GATA1/shGATA1 infected cells. The proportion of persistent CD34+ cells was slightly higher in mice engrafted with MYB-GATA1/shGATA1 (median: 21% *versus* 10%), as was the percentage of myeloid CD33+ cells (59% *vs* 19%), confirming results obtained previously with murine cells. Expression of the basophilic marker CD123 was similar in both groups (7 *vs* 5.5%), but other basophilic / mast cell antigens FcεRI (5.05 *vs* 1.45), CD203c (6.6 *vs* 1.15) and CD117 (20.85 *vs* 1.85) were significantly higher in cells expressing MYB-GATA1 and shGATA1.
Summary and Conclusions: Expression of the chimeric protein MYB-GATA1 in the context of low expression of GATA1 reproduces the conditions observed in leukemic blast cells. These conditions increase the self renewal of CD34 positive cells and favour a myeloid differentiation. They also promote the expression of basophilic and mast-cell markers FcεRI, CD203c and CD117. This validates the importance of MYB-GATA1 fusion and low GATA1 expression in basophilic leukemia, and suggests that additional anomalies are necessary for the full leukemic phenotype.

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THE IKAROS 6 ISOFORM COOPERATES WITH BCR-ABL1 TO INDUCE HUMAN ACUTE MYELOID LEUKEMIA IN XENOGRAFTS

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Background: Historically, our understanding of mechanisms underlying human leukemogenesis are inferred from genetically engineered mouse models. Relatively few models that use primary human cells recapitulate the full leukemic transformation as assayed in xenografts and myeloid transformation is infrequent.
Aims: We aimed to determine the functional consequence of combinatorial overexpression of *BCR-ABL1* and a dominant negative isoform of IKAROS, Ik6, in human lineage depleted cord blood (LIN-CB) cells.
Methods: LIN-CB cells were exposed to lentiviruses, and then to retroviral supernatants. Four independent cultures were generated: control, Ik6, *BCR-ABL1* and *BCR-ABL1*-Ik6. Cells were seeded after infection into lympho-myeloid promoting culture conditions. NSG mice were intrafemorally transplanted with transduced LIN-CB and sacrificed at 16-20 weeks post-transplantation or when sick. Engrafted human cells were characterized by flow cytometry and extensive histopathological analysis. Gene expression, gene set enrichment, pathway and network analysis were performed on purified double-transduced cells cultured *in vitro*.
Results: We report a humanized experimental leukemia model where xenografts develop aggressive acute myeloid leukemia (AML) with disseminated myeloid sarcomas within 4 weeks following transplantation of cord blood transduced with vectors expressing *BCR-ABL1* and Ik6. Ik6 induced transcriptional programs in *BCR-ABL1* transduced progenitors that contained repressed B cell progenitor programs, along with strong stemness, proliferation, and granulocyte-monocytic progenitor signatures; a novel combination not induced in control groups.
Summary and Conclusions: Thus, wild type IKAROS restrains stemness properties and has tumor suppressor activity in *BCR-ABL1* initiated leukemia. Although IKAROS mutations/deletions are common in lymphoid transformation, they are found also at low frequency in AML that progress from a prior myeloproliferative neoplasms (MPN) state. Our experimental system provides an excellent model to gain insight into these rare cases of AML transformation and the properties conferred by IKAROS loss of function as a secondary mutation. More generally, our data point to the importance of deregulated stemness/lineage commitment programs in human myeloid leukemogenesis.

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SERIAL TRANSPLANTATION AND *IN VIVO* MONITORING OF PATIENT-DERIVED XENOGRAFTS: AN ADVANCED PRECLINICAL MODEL TO STUDY DIVERSE GENETIC AML SUBGROUPS IN MICE

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Background: Despite recent improvements in diagnosis and treatment of acute myeloid leukemia (AML), the majority of patients suffer from a persistently poor prognosis. Preclinical research can improve the understanding of AML biology and help to develop novel therapeutic approaches. In recent years, primary human AML cells have been engrafted successfully in immuno-compromised mice to study stem cell features and to analyze treatment response on AML cells *in vivo*. However, until now, re-engraftment of patient-derived cells has only been analyzed to assess self-renewal capacity.
Aims: We aimed at improving the individualized xenograft mouse models of AML by establishing patient-derived xenografted (Pdx) lines and introducing *in vivo* monitoring. To confirm the validity of this model, we evaluated the consequences of sequential transplantation and transgene expression on genetic and functional characteristics of Pdx cells, and efficiency of *in vivo* bioluminescence imaging (BLI).
Methods: Patient-derived AML cells were injected into NOD/scid IL2 receptor γ chain knockout (NSG) mice. After successful engraftment, human cells were re-transplanted into next generation recipients for up to six passages to establish Pdx lines. Pdx cells and lines were genetically characterized by targeted resequencing of 43 AML-related genes. Furthermore, cells were transduced with lentiviral constructs containing enhanced firefly luciferase to facilitate BLI.
Results: Serial transplantation of Pdx cells was feasible in 6/12 of initially engrafted samples. Growth and sample characteristics like passaging time (indicating time from cell injection until overt disease), percentage of human cells within mouse bone marrow (BM) or peripheral blood (PB), and immunophenotype remained stable in Pdx lines. Targeted resequencing confirmed that clonal and subclonal mutations in patient specimens were preserved in the matched Pdx lines. However, subclones present in the primary sample became a major clone in two of six Pdx samples, and in another two samples, subclones within the patient were not detectable within Pdx cells. Therefore, we conclude that some subclones may have engraftment and/or growth advantages within the mouse. Nevertheless, the genetic characteristics of the primary samples were preserved within Pdx cells to a high extent. Until now, disease monitoring within the individualized xenograft mouse model of AML was challenging. Human cells are secreted into PB only at late disease stages. BM biopsies can be performed only infrequently due to animal welfare. Therefore, most data is determined by post mortem analysis. Here we show that transgenic Pdx (t-Pdx) cells expressing luciferase can be visualized by BLI. BLI was very sensitive, visualizing t-Pdx cells on a 10^{-4}, level of mouse BM cells on day one after cell injection. Additionally, growth of t-Pdx cells could be followed over time in an exponential manner. Furthermore, we performed limiting dilution transplantation assays, and found that the frequency of leukemia initiating cells could be determined within four to six weeks. Importantly, sample characteristics of t-Pdx cells still maintain immunophenotypic and mutational characteristics of the primary patient samples.
Summary and Conclusions: We conclude that Pdx lines faithfully represent the AML sample they originate from. Prospectively, these advancements enable repetitive, clinically relevant studies on AML biology and preclinical treatment trials on genetically defined and heterogeneous subgroups.

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LEUKEMIC STEM CELLS OF ACUTE MYELOID LEUKEMIA PATIENTS CARRYING NPM1 MUTATION ARE CANDIDATES FOR TARGETED IMMUNOTHERAPY

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Background: The nucleophosmin 1 (*NPM1*) gene is an interesting candidate for targeted immunotherapy in acute myeloid leukemia (AML). Mutations in *NPM1* are one of the most frequent molecular alterations in AML. Immune responses against the mutated protein of *NPM1* were described and might contribute to the favourable prognosis of AML patients carrying $NPM1^{mut}$. However, underlying mechanisms are still not clear. Leukemic stem cells (LSC) might be the source for leukemic self-renewal and might account to disease relapse after treatment.
Aims: We were interested in expression differences of the LSC enriched cell fraction descendent of $NPM1^{wt}$ and $NPM1^{mut}$ primary AML patients. We suggest that expression differences between the patients groups could be a factor for the better overall survival of $NPM1^{mut}$ patients. In addition we aimed to study new targets on $NPM1^{mut}$ LSC for new therapeutical purposes.
Methods: We enriched the CD34+CD38- fraction of primary AML peripheral blood mononuclear cells (PBMC), by Fluorescence-activated cell sorting (FACS). We sorted 21 AML patient samples; 12 $NPM1^{wt}$ and 9 $NPM1^{mut}$. We also enriched healthy donor samples for HSC purification, in order to compare expression levels. Enriched CD34+CD38- cells in $NPM1^{mut}$ AML samples harbor cytoplasmic NPM1 demonstrated via immunocytochemical staining. Functional assays, confirming the biological importance of these factors have been performed.
Results: We showed that enriched CD34+CD38- cells in $NPM1^{mut}$ AML samples harbor cytoplasmic *NPM1* via immunocytochemical staining, indicating that these cells belong to the leukemic clone. The cell number and RNA quality was sufficient for further Microarray studies in which we analyzed the CD34+CD38- enriched compartments descendent from $NPM1^{mut}$ and $NPM1^{wt}$ patients. Those showed significant differences in gene expression patterns which noticeably are immunologically coined, for example: immunoglobulin superfamily, member 10 (p=0.0003405; fold change: 6.22) and the interleukin 12 receptor, β 1 (p=0.000834, fold change: 1.87). This impression was confirmed by pathway analysis indicating deregulation of pathways like the NO2-dependent IL 12 Pathway in NK cells and the Th1/Th2 Differentiation and the Platelet Amyloid Precursor Protein Pathway. Furthermore we screened our data for new potential therapeutic target structures, specifically on enriched CD34+CD38- cells of $NPM1^{mut}$ patients, comparing the expression level of target genes to $NPM1^{wt}$ CD34+CD38- cells, and the expression level on HSC. Amongst others, our most promising candidates are SERPINA1 (p=0.0062344, fold change: 14.32), OSCAR (p=6.11E-05, fold change: 9.03) and several further interesting genes. These genes could be used in order to target CD34+CD38- cells of $NPM1^{mut}$ patients in a therapeutic manner.
Summary and Conclusions: Taken together, we could demonstrate that leukemic progenitor cells of $NPM1^{mut}$ and $NPM1^{wt}$ AML patients can be effectively separated for array analysis. The frequency of CD34+CD38- enriched LSC population in $NPM1^{mut}$ AML patients is significantly lower. Markedly, genes with immunological functions seem to play an important role in the $NPM1^{mut}$ AML subtype. Besides peptides derived from $NPM1^{mut}$, which were already described as an immunogenic target for specific immunotherapy, further interesting targets have been detected, like the cell surface marker CD96 and *IL12RB1*. Our functional assays suggest that these detected targets might have a therapeutic potential that has to be elucidated in clinical trials.

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MESENCHYMAL STEM CELLS (MSCS) OF AML PATIENTS HARBOR CHROMOSOMAL DEFECTS DISTINCT FROM THOSE OF LEUKEMIC CELLS

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Background: Various studies have demonstrated that the bone marrow microenvironment (the niche) is involved in leukemia development. As MSCs are key components of the niche, it was questioned whether MSCs from AML patients (pts) carry the same chromosomal defects of leukemic cells.
Aims: Thus, this possibility was tested by the present study which examined leukemic and MSCs from fourteen AML pts with three different technologies, conventional cytogenetics (CC), FISH and aCGH/SNPa.
Methods: At disease onset after informed consent all the fourteen pts were submitted to bone marrow (BM) aspiration. BM cells were submitted to CC and FISH analyses. In addition, MSC were isolated from BM cell suspension (10-15 ml) as previously described. Briefly, mononucleated cells were isolated from BM by density gradient centrifugation using Lympholyte®-H and seeded in 75 cm^2 cell culture flasks at a cell density of 10^6 cells/cm^2. Cells were cultured at 37°C, 5% CO2 in MEM-alpha medium containing 1% Penicillin/Streptomycin, 1% L-Glutamine and 10% fetal bovine serum. After 48-h adhesion, non-adherent cells were removed and culture medium replaced. Growth medium was changed every three days. MSCs were examined after the first passage and their phenotype was evaluated by flow cytometry. Cells were detached from culture using Tripsin-EDTA and after washing stained for ten minutes with the following fluorochrome-conjugated antibodies: anti-CD90-FITC, anti-CD105-PE, anti-CD14-FITC, anti-CD73-PE, anti-CD34-FITC, anti-CD80-PE, anti-CD133-APC, anti-CD31-PE and anti-CD45-APC-Alexa750. Stained cells were acquired with a Beckman Coulter Navios instrument and data analyzed with Kalooza software. The commercial FISH probes used were LSI D7S486/CEP7, LSI AMLE-TO from Abbot Molecular Inc. (Chicago, Il, USA) and ON c-Myc/SE8, SE10(D10Z1) from Kreatech (Amsterdam, NL). Their cut-off values were determined by applying a one-sided 95% confidence interval. aCGH/SNPa was carried out with the SureScan Microarray Scanner G4900DA (Agilent Technologies Inc. Santa Clara, CA).
Results: In leukemic cells CC revealed a chromosomally normal pattern in seven pts, a -7 in two, a del(7)(q31) in one, a +8 and a +10 in one each, a t(8;21)(q24,q22) in one and a complex karyotype in the last pt. All these defects were confirmed by FISH. In order to establish whether leukemic cells and MSCs shared the same pattern, MSCs cultures were tested with FISH. Flow-cytometry revealed that MSCs purity was 50-87%. FISH showed a normal pattern in all the cultures examined. In contrast, aCGH/SNPa revealed an amplification of the entire chromosome 5 in one pt, a LOH of a 3.8 Mb sized region located on 13q31.1 in one, a LOH of a 4.3 Mb region mapped on chromosomes 6 and 18 in two, an amplification of three 71Kb, 322Kb and 47Kb sized regions of chromosomes 5, 18 and 20 in one and an amplification of three 17Kb, 40Kb and 70Kb sized regions of chromosomes 9, 11 and 15 in another pt. In this last an amplification of six genes including *JAK1, ELN, FGFR2* was observed. FISH revealed that chromosome 5 amplification, the only defect tested by this technique, was produced by a true trisomy.
Summary and Conclusions: In conclusion i) MSCs from chromosomally abnormal AML pts may have a normal FISH pattern, but may contain on aCGH/SNPa analysis LOH or amplifications different from those of leukemic cells; ii) usually, these lesions are uncommon in AML; iii) MSCs defects may flag a leukemogenic-induced genomic instability which not only affects the hematopoietic tissue but also the niche; iv) aCGH/SNPa is a powerful technique for identifying clonal markers.

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ALTERATIONS IN MESENCHYMAL STROMAL PRECURSOR CELLS FROM THE BONE MARROW OF THE ACUTE MYELOID LEUKEMIA PATIENTS: NEWLY DIAGNOSED, AND BEFORE AND AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Stromal microenvironment is essential for normal hematopoiesis, as it forms niches for hematopoietic precursors. Leukemia and high dose chemotherapy affect both hematopoietic and stromal precursor cells. Changes in the hematopoiesis that occur during acute myeloid leukemia (AML) can cause some variations in the composition of the stromal microenvironment.
Aims: The goal of this study was to analyze the alterations in the characteristics of human multipotent mesenchymal stromal cells (MSC) and their more differentiated progeny - fibroblastic colony forming units (CFU-F), derived from the bone marrow (BM) of AML patients.
Methods: Eleven newly diagnosed cases and 20 patients before and during 1 year after allogeneic hematopoietic stem cell transplantation (alloHSCT) were involved in the study after informed consent. BM was aspirated prior to any treatment in the newly diagnosed group and before the conditioning and at 6 time points during 1st year after the alloHSCT in the group of patients with alloHSCT. MSC were cultured in aMEM with 10% fetal calf serum. Cumulative MSC production was counted after 3 passages. CFU-F concentration was analyzed in standard conditions. The relative expression level (REL) of different genes was measured by RT2 Profiler PCR Array (Qiagen) and TaqMan RQ-PCR. As a control MSC and CFU-F from 50 healthy donors of BM for alloHSCT were used.
Results: The CFU-F concentration in the BM of patients at the moment of AML diagnostics was half of the donor's (13.9±8.1 *versus* 32.2±4.3 per 10^6 nucleated cells in donor BM, p=0.06). Most of the patients assigned to alloHSCT were in the remission and at that moment CFU-F concentration in their BM was slightly lower than in donors (26.7±7.2 per 10^6 BM nucleated cells). After the alloHSCT CFU-F concentration in patients' BM decreased 3-9 folds for the next year of observation. The decrease at each time point was highly significant comparing to donors. Similar picture was observed in MSC analysis. Cumulative cell production in MSC of newly diagnosed AML patients was also half of

the donors (3.8±1.1 x10^6 *versus* 7.6±0.8x10^6 for donors' MSC, p=0.02). MSC from the BM of patients before alloHSCT were also slightly and insignificantly lower than from healthy donors (5.9±1 x10^6), and after alloHSCT cumulative MSC production decreased 1.3-5.2 folds for the next year. The decrease at almost each time point was significant comparing to donors. All these effects were reflected in the proliferation rate of studied MSC: time of reaching confluent monolayer at Po of MSC from newly diagnosed patients and the ones after alloHSCT was significantly prolonged comparing to donors. Gene expression analysis revealed that in MSC at the moment of AML diagnosis then REL of FGF2, IL1B, IL6, JAG1, PDGFB, VCAM1, VEGFA decreased more than 2 fold. Prior to alloHSCT the REL of IL6 and IL1B were still very low, following the transplantation the REL of IL6, JAG1, PDGFB increased, while IL1B stayed at the low level at least for 6 months.

Summary and Conclusions: During the AML development leukemic cells alter the stromal precursor cells leading to the decrease in their proliferative ability, in the REL of many regulatory genes and in the number of more differentiated stromal precursor cells in the BM. Chemotherapy used for induction of the remission in these patients did not alter the stromal precursor cells significantly. Conditioning regiments used for the alloHSCT significantly damage both types of studied stromal precursors, and the effect lasted at least for 1 year. So, both AML cells and chemotherapy affect BM hematopoietic microenvironment.

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COMPREHENSIVE ASSESSMENT OF BONE MARROW STROMA-DERIVED SOLUBLE FACTORS AND IMPACT ON *EX VIVO* DRUG SENSITIVITIES OF PRIMARY AML CELLS

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Background: The bone marrow (BM) microenvironment is composed of multiple cell types that via direct cell-to-cell interactions and soluble factors support the survival of leukemic cells and influence their response to therapeutic agents. Thus, survival signals from the BM microenvironment may contribute to the development of drug resistance. However, failure to mimic these interactions *ex vivo* leads to poor translation of results from drug sensitivity testing assays. More reliable prediction of drug sensitivity is needed and recent focus has been directed towards methods that take into account the supporting impact of the tumor microenvironment.

Aims: In these studies we aimed to develop a more predictive high throughput assay mimicking the *in vivo* tumor microenvironment in order to assess drug sensitivities of primary acute myeloid leukemia (AML) cells *ex vivo*.

Methods: Conditioned medium (CM) was collected from the HS-5 human BM stromal cell line (American Type Culture Collection, ATCC) and combined with RPMI medium for drug sensitivity testing. Mononuclear cell medium (MCM, Promocell) was used as the standard medium comparison. Sensitivity of primary AML (n=13) or healthy (n=5) BM mononuclear cells to 306 approved and investigational drugs was measured at five different concentrations covering a 10,000-fold concentration range. Cell viability was measured after 72 h and dose response curves generated for each tested drug. Drug sensitivity scores (DSS) were calculated based on the area under the dose response curve. The same cells were cultured in 25% CM or in MCM and the drug sensitivities compared. Drug sensitivities of matched fresh and vitally frozen biobanked AML cells were also assessed. Growth factors and cytokines present in the CM were detected by an antibody array (RayBioTech).

Results: HS-5 CM supported fresh and biobanked primary AML cells, promoting their overall survival. Freshly isolated AML cells had a mean viability of 128% after 3 days in CM compared to 63% in MCM. The viability of biobanked cells was 85% in CM *vs.* 20% in conventional medium. The supportive effect of the CM was seen with *ex vivo* drug sensitivity testing where sensitivities differed significantly when comparing CM to standard medium results. When cultured in CM, AML cells positive for the FLT-ITD mutation exhibited reduced efficacy to multiple tyrosine kinase inhibitors (TKIs) targeting VEGFR, PDGFR, ABL KIT, as well as FLT3. However the FLT3-ITD AML cells were sensitive to these inhibitors when cultured in the conventional medium. Comprehensive assessment of soluble factors in the HS-5 CM detected several inflammatory cytokines and chemokines, including high levels of IL-6, IL-8, GM-CSF, G-CSF, GRO, and MCP-1 that can activate adaptive survival signals, such as the JAK/STAT pathway. Corresponding to these findings, we observed increased sensitivity of AML cells to the JAK1/2 inhibitor ruxolitinib when cultured in CM (DSS 14 *vs.* 3).

Summary and Conclusions: Our data indicate that conditioned medium from stromal cells supports both fresh and biobanked AML cells, providing soluble factors present in the BM niche necessary for cell survival. Inflammatory cytokines present in the CM reduce the efficacy of multiple TKIs by activating adaptive survival pathways. Taken together, our results suggest that using conditioned medium from a stromal cell line in an *ex vivo* drug sensitivity assay, may better recapitulate the bone marrow microenvironment and the development of resistance to TKIs often observed in leukemia patients. This setting could therefore potentially provide better prediction of the *in vivo* response.

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THE CATS (FAM64A) PROTEIN IS IMPORTANT FOR CLONOGENICITY OF THE MONOCITIC LEUKEMIA CELL LINE U937

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Background: The CATS (FAM64A) protein was described as an interacting partner of both CALM and the leukemic fusion protein CALM/AF10. CATS localizes in the nucleus and nucleoli, causing both CALM and CALM/AF10 accumulation in those structures. It has been suggested that CATS could participate in leukemogenesis.

Aims: The aim of this study was to analyze CATS expression during erythroid, megakaryocytic and granulocytic differentiation of leukemia cell lines and to investigate CATS function upon its depletion in the CALM/AF10-containing U937 cell line.

Methods: Leukemia cells differentiated into erythroid (HE-HU+KU812), megakaryocytic (PMA+K562) and granulocytic (ATRA+NB4 or U937) lineages were analyzed for *CATS* expression by qRT-PCR. Lentivirus targeting CATS silencing (Sta Cruz Biotechnologies) was used to transduce the U937 cells. Depletion was confirmed at RNA (qRT-PCR) and protein (Western blotting) levels. CATS depleted (shCATS) and control cells (shControl) underwent functional *in vitro* assays. Cell proliferation was assessed by MTT assay and BrdU incorporation. Apoptosis was accessed by Annexin-V/PI staining of normal cultured, serum starved and UV-irradiated cells. Cell cycle was accessed by FACS analysis of the DNA content using PI. Migration was analyzed in 8µm pore-sized transwell plates, in which cells migrated towards the chemotatic stimulus of 10% FBS or CXCL12-containing medium. To access the clonogenicity cells were cultivated in semi-solid media without growth factors and colonies were counted after 7 days. Proliferation was also assessed *in vivo* using xenotransplant model of tumor growth, in which cells were inoculated subcutaneously into NOD-SCID mice. Tumors were excised after 12 days of growth, measured and weighted. Expression analysis of cell cycle- and migration-related proteins were performed by Western blotting; and of self-renewal-related genes by qRT-PCR.

Results: *CATS* expression decreased by 58% and 43%, during erythroid and megakaryocytic differentiation, respectively. Interestingly, *CATS* transcript levels increased by 2 fold during ATRA induced granulocytic differentiation of NB4 (4 days of differentiation) and U937 cells (2 days of differentiation); in which *CATS* expression decreased after 4 days of differentiation. CATS depletion was about 80% in shCATS cells. Proliferation of these cells was reduced by 20% when compared to control whereas no effect was observed on cell death. Accordingly, a significant decrease of 12% in the percentage of cells in S phase of the cell cycle was observed forCATS. Moreover, CATS silenced cells migrated less towards serum condition (15%) than control, while no difference was observed towards the chemoatractant CXCL12. Most notably, shCATS cells formed significantly less colonies (66%) on semi-solid medium than control cells. No difference in tumor growth was observed by shCATS cells compared to those formed by shControl cells. Finally, CATS silencing downregulated *GLI1* transcript expression (85%) and altered expression of the cell cycle protein CYCLIN E and the expression and stability of the microtubule dynamic-related protein a-TUBULIN.

Summary and Conclusions: *CATS* seems to be responsive to the differentiation inducer retinoic acid. Moreover, CATS seems to play a role in controlling cell proliferation, migration, and most importantly, the clonogenicity of U937 cell line. More studies are needed in order to investigate whether these functions are specific to the CALM/AF10-containing U937 cells or if it is extendable to other cell lines.

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LEUKEMOGENIC FLT3-ITD AND KIT D814V DEPEND MORE ON CALM FUNCTION THAN THEIR WILD TYPES TO TRANSMIT GROWTH/SURVIVAL SIGNALS: IDENTIFICATION OF CALM AS A NEW THERAPEUTIC TARGET

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Background: Clathrin Assembly Lymphoid Myeloid leukemia protein (CALM)

is implicated in the formation of clathrin-coated vesicles which mediates endocytosis and intracellular trafficking of growth factor receptors and nutrients. We previously reported that CALM regulates intracellular trafficking of receptor tyrosine kinases (RTKs), such as FLT3 and KIT, thereby regulating the growth and survival of hematopoietic stem/progenitor cells (18th Congress of EHA 2013). Meanwhile, it has been reported that oncogenic RTKs are mislocalized in cytoplasm, where they transmit aberrant signals to downstream molecules. However, the precise mechanisms how they are transported in cytoplasm and transmit their signals are not fully understood. Also, the roles of CALM in this system remain unknown, Among mutated RTKs, FLT3 internal tandem duplications (ITD) and a point mutation of KIT at D816 (corresponding to murine KIT D814) have been shown to be involved in the pathogenesis of acute myeloid leukemia (AML).

Aims: In the present study, we investigated the roles for CALM in their intracellular trafficking and leukemogenic activity of these mutated RTKs.

Results: We introduced murine FLT3 wild-type (WT) and ITD and KIT (WT and D814V) into murine embryonic fibroblasts (MEFs) isolated from CALM$^{-/-}$ and WT mice, respectively, and examined the role of CALM in their intracellular trafficking. In response to each ligand, FLT3 WT and KIT WT were internalized from membrane to cytoplasm and transported from early to late endosomes and consequently to lysosome in WT MEFs. In these processes, the trafficking from early to late endosomes was impaired in CALM$^{-/-}$, MEFs. In contrast, FLT3 ITD and KIT D814V were distributed at close to endoplasmic reticulum (ER) and Golgi in cytoplasm with a punctate pattern irrespective of ligand stimulation in WT MEFs, from where they transmitted the signals. However, in CALM$^{-/-}$ MEFs, these RTK mutants were predominantly localized apart from ER and Golgi with a diffused pattern, indicating that the system retaining mutated RTKs at ER or Golgi is disrupted by CALM deficiency. Next, we knocked down (KD) the expression of CALM by stably transfecting shRNA against CALM into an IL-3-dependent cell line Ba/F3 engineered to express FLT3 (WT and ITD) and KIT (WT and D814V), respectively. Ba/F3 cells transfected with FLT3 WT and KIT WT dose-dependently proliferated in response to each ligand. However, CALM shRNA partially suppressed their growth, while it hardly affected IL-3-dependent growth. Although FLT3 ITD and KIT D814V enabled Ba/F3 cells to grow independently of IL-3, the growth mediated by FLT3 ITD and KIT D814V was severely inhibited by CALM KD. As for this mechanism, we found that tyrosine autophosphorylation of FLT3 ITD and KIT D814V located in cytoplasm was suppressed by CALM KD, while ligand-induced tyrosine phosphorylation of membrane-bounded FLT WT and KIT WT was hardly influenced. These results indicate FLT3 ITD and KIT D814V depends more on CALM function than respective WTs to transmit their signals. In accord with these *in vitro* results, tumorigenic activities of FLT3 ITD and KIT V816V were severely suppressed by CALM KD in mouse transplantation model using these Ba/F3 clones.

Summary and Conclusions: Intracellualr localization and transport of FLT3 ITD and KIT D814V are different from those of WTs, where the roles of CALM were also distinct between these mutants and WTs. In addition, our results indicate that CALM plays a critical role in leukemogenic activity of FLT3 ITD and KIT D814V and would be a promising therapeutic target.

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TARGETING THE HOXA CLUSTER IN MLL-FUSION LEUKEMIAS

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Background: *Hox* genes are highly expressed in normal hematopoietic stem and progenitor cells but rapidly decrease during normal differentiation, however elevated levels are maintained in leukemia. *Hox* expression is epigenetically regulated by polycomb repressor complexes (PRCs) and histone modifiers *e.g.* Mixed Lineage Leukemia (MLL). Translocations involving MLL are frequent in acute leukemia however the role and necessity of HOX-TALE expression in disease maintenance is not fully elucidated.

Aims: To identify the criticality of the HoxA cluster in MLL-fusion leukemia maintenance.

Results: A transgenic mouse line in which the complete HoxA cluster is flanked by loxP sites (HoxA$^{flox/flox}$) has been established and crossed with MxCre mice to generate a MxCre/HoxA$^{flox/flox}$ (MAFF) mouse line. Ectopic overexpression of MLL-fusion oncogenes in sorted primary MAFF bone marrow cells result in increased colony formation and growth in liquid culture. Transformed bone marrow cells were grown in methylcellulose culture to form colonies and serially replated after 5-7 days. Cells were transplanted into irradiated recipient mice from each stage of plating to give rise to primary MLL-fusion leukemias.

Leukemias generated in the MAFF background had one of the HoxA cluster alleles deleted due to background activation of the *Mx1* promoter. Haploinsufficiency of the HoxA cluster was still capable of initiating primary leukemia, with limited compensation from the intact HoxA cluster. Single colonies selected from methylcellulose and expanded in liquid culture demonstrated deletion of the HoxA cluster by PCR, validated by DNA sequencing.

Exposure of the MAFF MLL-fusion leukemias to Interferon-α *in vitro* activated the *Mx1* promoter and resulted in deletion of the HoxA cluster. Preliminary results indicate that loss of HoxA has no anti-proliferative effects in liquid culture and a moderate decrease in colony formation in methylcellulose.

Summary and Conclusions: Studies are ongoing to assess the complete deletion of the HoxA cluster in MLL-fusion leukemias, to determine their necessity in maintaining the leukemic phenotype in pre-established disease.

It is anticipated that models generated will provide a platform to further examine the role of HoxA in MLL-fusion leukemia maintenance and help identify key underlying molecular mechanisms in this disease subtype for future drug discovery.

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LOW MST1 HIPPO KINASE EXPRESSION CONTRIBUTES TO DIFFERENTIATION DEFICIENCY IN AML CELLS

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Background: The Hippo pathway represents a signal transduction pathway that is involved in regulation of organ growth, regeneration, differentiation and gene expression control. The Ser/Thr kinases mammalian sterile 20-like kinase 1/2 (MST1/2; Hippo in Drosophila) at the top of the cascade activate the LATS1/2 kinases, which in turn inhibit the transcriptional co-activators YAP and TAZ (Yorkie in Drosophila). Unphosphorylated YAP and TAZ interact with the TEAD transcription factor to drive the expression of mainly growth-promoting and apoptosis-inhibiting genes. Thus, the growth inhibitory activity of the Hippo pathway functions by inhibiting nuclear accumulation of active YAP and activation of growth-promoting genes. In this context, active MST1 is considered a tumor suppressor and its expression is significantly repressed in breast, non-small cell lung and gastric cancer.

Aims: In our study we aimed at quantifying expression levels and testing a possible function of MST1 in the pathology of acute myeloid leukemia (AML), a disease characterized by a block of myeloid differentiation and increased cell survival.

Methods: mRNA expression of MST1 was measured by low density array in clinical AML patient samples (n=98) among FAB subtypes M0 to M4, CD34$^+$ progenitor cells, macrophages and granulocytes of healthy individuals.

Neutrophil differentiation of CD34$^+$ progenitor cells was induced using granulocyte colony stimulating factor (G-CSF) and MST1 mRNA expression was measured at day 3, 6 and 12. The APL cell lines NB4, HT93 and the AML cell line HL-60 were differentiated towards granulocytes by 1μM all-trans retionoic Acid (ATRA) treatment for 6 days. Gene expression of MST1, G-CSF-R was determined by quantitative real-time RT-PCR. Western Blotting was used to measure protein levels of MST1, YAP1 and YAP1-PH. NB4 cells were transduced with a lentiviral vector expressing a small hairpin (sh)RNA targeting MST1 mRNA.

Results: Firstly, we quantified MST1 mRNA expression in clinical AML patient samples (n=98; FAB subtypes M0 to M4). We observed a highly significant ($p<0.001$) about 7-fold downregulation of MST1 in AML blasts, normal CD34$^+$ progenitor cells and macrophages of healthy individuals as compared to mature neutrophils from healthy donors (Figure 1 a). Accordingly, neutrophil differentiation of CD34$^+$ progenitor cells using granulocyte colony stimulating factor (G-CSF) resulted in increased MST1 mRNA expression (Figure 1 b). We next analyzed MST1 mRNA and protein expression during *in vitro* neutrophil differentiation of NB4 and HT93 acute promyelocytic leukemia (APL) cell lines. APL t(15;17) cells express the leukemic fusion protein PML-RARA, which represses genes important for neutrophil differentiation. Pharmacological doses of all-trans retinoic acid (ATRA) can overcome this differentiation block. ATRA-induced neutrophil differentiation of both APL cells resulted in a significant 10-fold induction of MST1 mRNA paralleled by a marked increase in MST1 protein expression (Figure 1 c). Importantly, in ATRA-resistant NB4-R2 MST1 expression was not upregulated indicating that MST1 induction is not due to an unspecific ATRA-response. Increased MST1 expression was also observed during neutrophil differentiation of PML-RARA negative HL60 AML cells. Next, to address the functional involvement of MST1 in neutrophil differentiation, we generated NB4 MST1 knockdown cells using a lentiviral vector expressing small harpin (sh) RNA targeting MST1. Knockdown efficiency as assessed by qPCR and Western blotting was 80%. Inhibition of MST1 resulted in significantly attenuated neutrophil differentiation as shown by significantly reduced CD11b surface (Figure 1 d) and G-CSF-R mRNA expression. Of note, preliminary data show decreased YAP1 phosphorylation in NB4 MST1 knockdown cells supporting functionality of the MST1 knockdown.

Summary and Conclusions: Collectively, our findings suggest that MST1 expression is associated with an immature myeloid phenotype and define a novel regulatory, MST1-mediated (Hippo) pathway required for neutrophil differentiation of APL cells.

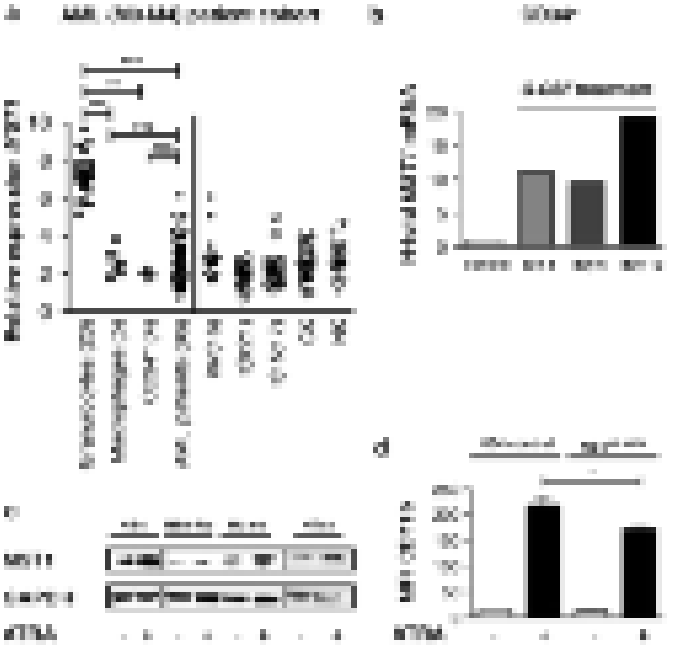

Figure 1.

P158

NOVEL FUNCTION FOR THE RNA BINDING PROTEINS RBM38 AND DND1 IN AML CELL DIFFERENTIATION

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Background: The RNA binding proteins RBM binding motif protein 38 (RBM38) and DEAD END 1 (DND1) are known to selectively stabilize mRNAs by attenuating RNAse activity or protecting them from miRNA-mediated cleavage (Figure 1 a). Both proteins can cause cell cycle arrest in G1 phase by, for example, stabilizing the mRNA of the cell cycle inhibitor $p21^{CIP1}$. Low expression of these genes may lead to decreased stability of $p21^{CIP1}$ or increased vulnerability to miRNAs.
Aims: In our study we aimed at quantifying expression levels and testing possible functions of RBM38 and DND1 in the molecular pathology of acute myeloid leukemia (AML), a disease characterized by a block of myeloid differentiation and increased cell survival.
Methods: mRNA expression of RBM38 and DND1 was measured by low density array in clinical AML patient samples (n=98) among FAB subtypes M0 to M4, $CD34^+$ progenitor cells, macrophages and granulocytes of healthy individuals. The APL cell line NB4 and its resistant subline NB4-R2 were differentiated towards granulocytes by 1μM all-trans retionoic Acid (ATRA) treatment for 6 days. Successful neutrophil differentiation was evidenced by CD11b FACS analysis or increased of granulocyte colony-stimulating factor receptor (G-CSF-R) mRNA expression. Gene expression of RBM38, DND1, G-CSF-R and $p21^{CIP1}$ was measured by quantitative real-time RT-PCR. Protein expression was assesed by Western Blotting. NB4 cells were transduced with a lentiviral vector expressing a small hairpin (sh)RNA targeting RBM38 and DND1 mRNA. Knock-down efficiency was validated by qPCR and Western blotting. Differences between two groups were assessed using the non-parametric Mann-Whitney-U test. P-values <0.05 were considered to be statistically significant.
Results: In a first attempt to investigate a possible role for RBM38 and DND1 in AML pathology, we quantified their mRNA expression in clinical AML patient samples (n=98). We observed a highly significant down-regulation of RBM38 and DND1 mRNA expression in AML blasts, normal $CD34^+$ progenitor cells, macrophages of healthy individuals as compared to mature neutrophils from healthy donors. These findings clearly indicate that low RBM38 and DND1 mRNA expression is associated with an immature myeloid phenotype. To investigate the function of RBM38 upon differentiation, $CD34^+$ progenitor cells were treated with G-CSF to induce granulocyte differentiation. RBM38 mRNA was 6-fold induced compared to day 0 of differentiation. Next, we analyzed RBM38 and DND1 expression during *in vitro* neutrophil differentiation of NB4 acute promyelocytic leukemia (APL) cells as a model for neutrophil differentiation. The PML-RARA-mediated differentiation block at the promyelocyte stage in APL cells can be overcome by treating them with pharmacological doses of all-trans retinoic acid (ATRA). Neutrophil differentiation of NB4 APL cells resulted in a 12- and 4-fold induction of RBM38 and DND1 mRNA expression, respectively (Figure 1 b). Increased mRNA levels were paralleled by protein expression. NB4-R2 ATRA-resistant cells did not show increased RBM38 and DND1 expression indicating that the activation of gene expression is not due to an unspecific ATRA-response. Next, to address the function of RBM38 and DND1 in neutrophil differentiation, we generated two independent NB4 RBM38 as well as DND1 knock-down cell lines. Generally, RBM38 and DND1 NB4 knock-down cells displayed significantly attenuated neutrophil differentiation compared to control transduced cells as assessed by CD11b (Figure 1 c) and G-CSF-R expression. Of note, inhibiting RBM38 and DND1 also resulted in decreased $p21^{CIP1}$ mRNA (Figure 1 d) and protein expression due the lack of $p21^{CIP1}$ mRNA stabilization in the absence of these proteins.

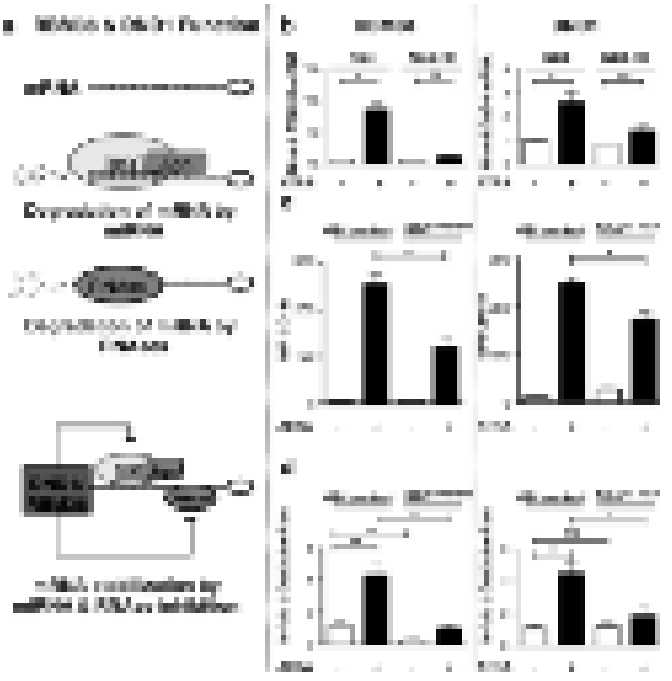

Figure 1.

Summary and Conclusions: Our results clearly indicate that RNA binding proteins RBM38 and DND1 play significant new roles in neutrophil differentiation of leukemic cells, and we propose that low expression levels of these genes contribute to the immature AML phenotype.

Acute myeloid leukemia - Clinical 1

P159

EARLY PERIPHERAL BLAST CELL CLEARANCE ASSESSED BY FLOW CYTOMETRY IN INDUCTION IS A NOVEL POWERFUL PROGNOSTIC INDICATOR IN ACUTE MYELOID LEUKEMIA: A NORTHERN ITALY LEUKEMIA GROUP (NILG) STUDY

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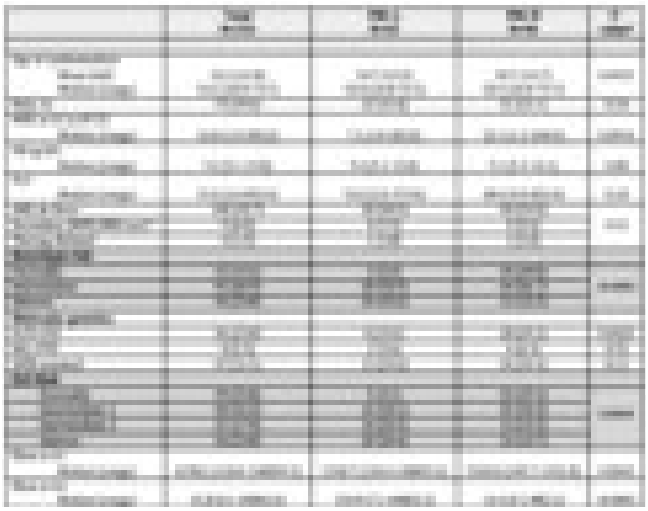
Background: In acute myeloid leukemia (AML), genetics is the most relevant prognostic factor representing the framework of European Leukemia Net (ELN) risk stratification system. It defines subgroups with high likelihood to achieve complete remission (CR) and long survival (ELN-favorable) and at the opposite a category with poor response to chemotherapy (ELN-adverse). However, in the absence of genetic determinants, ELN system merges patients (pts) with heterogeneous diseases. At any rate, the response to remission induction therapy remains a powerful prognostic parameter, expressing chemosensitivity of AML cells. In responsive pts, minimal residual disease (MRD) is emerging as an accurate tool to refine risk category. Typically MRD is evaluated after one or two consolidation cycles, however an earlier assessment has been shown to correlate with CR achievement and survival.
Aims: To predict the outcome to induction therapy as early as possible, we studied the peripheral blast cell clearance (PBC) after the first 3 days of induction therapy.
Methods: Eligible pts had untreated non-M3 AML according to the World Health Organization criteria and participated into the NILG AML 02/06 clinical trial, in which they were randomized between a standard *versus* an experimental induction therapy. At diagnosis, bone marrow aberrant leukemia-associated immunophenotypes (LAIPs) were identified by flow cytometry. We quantified LAIP cells by single platform before treatment on day 1 and then following the first 3 days of therapy (day 4). PBC of each individual pt was expressed as the ratio between absolute LAIP cell count on days 1 and 4 converted to a logarithmic scale.
Results: Between 2007-2012, 178 pts were enrolled in PBC study, of whom 27 were excluded because of the lack of circulating blasts (n=7, 3.9%), undetectable LAIP (n=17, 9.6%) or less than 100/μL LAIP cells at day 1 (n=3, 1.7%). Thus, 151 pts (84.8%) were assessable. The median value of LAIP cells decreased from 4.198/μL (range 120-148.859) on day 1 to 41.8/μL (0.07-44,802.3) on day 4, for a median PBC index of 1.9 (0.40-4.50). After the first induction course, 108 pts (71.6%) achieved CR, 36 (23.8%) were refractory and 7 (4.6%) died of complications. Although no difference in day 1 LAIP cells was noted between CR and refractory pts, the latter exhibited a significantly higher LAIP cell count at day 4 and a lower PBC index (1.0) compared with CR pts (2.3; p<0.0001). By ROC curves we identified 1.5 log as the most accurate cut-off separating pts with high and low PBC index, respectively (PBC-H, PBC-L), also correlating significantly with the main prognostic factors (Table 1). Among the evaluable pts, 84/92 PBC-H (91.3%) achieved CR after a single course compared to 24/52 PBC-L (46.2%; p<0.0001). In multivariate analysis, PBC index was the only predictive factor for CR (p=0.0004) and a significant predictive factor for event-free survival (p=0.0001).
Summary and Conclusions: PBC is a new very early and powerful outcome predictor in AML. This analysis is simple and minimally invasive, providing a real time quantification of AML burden reduction in the first days of chemotherapy. Along with other prognostic markers, it could allow to customize AML induction treatment.

Table 1. Characteristics of patients according to PBC.



P160

PROPENSITY SCORE MATCHED COMPARISON OF INTERMEDIATE INTENSITY CHEMOTHERAPY INDUCTION *VERSUS* INTENSIVE CHEMOTHERAPY INDUCTION IN ELDERLY PATIENTS (AGES 60 YEARS OR OLDER) WITH AML

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Background: A majority of patients with acute myeloid leukemia (AML) age ≥60 are unable to tolerate intensive chemotherapy (IC) induction. Front-line therapy with clofarabine plus low dose cytarabine (CLDA) has been used for this subset of patients. We hypothesized that CLDA provides equivalent outcome on elderly patients with AML compared to conventional IC induction with less toxicity.
Aims: To compare treatment related toxicity and outcome of CLDA induction *vs.* conventional IC induction in elderly patients with AML.
Methods: Previously untreated patients with AML, age ≥60, who received front-line therapy between 2002 and 2012 by one of the following regimens were matched by the propensity score to adjust pre-treatment confounding factors: 1) clofarabine and low dose cytarabine (CLDA group: clofarabine 20 or 30 mg/m^2 intravenous daily for 5 days+cytarabine 20 mg/m^2 subcutaneous once or twice daily for 10 to 14 days, N=142) or 2) idarubicin and high dose cytarabine (IA group: idarubicin 12 mg/m^2 intravenous once daily for 3 days and cytarabine 1.5 gram/m^2 intravenous over 24 hours for 3 to 4 days, N=104).
Results: Propensity matching resulted in 66 patients from each group to be matched for their pre-treatment characteristics including age, performance status, organ function, cardiac function, and cytogenetics. Proportion of patients who achieved complete remission (CR) within 2 course of induction were similar between 2 groups (CLDA *vs.* IA, 59% *vs.* 53%, P=0.48). For responders, 95% of the patients in CLDA group achieved CR within 2 cycles of treatment, whereas 100% of patients in IA group achieved CR within 2 cycles of treatment. Proportion of patients who experienced grade 3 or more toxicity during induction was significantly higher in IA group (CLDA *vs.* IA, 48% *vs.* 73%, P=0.004). The median length of hospital stay during the induction was similar between 2 groups (CLDA *vs.* IA, 27 *vs.* 26 days, P=0.85). Mortality at 4 and 8 weeks were similar between 2 groups (CLDA *vs.* IA, 5% *vs.* 11% at 4 weeks and 14% *vs.* 15% at 8 weeks, P=0.32 and 0.80, respectively). Comparable proportion of patients were bridged to stem cell transplant during the first CR (CLDA *vs.* IA, 6% *vs.* 5%, P=1.00). There was no statistical difference in overall survival (OS) between 2 groups (CLDA *vs.* IA, 11.4 months [95% CI: 7.0-15.8] *vs.* 10.4 months [95% CI: 4.9-15.9], P=0.60, Figure 1). In an analysis sub-grouped by clinical characteristics, no difference in OS was observed between 2 groups in any of the subgroups (by age ≥70 *vs.* <70, adverse risk cytogenetics *vs.* favorable or intermediate risk, *FLT3*-ITD *vs.* wild type, performance status ≥3 *vs.* <3, bone marrow blast >30% *vs.* ≤30%, white blood cell count >20x10^3/μL *vs.* ≤20x10^3/μL). Multivariate Cox proportional hazard regression for OS showed that adverse risk cytogenetics, age ≥70, bone marrow blast >30%, and performance status ≥3 adversely affected OS but induction regimen did not affect OS.

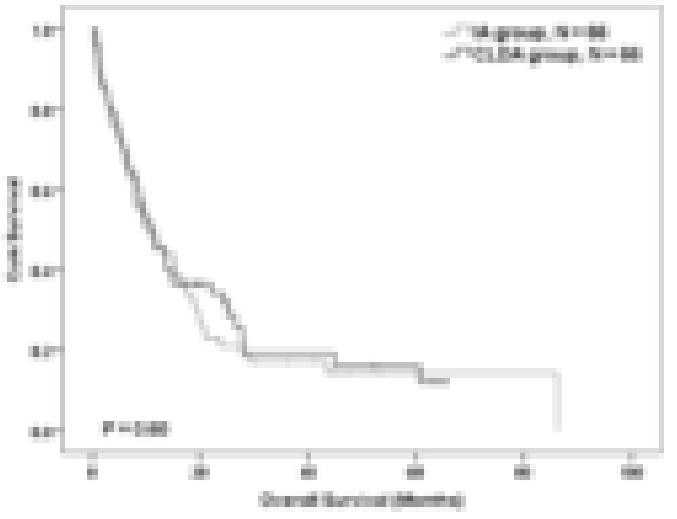

Figure 1.

Summary and Conclusions: In this clinically well matched cohort, induction chemotherapy with CLDA showed equivalent treatment response and survival in patients with AML age ≥60 but was associated with significantly less toxicity compared to conventional IC induction. Intensity of induction therapy in elderly AML patients may be attenuated without compromising outcome. Prospective randomized trial to confirm these findings is warranted.

P161

PROGNOSTIC SIGNIFICANCE OF THE MRC CYTOGENETIC CLASSIFICATION COMPARED WITH THE EUROPEAN LEUKEMIANET GENETIC RISK CLASSIFICATION SYSTEM IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: The prognostic relevance of cytogenetic and molecular aberrations in patients with acute myeloid leukemia (AML) is well established. Recent reports by the Medical Research Council (MRC) and by the European LeukemiaNet (ELN) have further established the predictive value of both cytogenetic abnormalities and a number of recurring gene mutations in determining disease free and overall survival of patients.
Aims: We conduct this study to validate the prognostic significance of these classification systems in a cohort of patients treated relatively uniformly at our institution. Patients younger than 65 were treated mainly with induction regimens containing high dose cytarabine and idarubicin and patients over 65 years of age received mainly non intensive regimens.
Methods: Patients with newly diagnosed AML treated at our institution from 1993-2012 were included in this study. Patients with available cytogenetics and molecular data were then classified according to the MRC as well as the ELN criteria and were evaluated for outcome.
Results: 2070 patients with a median age of 60 (range, 12-89) had complete cytogenetic data, of which 1196 (55%) were male and 972 (47%) were <60 years of age. 766 patients (37%) had normal karyotype including 330 (34%) patients <60 years. The remainder had at least 1 cytogenetic abnormality. 27%, 10%, 5%, 3%, and 17% of patients had at 1, 2, 3, 4, or 5+ abnormalities, respectively. In patients <60, the proportions were 31%, 11%, 5%, 2%, and 13%, respectively. The outcome by various cytogenetic abnormalities (including the uncommon karyotypes, but excluding APL) defined by the MRC was as predicted (Figure 1). Among the patients <60, 20%, 48%, and 32% were in the favorable (FAV), intermediate (INT), and adverse (ADV) prognostic categories of the MRC-C, respectively. FLT3 and NPM1 mutation data was available in a subset of 1026 patients (50%), allowing classification by ELN-C; 487 patients (47%) were <60 yrs of age and 539 patients (53%) were ≥60. The proportion of patients in each of the ELN-C categories were: FAV: 26%, INT-1: 18%, INT-2: 24%, and ADV: 32%. Overall survival in the same cohort of patients, for <age 60 (Figure 1A and IC) and ≥age 60 (Figure 1B and 1D) by MRC-C and ELN-C risk groups are shown below.

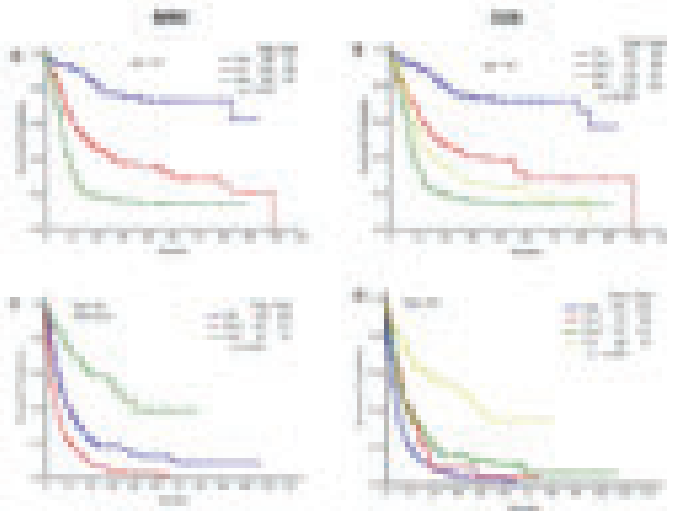

Figure 1.

Summary and Conclusions: The revised MRC-C and the ELN-C were able to distinguish separate prognostic groups for patients with AML based on their genetic abnormalities. Among the patients >60 years, the MRC-C was more predictive of the outcome, particularly for patients with intermediate risk disease where INT-1 and INT-2 were not able to discriminate prognosis.

P162

WT1-ASSOCIATED MISSPLICING IN ACUTE MYELOID LEUKEMIA

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Background: While about one third of expressed genes are abnormally spliced in AML, the causes, mechanisms, and consequences of missplicing remain incompletely understood in this disease. WT1 is overexpressed in the majority of AML while it represses the splicing-factor kinase SRPK1 and physically interacts with the splicing factors WTAP, RBM4, and U2AF1/U2AF2.
Aims: To determine whether and how WT1 modifies alternative exon usage (AEU) in AML and to test if these changes have a clinical impact.
Methods: The exon expression profiles of 3 AML cell lines (MOLM13, Kasumi, and KG1) knocked down (KD) or not for WT1 gene expression were examined using Affymetrix HTA2 exon arrays. Four WT1-specific AEUs involving 4 ATP-binding cassette transporter genes (ABC-A2, -A3, -A5, and -C3) were quantified by quantitative exon specific PCR (qESPCR) in fresh AML cells deriving from 132 AML patients of whom 106 have been treated with intensive chemotherapy (median follow-up 27 months). Overall survival (OS) and disease-free survival (DFS) were compared with the log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) were estimated by Cox proportional hazards regression models to determine independent risk factors associated with survival in multivariate analyses. The 25 patients who received allogeneic HSCT were censored on the day of cell infusion.
Results: WT1-KD was confirmed by western blot analysis for the 3 cell lines. Upon WT1-KD, 954 AEUs were identified in 846 genes, of which 337 (40%) were altered at the whole gene expression level. 73/100 array-predicted exon usages were validated by ESPCR. Gene ontology analysis showed a significant gene enrichment in pathways such as hematopoietic cell lineage, calcium signaling, ABC transporters, DNA replication, and pyrimidine and purine metabolism. To test the relationship between WT1 expression and AEUs *in vivo*, 4 WT1-induced AEUs identified in 4 ABC transporters were quantified through qESPCR in 132 AML. A statistically significant correlation linked WT1 expression and the distribution of the 4 ABC transporter isoforms in the 132 AML diagnostic samples ($p<0,01$ for each, Spearman Rank Correlation) but not in control samples. As an additional control experiment, qESPCR was carried out with the 132 AML for 2 AEUs of the TET2 gene mRNA that were not found to be linked with WT1 expression *ex vivo*. In contrast to ABC transporters, no correlation could be evidenced between WT1 expression and the distribution of the 4 TET2 isoforms in fresh AML samples. By univariate analysis, ABC-A3 exons 18-20 expression, *i.e.* exon skipping of exon 19 significantly affected both OS and DFS; higher the level of 18-20 isoform expression, poorer the outcome. Alternatively, an elevated expression level of the alternative ABC-A3 19-20 isoform was associated with statistically significant better OS and DFS. In multivariate analysis, age cytogenetic, and ABC A3 exon 19 skipping were identified to be independent prognostic factors for OS and DFS (Table 1). Age and ABC-A3 exon exclusion were identified to be independent prognostic factor for OS in the 49 patients with normal karyotype.

Table 1. Multivariate analyses of survival outcomes in AML treated with intensive chemotherapy.

Summary and Conclusions: In AML, WT1 affects AEUs of numerous key genes involved in hematologic differentiation, leukemogenesis, and resistance to chemotherapy. 4/4 tested AEUs displayed the same correlation with WT1 expression *in vivo* and *ex vivo*. This correlation was specific because it was not observed in control samples or in AML with 2 TET2 AEUs found WT1-independent *ex vivo*. These results help explain the deregulated pattern of AEUs

observed in AML cells where spliceosome gene mutations represent a rare event. ABC-A3 missplicing possesses a strong prognostic impact indicating that besides whole gene transcription, qESPCR might represent a promising tool for assessing AML aggressiveness at the time of diagnostic in patients with normal or abnormal karyotype.

P163

VALIDATION OF EUROPEAN LEUKEMIA NET (ELN) GUIDELINES FOR CYTOGENETICALLY NORMAL AML IN ASSOCIATION WITH NPM1 AND FLT3-ITD MUTATIONS: FURTHER STRATIFICATION FOCUSING ON WT1 EXPRESSION

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Background: According to the risk-stratifications of the karyotypes of adult AML, intermediate-risk group is mostly comprised of cytogenetically-normal (CN)-AML and other undefined karyotypes. According to the European Leukemia Net (ELN) guidelines, CN-AML is divided into four molecular subgroups based on the mutation statuses of *NPM1* and *FLT3-ITD*. Except isolated *NPM1*mut CN-AML, another 3 subgroups belong to the risk-group of 'Intermediate-I', but the guidelines suggest that most of the cases in the 'Intermediate-1' risk-group are associated with poor prognosis.
Aims: We validated the characteristics and identified clinical outcomes of CN-AML patients in a single center in Korea based on the ELN guidelines. We then further modified the stratification using *WT1* expression.
Methods: This single center retrospective study enrolled 223 adult CN-AML patients (median 55 yrs (18-83) from 2007 to 2011. Except 18 untreated patients, 156 were treated with standard chemotherapy and 49 were treated with attenuated chemotherapy. After achieving complete remission (CR), patients with available donor were treated with allogeneic (allo)-HSCT (n=98) and the rest were treated with auto-HSCT (n=13) or chemotherapy alone (n=48). We calculated clinical outcomes after dividing the CN-AML patients into 4 subgroups based on the ELN guidelines; isolated *NPM1*mut CN-AML (n=47), *NPM1*wt/*FLT3-ITD*-neg (n=125), *NPM1*mut/*FLT3-ITD*-pos (n=35), and *NPM1*wt/*FLT3-ITD*-pos (n=16). We then used diagnostic *WT1* expression measured by RQ-PCR (*WT1* ProfileQuant™ kit, Ipsogen, France) method for additional stratification of the 'Intermediate-I' risk-group and also calculated the clinical outcomes. The significant cut-off of *WT1* expression was calculated with ROC curve analysis.

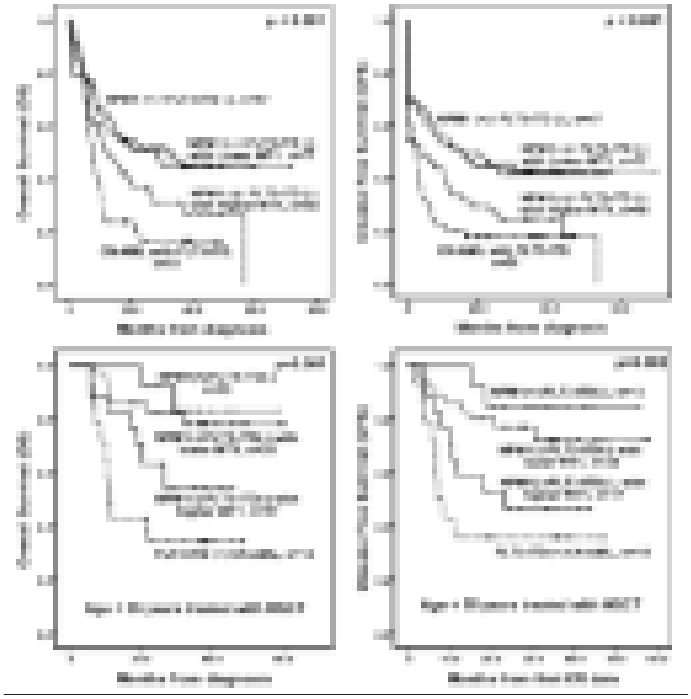

Figure 1.

Results: We identified that *NPM1*mut was 36.8% among CN-AML, and *NPM1*mut CN-AML patients were older (54.4yrs *vs.* 49.8yrs) and showed leukocytosis, higher blast counts and higher *WT1* expression compared to *NPM1*wt CN-AML. Higher proportion of *FLT3-ITD* mutation was also identified in *NPM1*mut CN-AML (42.7% *vs.* 11.3%). Isolated *NPM1*mut CN-AML showed the most favorable outcomes with higher CR rate (80.9% *vs.* 67.5%) and significantly lower refractory cases (4.7% *vs.* 27.5%) compared to the other 3 subgroups. Isolated *NPM1*mut CN-AML also showed significantly superior 4-year OS at 70% especially in the younger patient subgroup. Without regarding *NPM1* mutation, the presence of *FLT3-ITD* showed the worst 4-year OS <20% with higher incidence of relapse. In the intermediate-I risk-group, *NPM1*wt/*FLT3-ITD*-neg CN-AML could be further stratified with diagnostic *WT1* expression. *NPM1*wt/*FLT3-ITD*-neg CN-AML with lower *WT1* (n=77) showed favorable OS (p=0.060) and DFS (p=0.015) than the group with higher *WT1* (n=48), and the superior results were comparable with those of isolated *NPM1*mut CN-AML. We finally suggest that isolated *NPM1*mut or *NPM1*wt/*FLT3-ITD*-neg with lower *WT1* can be considered as a lower-risk group, while *FLT3-ITD*-pos or *NPM1*wt/*FLT3-ITD*-neg with higher *WT1* as a higher-risk group. And we found that survival outcomes of the higher-risk CN-AML could be improved with HSCT, presenting 4-year OS >35% in the younger patients subgroup.
Summary and Conclusions: Our modified stratification using *WT1* expression is considered reasonable for CN-AML and should be validated by larger studies in the future. Based on this study, higher-risk CN-AML should be treated with risk-adapted approach including HSCT.

P164

WT1 MUTATIONS ARE SECONDARY EVENTS IN AML, SHOW VARYING FREQUENCIES WITHIN GENETIC SUBGROUPS AND DIFFERENT IMPACT ON PROGNOSIS

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Background: Mutations (mut) in the *WT1* gene belong to the first genetic aberrations described in AML. In contrast to recurrent fusion genes *WT1*mut do not seem to be disease defining. However, the impact of *WT1*mut still is discussed controversially.
Aims: To analyze the frequency and prognostic impact of *WT1*mut in AML patients (pts).
Methods: 3,157 unselected AML pts were analyzed (*de novo*: n=2,699, s-AML: n=234, t-AML: n=224; 1,708 males, 1,449 females). Median age was 67.1 years (y) (range: 17.8-100.4 y; <60 y: n=1,108, ≥60 y: n=2,049). The mutational hot spot regions of *WT1* (exons 7 and 9) were analyzed by direct Sanger sequencing. Karyotype and *WT1* mutation status was available in all pts. Other mutations were assessed in subsets: *ASXL1* (n=1,951), *CEBPA* (n=2,670), *DNMT3A* (n=1,293), *FLT3*-ITD (n=3,149), *FLT3*-TKD (n=3,004), *IDH1*R132 (n=2,431), *IDH2*R140 (n=2,380), *IDH2*R172 (n=2,412), *KRAS* (n=1,409), *NRAS* (n=1,780), *NPM1* (n=3,003), *MLL*-PTD (n=2,961), *RUNX1* (n=2,390), *TET2* (n=1,016) and *TP53* (n=1,215).
Results: 189 *WT1* mutations were detected (exon 7: n=151, exon 9: n=38). Most were disrupting frameshift (n=142) or nonsense (n=14) mutations, whereas 24 were missense (mostly in exon 9: n=19), only 2 indel and 7 splice site mutations. 14 patients had 2 mutations. Thus, the total frequency of *WT1*mut pts was 175/3,157 (5.5%). With regard to genetic subtypes, significantly higher frequencies were detected in biallelic *CEBPA*mut (15/110, 13.6%, p=0.001), followed by t(15;17)/*PML-RARA* (18/164, 11.0%, p=0.004), and *FLT3*-ITD (58/682, 8.5%, p<0.001). Lower frequencies were observed in *DNMT3A*mut (18/412, 4.3%, p=0.014), *ASXL1*mut (6/355, 1.7%, p<0.001), *IDH2*R140 (5/286, 1.7%, p=0.001), and *IDH1*R132 (2/222, 0.9%, p<0.001). *WT1*mut were never detected in pts with complex karyotypes (0/175, p=0.047). *WT1*mut were more frequent in females (95/1,449, 6.6%) than in males (80/1,708, 4.7%) (p=0.014) and in younger pts (<60 y: 102/1,108, 9.2% *vs.* ≥60 y: 73/2,049, 3.6%, p<0.001). Median age of pts with *WT1*mut was 55.5 *vs.* 63.6 y in *WT1*wt (p<0.001). Stability of *WT1*mut was analyzed in 35 paired diagnostic and relapse samples. In 23/35 (65.7%) *WT1*mut was retained at relapse and in 12/35 (34.3%) it was lost. In 5 cases a sample at 2nd relapse was available. 3 of these retained and 2 lost the *WT1*mut. Survival was calculated for intensively treated pts (n=1,936, *WT1*mut: n=132, 6.8%). In the total cohort, *WT1*mut had no impact on prognosis. In pts ≥60 y there was a trend to shorter event free survival (EFS) for *WT1mut* (9.3 *vs.* 12.3 m, p=0.052). In prognostically favorable subgroups with high *WT1*mut incidences (biallelic *CEBPA*mut and *PML-RARA*) no effect on outcome was seen. Restricting to normal karyotype AML (*WT1*mut: n=85, *WT1*wt: n=1,093) *WT1*mut pts had shorter EFS (10.8 *vs.* 17.9 m, p=0.008). This was true for younger (12.2 *vs.* 29.0 m, p=0.007) as well as for older pts (9.3 *vs.* 13.9 m, p=0.016). In multivariate analysis, *WT1*mut had an independent adverse impact on EFS (p=0.002, HR: 1.64) besides *FLT3*-ITD status (p<0.001, HR: 1.71) and age (p<0.001, HR: 1.28), but only in normal karyotype AML.
Summary and Conclusions: *WT1* mutations are more frequent in females and younger patients, t(15;17)/*PML-RARA*, biallelic *CEBPA*mut, and *FLT3*-ITD mutated AML. *WT1* mutations were nearly mutually exclusive of *ASXL1*, *IDH1*, *IDH2* and complex karyotypes. The distribution pattern in different genetic subtypes and the instability during follow-up as shown by paired sample analyses clearly emphasize a secondary character of *WT1*. In normal karyotype AML, an independent adverse impact of *WT1*mut on EFS was shown.

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A RAPID BACTERIAL-BASED BIOLUMINESCENT ASSAY FOR IN-VITRO TESTING OF CHEMOTHERAPY SENSITIVITY IN ACUTE MYELOID LEUKAEMIA.

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Background: Patients with Acute Myeloid Leukaemia (AML) are routinely given chemotherapy regimens containing cytosine arabinoside (Ara-C), although up to 30% of intensively treated patients fail to respond/relapse, due to non-uptake of Ara-C or lack of conversion to the active form (Ara-CTP) as a possible cause. Patients aged over 70 years are more commonly being given low-dose Ara-C where remission rates are low (~20%).
Aims: A whole cell bacterial biosensor has been constructed for rapid detection of Ara-CTP within leukaemic cells. The assay incorporating this biosensor enables same-day prediction of patient response to chemotherapy before the start of treatment. Verification and clinical utility of the assay have been assessed herein.
Methods: The assay uses a genetically modified *E. coli,* expressing the human deoxycytidine kinase (dCK) gene responsible for conversion of Ara-C to Ara-CTP, and a *lux*-expression cassette, which enables an increase in bioluminescence in response to Ara-CTP.
Results: For validation, the minimum detection level of Ara-CTP was determined in seven AML cell lines using both the biosensor and HPLC. The limit of detection of Ara-CTP was found to be 25 nM using the biosensor assay (requiring 2 million cells), compared to 50 nM with HPLC (requiring 20 million cells). Results from the two methods of analysis correlated statistically across the seven cell lines (R=0.9722, p=0.0028), indicating that the biosensor is accurately detecting Ara-CTP. An MRC-funded retrospective study is currently evaluating the biosensor in clinical samples to determine the uptake and metabolism of Ara-C by the leukaemic blast cells, and shows promising correlation with outcomes examined to date (n=50).
Summary and Conclusions: This technology is currently being extended to other chemotherapy agents in combination with Ara-C, and could allow provision of an individual chemo-sensitivity profile to augment the clinical information available at the point of treatment for these patients.

P166

MULTICENTER SURVEY ON OUTCOME OF AML REFRACTORY TO FIRST INDUCTION CHEMOTHERAPY IN THE LAST 4 YEARS (2010-2013)

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Background: Despite the development of a variety of new investigational therapies, Acute Myeloid Leukemia (AML) refractory to first induction chemotherapy remains a challenge and there are only few recent epidemiologic data regarding the outcome of this group of patients.
Aims: In this multicenter survey we evaluate the prognosis and outcome of 76 patients (pts) with AML, diagnosed and treated in four Institutions (Udine, Verona, Treviso, Padova), during the last 4 years (between 2010 and 2013).
Results: All 76 AML cases were refractory to first induction course of chemotherapy. Data were updated as of January 2014. There were 43 male and 33 females with a median age 63 years, (range: 19-79); 57% (43/76) of pts were younger than 65 yrs and 39% (30/76) had AML secondary to MDS. At onset 49% of cases presented an adverse karyotype. According to the risk stratification at diagnosis based on cytogenetic/molecular profile (Dohner *et al.*, Blood 2010), 20/76 (26,3%) of pts were classified as a Favorable/Intermediate-I risk group and 45/76 (59,2%) as Intermediate-II/Adverse risk group; in 11/76 (14,5%) cases cytogenetic/molecular profile were not available. After a median follow up of 9 months (range 1-40), 56/76 (74%) of pts had died and only 20/76 (26%) were alive (12/20 with active AML and 8/20 in cytologic Complete Remission-cCR). Twenty-four pts (32%) underwent allogeneic hematopoietic stem cell transplantation (HSCT) and of these 21% (5/24) were alive at last follow up (January 2014). The 12 and 21 months probability of Overall Survival (OS) of the whole population was 48% and 21%, respectively. The probability of OS was significantly improved by HSCT procedure (32% *vs* 6% at 25 months; log-rank, p=0,0007) and was better in those pts with Favourable/Intermediate-I cytogenetic/molecular risk at diagnosis (34% *vs* 17% at 20 months; log-rank, p=0,018). A pre-transplant cCR status was a significant factor (P=0,0007) to predict a favourable outcome after HSCT.
Summary and Conclusions: Treatment options of AML refractory to first induction course are still limited and the prognosis of these pts remains currently extremely poor. This survey shows that these pts are rarely cured without undergoing allogeneic HSCT and confirms the importance of initiating an urgent unrelated donor search in AML pts with no matched sibling donor and without response to first induction chemotherapy. Moreover, the outcome of HSCT procedure is better in patients who achieve HSCT with a good AML debulking. To reach this goal, new and tailored protocols of salvage therapy (with new drugs or new combinations) would need to be developed.

P167

PRETREATMENT RISK SCORE FOR PREDICTION OF EARLY DEATH AFTER INDUCTION THERAPY IN PATIENTS WITH NEWLY DIAGNOSED MYELOID LEUKEMIA

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Background: Outcome in acute myeloid leukemia (AML) worsens with age due to the higher rate of early death (ED) in older patients. Eligibility for intensive AML treatment protocols is therefore typically based on age as the implied principal predictor of ED. However, it was recently shown that other health and disease-related pretreatment factors can affect the outcome of AML patients after induction therapy.
Aims: To elaborate original prognostic score system for prediction of ED after induction therapy in AML patients based on analysis of pretreatment risk factors.
Methods: This single-centre study included 376 adult patients with nonpromyelocytic newly diagnosed AML, aged ≤60 years. All patients were given induction therapy, which consisted of doxorubicin at 60 mg/m2 daily on days 1-3, in combination with cytarabine 200mg/m2 daily as a continuous intravenous infusion for 7 days (3+7 regimen). The following parameters were estimated as risk factors for ED: age, leukocytosis (WBC <30x109/L *vs* ≥30x109/L), PS, cytogenetic risk group, comorbidities, elevated fibrinogen (>4g/L), decreased albumin (<40 g/L), elevated lactate dehydrogenase (LDH) >1.5xupper limit of normal (N), elevated bilirubin (1.5xN), elevated transaminase (1.5xN) and elevated creatinine (>176 µmol/l). Performance status (PS) evaluated by the Eastern Cooperative Oncology Group (ECOG), ranged between 0-4. The cytogenetic risk group was assessed according to the recommendation of the European LeukemiaNet (ELN). Comorbidities were evaluated using the haematopoetic cell transplantation-specific comorbidity index (HCT-CI). ED is defined as death occurring 30 days of initiation of treatment. Integer weights for the risk score were derived from Cox proportional hazards modeling. The prognostic score was validated via 10-fold cross validation.
Results: The mean age of the patients was 52 years (range 18-60). Our results showed that the most significant adverse factors for ED in univariate analysis were: age ≥55 years (p=0.007), ECOG PS ≥2 (p=0.001), leukocytosis (p=0.046), elevated LDH (p <0.001), elevated fibrinogen (p=0.004), elevated bilirubin (p <0.001), transaminase (p <0.001) and creatinine (p <0.001), decreased albumin (p <0.001) and HCT-CI ≥3 (p <0.001). Our prognostic scoring system was developed after analysis of the prognostic risk factors: elevated LDH, bilirubin and transaminase=1 point, elevated creatinine=1.5 points, decreased albumin=2 points and HCT-CI ≥3=3 points. According to this prognostic model, patients were classified into three risk groups for prediction of ED: low risk=0-1.5 points, intermediate risk=2-4 points, and high risk=>4 points. ED between these groups was highly significant (p <0.001).
Summary and Conclusions: The prognostic model developed in this study can distinguish between AML patients in different risk groups for prediction of ED. This model, based on analysis of pretreatment risk factors, indicates a subset of high risk patients not suitable for intensive chemotherapy, despite their eligibility regarding age (≤60 years).

P168

TRAIL AND ITS RECEPTORS EXPRESSION ON LEUKEMIC CELLS OF PATIENTS WITH AML1-ETO POSITIVE ACUTE MYELOID LEUKEMIA

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Background: TRAIL signaling pathway is an important pathway having broadly biological activities involving cell apoptosis, differentiation and proliferation. There are AML1 consensus bingding sites in the promoter of TRAIL and TRAIL receptor 2 which imply exsiting the relationship between TRAIL signaling pathway and AML1-ETO positive acute myeloid leukemia (AML). However, the expression of TRAIL and its receptors on leukemic cells of AML1-ETO positive acute myeloid leikemia patients is unknown.
Aims: To detect the expression of TRAIL, TRAIL receptor 1(DR4), TRAIL receptor 2 (DR5), TRAIL receptor 3 (Decoy receptor 1, DcR 1) and TRAIL receptor 4 (Decoy receptor 2, DcR2) on leukemic cells of patients with AML1-ETO positive AML and investigate its possible clinical significance.
Methods: The mean fluorescence intensity (MFI) of TRAIL, DR4, DR5, DcR1 and DcR2 expression were analysed by using flow cytometry on bone marrow

CD34+ cells from patient with AML1-ETO positive AML, AML1-ETO negative AML and non-hematological maliganancies (such as iron deficiency, idiopathic neutropenia and thrombocytopenia, the control group). The study was aproved by the hospital ethics committe and the infromed consent were obtained from all the patients.

Results: Comparing with AML1-ETO negative AML group and the control group, the MFIs of bone marrow CD34+ cells from patients with AML1-ETO positive AML were significantly higher ($p<0.01$, respectively). Regarding DR4 expression, the MFIs of both AML1-ETO positive AML and AML1-ETO negative patients were higher than the control group ($p<0.01$), but there were no significantly difference between the AML1-ETO positive and negative AML groups ($p>0.05$). The MFIs of DR5 expression in patients with AML1-ETO posivite AML were significantly higher than the AML1-ETO negative AML group ($p<0.01$) and the control group ($p<0.01$). The MFIs of DcR1 expression in both patients groups with AML1-ETO positive and negative AML were higher than the control group ($p<0.01$), but there was no difference between the two AML groups. The MFIs of DcR2 in AML1-ETO positive AML group was significantly lower than AML1-ETO negative AML group ($p<0.01$) and the control group ($p<0.01$) (Table 1 and Figure 1).

Table 1. The MFIs of TRAIL and its receptors expression on BM CD34+ cells from patients with AML1-ETO positive AML(A), negative AML(B) and the control(C) ($\bar{x}$±SD).

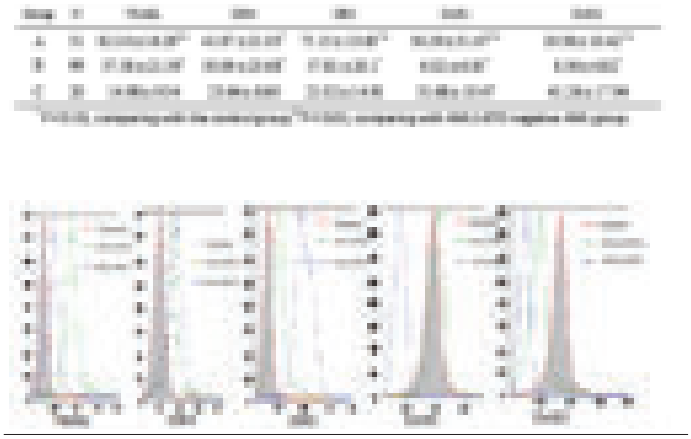

Figure 1. A representive cureve of MFI of TRIAL and its receptors on bone marrow CD34+ cells in three different groups.

Summary and Conclusions: The expression of TRAIL and its receptors in patients with AML1-ETO positve AML are significnatly different from the patients with AML1-ETO negative AML and the non-hematological maligancies patient which imply the TRAIL sigaling pathway involving the pathogensis of AML1-ETO positve AML and need to be further investigated.

P169

TWO-GROUP STUDY OF MEDIAL MICROSCOPY AS A ROBUST TOOL TO HARMONIZE AND STANDARDIZE HAEMATOLOGICAL DIAGNOSIS

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Background: Morphological blood cell evaluation through microscopic examination remains a cornerstone in the integrated diagnostic process in haematology. Many factors contribute to a lack of standardization of this diagnostic tool. According to literature, concordance rate between experienced morphologists from different Institutions is highly variable. The availability of a new technology, the medial microscopy (MM), a robotic scanner microscope capable of digitizing an entire or a selected part of a glass slide, makes available on the web the optical scanning of PB and BM smears and allows a virtual community to be trained and harmonized with the same diagnostic approach of the optical microscope routinely used for the diagnosis, reproducing same facilities of smear navigation and zooming and offering the possibility of field identification (FOVs) through a grid system.

Aims: To evaluate the reproducibility on morphologically critical cell classification through the assessment of the inter-observer agreement using MM.

Methods: A digitized smear was prepared from BM of an untreated patient with AML with monocytic differentiation. Two different selections of three FO*V*s (out of 1500) were submitted for cell identification to 2 different groups of expert morphologists (EM): an Italian (G1) and an International (G2) group composed by 8 and 16 EM respectively. G1 group evaluated 288 cells and G2 group evaluated 110 cells. All EMs were required to separately record in a pre-defined form the number of blasts listed in the subtypes (agranular, granular, monoblasts, promonocytes and unspecified[6]) and of immature/atypical and mature monocytes. Filled forms were evaluated anonymously and data were analyzed with standard statistics.

Results: In the G1 group 86% of the 288 cells were assigned to one of the different subgroups of blast with a consensus agreement ≥90% in 85% of EM and an overall consensus of 79%. In the G2 group the consensus agreement on 110 cells was verified at the 75% and 60% level of EM for all cell types. Agreement on the definition of a cell as "blast" or "non-blast" was 91/110 cells (83%). Agreement on the "blastic" nature of cells of monocytic lineage was also excellent (Figure 1). The Table 1 shows examples of classification for cells of the monocytic lineage with high level (D05, D10, D17) o lack of agreement (D106, D107).

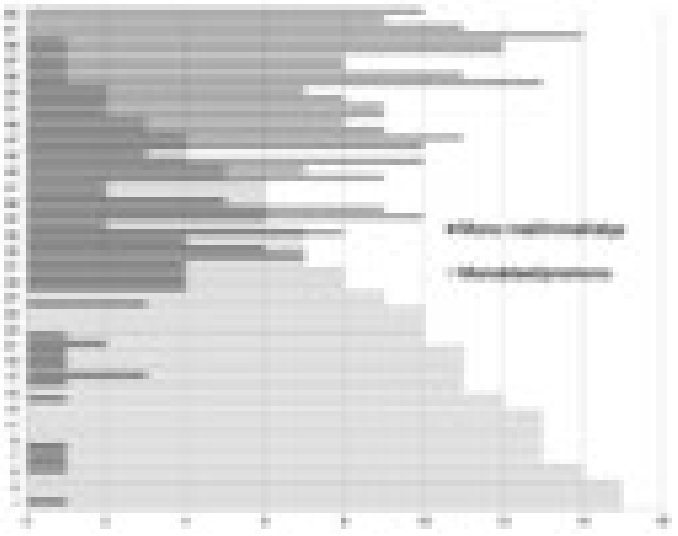

Figure 1. Agreement on the blastic nature of monocytic cells. Cell number (y-axis) *versus* number of participants who have classified each cell as monoblast/promonocyte or atypical-immature monocyte/mature monocytes.

Table 1. Examples of agreement and disagreement in the cytomorphological classification of five cells.

	Number of participants that identified the cell as:					
Cell Number	Mono-blast	Promo-nocyte	Myelo-blast	Atypical monocyte	Mature monocyte	Blast* (any type)
D05	14	1	0	0	0	15/15
D10	5	9	0	1	0	14/15
D17	4	11	0	1	0	15/16
D106	0	6	5	4	1	11/16
D107	0	7	4	4	0	11/16

**blasts=monoblasts+promonocytes+myeloblasts.*

Summary and Conclusions: Agreement on cell classification and definition of blast cells, monoblasts and promonocytes was excellent in both groups: better results in G1 may be explained by cultural homogeneity. In a previous study, first line consensus agreement on similar cell photographs submitted to international EMs was lesser (20-50%) (Zini *et al.* Br J Haematol 2010;151:359-64). One main reason for the excellent results obtained with MM is that cells are evaluated "in the company they keep" on the smear, as it happens in the routine diagnostic process at the microscope. MM does represent a robust method for standardization and harmonization in cytomorphological diagnosis, quality assurance and proficiency testing in haematology.

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HIGH EXPRESSION LEVEL OF PRAME GENE IS FAVORABLE PROGNOSTIC FACTOR IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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Background: An appearance of a chimeric oncogene *PML/RAR*α is a cause of acute promyelocytic leukemia (APL) development. This molecular marker is used for diagnosis and minimal residual disease monitoring in patients with APL. *PRAME* gene related to a group of cancer-testis genes is expressed in APL and in a lot of other malignancies.
Aims: We studied the ratio between *PRAME* and isoforms bcr1 and bcr3 of *PML/RAR*α expression levels and compared it with a clinical data of patients with APL.
Methods: We compared mRNA expression levels of gene *PRAME* and *PML/RAR*α isoforms bcr1 and bcr3. All expression levels was confirmed after equation to *ABL* level. As the detection method we used RQ PCR. To create positive control, we cloned the genes sequences into expression vectors. We have observed the condition of patients within 20 months after the confirmation diagnosis. Therapy of all patients carried out according to AIDA protocol. Mann-Whitney test was used for comparison of the genes expression level.
Results: We found out that the gene *PRAME* is expressed in bone marrow of all primary APL patients (n=72, 100%). According our data, we divided the APL patients into two groups: with a high (more then 5%) and low (less then 5%) *PRAME* expression level in relation to *PML/RAR*α isoforms expression level. There were 28 patients (male, n=19; female, n=9; median age 29 y.o.) in first group and 44 patients (male, n=23; female, n=21; median age 28 y.o.) in second group. During 20 months of observation, 11 (15% of the total) patients of the second group developed hematologic relapse of APL. Among them 2 patients had isoform bcr1 and 9 patients had bcr3 isoform of *PML/RAR*α gene. All patients from the first group had not relapse during the observation period.
Summary and Conclusions: Patients with a low *PRAME* expression level compared with *PML/RAR*α gene expression level had a leukemia relapse earlier than patients with the high ratio values (p=0,0023). The most unfavorable prognostic factor is the expression of the isoform bcr3 *PML/RAR*α gene and low expression level of gene *PRAME*. We suggest that the ratio between their expression levels accurately determines a risk of relapse.

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ASXL1 MUTATIONS DEFINE A SUBGROUP OF AML PATIENTS WITH DISTINCT GENE EXPRESSION PROFILE AND POOR PROGNOSIS: A META-ANALYSIS OF 3311 ADULT AML PATIENTS

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Background: *Addition of sex combs-like 1* (*ASXL1*) is a tumor-suppressor gene recurrently mutated in a broad spectrum of myeloid malignancies. In the last decade the elucidation of prognostic role of different somatic mutations in acute myeloid leukemia (AML) is gaining a growing clinical interest.
Aims: We performed a meta-analysis on published data from 6 independent reports of 3311 adult AML patients, including 385 *ASXL1* mutated cases to provide a robust evidence supporting *ASXL1* mutations testing in clinical setting for AML patients.
Results: Our meta-analysis showed that *ASXL1* mutations were associated with male gender and advanced age and were more frequent in secondary AML. Notably, *ASXL1* mutations were mutually exclusive with *FLT3* and *NPM1* mutations. *ASXL1* mutations appeared to be an independent adverse prognostic factor in all patients (OS (p<0.00001) and EFS (p=0.01)). This was also true for the subgroup of elderly patients over 60 years ((OS (p=0.002)). We also performed a meta-analysis of gene expression profiles of 3 publically available datasets. We were able to identify a signature of 92 genes, which were differentially expressed in *ASXL1* mutated *vs.* unmutated cases.
Summary and Conclusions: In conclusion, our data show that *ASXL1* mutations define a subgroup of AML patients with specific molecular profile and worse prognosis that justifies their testing in clinical settings.
Acknowledgements: This work is supported financially by Project ID_09_157 (Contract 5/16.12.2009) from the National Science Fund, Bulgaria.

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INCIDENCE AND PROGNOSTIC IMPACT OF FLT3 AND NPM1 MUTATIONS IN ACUTE MYELOID LEUKEMIA AND CKIT AND NRAS GENE MUTATIONS IN RUNX1/RUNX1T1 AML

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Background: Several genetic alterations such as translocations, gene mutations and deletions play an important role in myeloid leukemogenesis. Activating mutations of *FLT3* receptor and *NPM1* are one of the most common genetic abnormalities reported. However, the prevalence and prognostic significance of this mutations in AML patients is still controversial. Higher complete remission rate and lower incidence of relapse led the researches to consider AML with t(8;21) as a favorable prognostic group. Nevertheless, approximately 30–50% of patients carrying this translocation relapse. The reason may be the presence of the mutations of different genes, that can considerably influence the prognosis.
Aims: The aim of the study was to determine the incidence and prognostic impact of *FLT3* and *NPM1* mutations in AML patients; and *CKIT* and *NRAS* gene mutations in AML with t(8;21).
Methods: Cytogenetic studies were performed on bone marrow samples using standard GTG-method. Polymerase chain reaction with the following restriction or direct sequencing analyses was performed to detect mutational status of *FLT3, NPM1, CKIT* and *NRAS* genes.
Results: 280 patients with *de novo* AML in the age of 18 to 86 years (Me=55) were analyzed. The male/female ratio was 134/146. Mutations in *FLT3* and *NPM1* were detected in 90/280 (32.1%) patients. A total of 107 mutations were revealed in this group: 54 – *FLT3-ITD*, 21 – *FLT3-TKD* and 32 – in *NPM1* gene. 73 (26.1%) patients had single mutations: 41 (14.6%) – *FLT3-ITD*, 15 (5.6%) – *FLT3-TKD* and 17 (6.1%) – in *NPM1*. In 17 patients mutations occurred simultaneously: 11 (3.9%) with *FLT3-ITD* and in *NPM1*, 4 (1.4%) with *FLT3-ITD* and *FLT3-TKD*, 2 (0.7%) with *FLT3-TKD* and in *NPM1*. According to the cytogenetic analyses patients were separated in the next groups: with normal karyotype (NK) – 151 (53.9%), with complex karyotype – 39 (13.9%), with another chromosomal aberrations – 90 (32.2%). We found that mutations with the significantly higher incidence (p=0.02) were observed in the groups of patients with NK (57/151) and the intermediate risk group (28/90), whereas there were only 5/39 patients with mutations in the group with complex karyotype. As the result of overall survival (OS) analyses we revealed the significant unfavourable influence of *FLT3-ITD* mutation on the prognosis (p=0.012).
Mutations of 8, 11 and 17 (D816V) exons of *CKIT* and 12, 13 and 61 codons of *NRAS* were analyzed in 21 patients with *de novo* AML and t(8;21) with the median age of 53 years (range 17–70). Mutation D816V in *CKIT* gene was detected in 3 (14,3%) patients. There were no mutations of 8 or 11 exons of *CKIT* in the investigated group. Mutations G12D, G13D in *NRAS* were found in 2 (9,5%) patients. We detected the significant differences of overall (p=0.041) and relapse-free survival (p=0.009) in patients with and without D816V mutation. Mutations in *NRAS* didn't show the same correlation (p=0.086).
Summary and Conclusions: Mutations in *FLT3* and *NPM1* had a significantly higher incidence in the group of patients with intermediate risk comparing with the group with complex karyotype. *FLT3* mutations showed the adverse prognostic value. The incidence of mutations in *CKIT* and *NRAS* genes in patients with t(8;21) was relatively high. *CKIT* D816V mutation was associated with higher relapse incidence and a worse survival in *RUNX1/RUNX1T1* AML patients, while *NRAS* mutations showed lack of prognostic significance. It could be recommended to further accumulate clinical and molecular-genetic data while choosing therapy regimen in AML with t(8;21) patients, as it showed quite heterogeneity.

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THE NUP98 FUSION PROTEINS ARE RECURRENT ABERRANCIES IN ACUTE MYELOID LEUKEMIA: A REPORT FROM THE AIEOP AML-2002 STUDY GROUP.

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Background: The Nucleoporin 98 (NUP98) is part of a family of proteins that are involved in the nuclear pore complex known to control the trafficking between nucleus and cytoplasm. However, *NUP98* is discovered to play a critical role in gene regulation being involved in several chromosomal translocations in hematopoietic disorders. Thirty different *NUP98* partner genes have been identified among human myelodisplastic syndrome and acute myeloid leukemia (AML). The chimeric NUP98 protein has the N-terminal of NUP98 and the C-terminal of its partner gene. Partners, if belonging to the homeobox genes conserved the DNA-binding domain, if not, they maintain chromatin interaction domains, mediating in any case a transcriptional regulatory function of the chimera, as recently described for *NUP98-NSD1* and *NUP98-JARID1A* fusions.
Aims: Here, we aim to identify the more frequent NUP98 fusions at diagnosis of Italian AML.
Methods: We performed a molecular screening of 8 partner genes of *NUP98*, in particular *NSD1, HOXC11,PHF23, HOXA9, JARID1A, HOXD13, LEDGF, DDX10* in 169 patients affected by AML *de novo* enrolled in the AIEOP LAM 2002/01 protocol. All patients were previously found negative for known recurrent genetic abnormalities involving *MLL*, *CBFB*, and *FLT3* genes.
Results: We found 21 out of the 169 negative patients (14%) harboring one of the *NUP98* chimeric fusion studied, identifying a new AML subgroup. By considering the Italian pediatric AML 2002/01 protocol 21 out of 482 enrolled patients had

a NUP98 rearrangement confirming a final frequency of NUP98 aberrancies of the 4.3% at diagnosis. In particular, 6 out of 169 patients were found positive for t(5;11)(q35;p15.5)*NUP98-NSD1*, 5/169 for t(11;12)(p15;q13)*NUP98-HOXC11*, 3/169 for t(11;17)(p15.5;p13)*NUP98-PHF23*, 2/169 for t(7;11)(p15;p15)*NUP98-HOXA9*, 2/169 for t(5;11)(q35;p15.5)*NUP98-JARID1A*, one patient harbored the t(2;11)(q31;p15)NUP98-HOXD13, one the t(9;11)(p22;p15.5)*NUP98-LEDGF*, and one the inv (11)(p15q22)*NUP98-DDX10*. Same rearrangements were screened in a group of 34 FLT3ITD patients and revealed a strong association (50%) exclusively with the *NUP98-NSD1* translocation as previously described. We then considered the *NUP98* rearrangements for their clinical impact and revealed that NUP98-rearranged patients event free survival (EFS) was significantly dismal (n=21, EFS at 10 years=15.1%, SE 8.8), than the rest of *NUP98*-negative patients (n=148, EFS=51.1%, SE 4.4). These data support the hypothesis that all *NUP98* rearrangements mediated a similar leukemia phenotype by controlling the expression of a series of common genes. In fact, partner genes with DNA binding domains (ie. *HOXA9*, *HOXC11*) are shown to interact with typical loci, such as the *HOX* locus, that are the same affected at the chromatin levels by methylation and acetylation (ie. *JARID1A*, *NSD1*, *PHF23*), or by RNA helicase domains (ie. *DDX10*) of those partner genes. Furthermore, the aberrant transcriptional regulation mediated by *NUP98*-fusions is shown to be enforced by their ability to sequester the wild type *NUP98*, which plays a critical role during normal hematopoiesis.

Summary and Conclusions: Finally, we described that *NUP98* is not rarely involved in somatic translocations causing AML in childhood. Furthermore, *NUP98* rearrangements identify a new subgroup of AML with a similar destiny of very poor prognosis, opening for further consideration in novel biological studies in order to attempt to a future targeting of NUP98 to improve their outcome.

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SUPER-SILAC BASED QUANTITATIVE PROTEOMICS IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is an aggressive hematopoietic cancer of the myeloid lineage, where immature myeloblasts accumulate in the bone marrow. The disease is heterogeneous in both biological and clinical aspects. This complicates prognostication for patients receiving conventional intensive chemotherapy, particularly for the intermediate risk group, which lacks risk-stratifying factors. The treatment regime and therapy outcome thus depends on age, cytogenetics, molecular genetic abnormalities, and also previous clinical history. Quantitative mass-spectrometry based proteomics is a powerful analytical technique with potential to improve clinical evaluation in AML, providing complementary information to cytogenetic studies. Here, we applied the super-SILAC approach in which the cell lines used as internal standard are cultured in a modified medium where the endogenous light isotopes (^{12}C and/or ^{14}N) of the amino acids arginine and lysine are exchanged with heavy isotopes (^{13}C and/or ^{15}N, respectively), inducing a controlled mass increase of the amino acids which is measurable by mass spectrometry and allows distinguishing the internal standard from patient samples.

Aims: Using mass-spectrometry based quantitative proteomics, we aimed at gaining deeper clinical and biological insight into the leukemic proteome by comparing the blast cell proteome of 13 AML patients sampled at 1) time of diagnosis and 2) during relapse, after conventional intensive chemotherapy. Proteins with altered expression pattern between these stages of the disease could provide new biomarkers for improved prognostication and risk stratification of patients.

Methods: To obtain accurate protein quantification of AML patient blast cells, and at the same time cover the heterogeneity of the disease, we first generated an internal standard of five AML-derived cell lines, selected based on their diversity in regard to clinical, cytogenetic and molecular risk factors used for prognostication of AML patients. The five metabolically labeled cell lines were combined in order to generate the internal standard, which was spiked into each of the lysates obtained from individual AML patient blast cells in ratio 1:1. Protein separation was obtained with SDS-PAGE, followed by tryptic digestion, peptide separation by liquid chromatography and measurement by tandem mass spectrometry. Based on the acquired spectra, proteins were identified and quantified using MaxQuant coupled to its integrated search engine Andromeda. Statistical analysis was conducted using the Perseus software. Informed consent was obtained from all patients used in this study.

Results: The relative protein abundance was obtained between each patient (light isotope) and the internal standard (heavy isotope), enabling relative quantification of thousands of proteins between the individual samples. Approximately 3000 proteins could be quantified accurately in the AML patient blast cells proteome. Comparing how protein expression changed in 13 patients from time of diagnosis to relapsed leukemia revealed significantly altered expression of several hundred proteins, including proteins previously described as altered during relapse. Proteins involved in *e.g.* transcription and mitochondrial processes were expressed in a significantly higher level at relapsed disease, compared to the primary disease. Oppositely, some protein phosphatases and proteins involved in immune defense processes were higher expressed during primary disease. Interestingly, the significantly regulated proteins allowed us to segregate the relapsed leukemia patients into subgroups, revealing biomarker candidates that could potentially be used for improved risk stratification.

Summary and Conclusions: By using our super-SILAC proteomic approach we were able to quantify thousands of proteins in the leukemic proteome derived from 13 AML patients. By comparing the blast cell proteome from 13 patients sampled 1) at time of diagnosis and 2) relapsed disease, we were able to reveal AML-related proteins and pathways that were significantly regulated before and after conventional chemotherapy. We propose that new risk stratification markers and an improved understanding of chemoresistance mechanisms can be obtained from these data.

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EARLY ABSOLUTE LYMPHOCYTE COUNT IS NOT A PREDICTOR OF RELAPSE OR SURVIVAL IN ACUTE MYELOID LEUKEMIA

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Background: The absolute lymphocyte count (ALC) recovery is an independent predictor for survival in acute myeloid leukemia (AML) post autologous transplantation or in childhood acute lymphoblastic leukemia after induction chemotherapy. However, the role of ALC recovery after induction chemotherapy in adult AML is controversial.

Aims: We examined the ALC recovery after induction chemotherapy to assess survival in adult AML.

Methods: We performed a retrospective study involving 303 adult patients newly diagnosed with AML excepted acute promyelocytic leukemia between 2001 and 2012 who received either daunorubicin or idarubicin in combination with cytarabine as induction therapy. 230 patients (75.9%) obtained complete remission (CR). Data was analyzed for survival in these 230 patients.

Results: The study included 140 males and 90 females, with median age at diagnosis of 48 years (range, 15-77 years). Pretreatment cytogenetics was determined in 228 (99%): 57 (25%) were categorized as favorable (t(8;21) or inv(16)/t(16;16) with or without other abnormalities), 23 (10%) had abnormalities of chromosome 7 and/or complex karyotype defined as ≥3 aberrations (adverse), while the remaining 148 (64%) were categorized as intermediate with other findings, primarily composed of a normal karyotype (45%). 171 patients obtained CR with 1 course of induction chemotherapy, while 46 needed 2 courses, and 13 needed 3 courses. Sixty-eight patients received an allogeneic transplantation in their first CR, with 53 patients receiving it after their first relapse. With a median follow-up of 3.7 years (range, 0.3 - 11.7 years), relapse and death occurred in 105 and 87 patients, respectively. Disease-free survival (DFS) and overall survival (OS) for all patients are 42.4% and 64.1% at 3 years, respectively. The ALC at 28 days after first induction chemotherapy initiation was 705/μl (range 56-3781/μl). We divided ALC at time of day 28 into quartiles and then assessed DFS and OS. Lower quartile was 442.5/μl and upper quartile was 1043/μl in ALC of our cohort. Neither DFS nor OS have significant differences between the top and bottom quartile of ALC at day 28. Survival curves were superimposed in these groups. Same result was shown in the subgroup of patients who obtained CR with 1 cycle of induction therapy. Multivariate analysis showed the recovery of platelet count and hemoglobin level at day 28 and favorable cytogenetic abnormalities were significant predictors for DFS, while favorable cytogenetics and age at diagnosis were significant predictors for OS (Figure 1).

Figure 1. Survival according to ALC at day 28.

Summary and Conclusions: ALC at 28 days from therapy initiation is not predictive of DFS or OS for adult AML.

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TREATMENT WITH RADIATION, CHEMOTHERAPY, OR BOTH DEMONSTRATES SIMILAR OUTCOMES IN PATIENTS WITH ISOLATED MYELOID SARCOMA THAT MAY NOT BE DIFFERENT THAN *DE NOVO* ACUTE MYELOID LEUKEMIA

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Background: The presentation of acute myeloid leukemia as an isolated extramedullary tumor without bone marrow involvement is an uncommon event but one that poses difficulties in clinical decision-making. Due to its rarity, the treatment of isolated myeloid sarcomas has never been validated in a randomized clinical trial, and treatment paradigms have been developed based largely on case reports and clinician experiences. Various treatment modalities, such as systemic chemotherapy and radiation therapy have been employed in the treatment of this condition, however there is a paucity of data to guide the utilization of these different approaches in the treatment of myeloid sarcoma.
Aims: To determine the impact of chemotherapy, radiation therapy, and the combination of chemotherapy and radiation therapy on the survival and disease response in patients with isolated myeloid sarcoma.
Methods: We performed a retrospective review to compare radiation therapy (RT), chemotherapy (CT) and combination radiation and chemotherapy. A PubMed search was completed that identified seven publications consisting of large case reports or reviews where individual patient characteristics (including treatment course, site of disease, overall survival and time to development of bone marrow involvement) were defined. We included adult patients who had extramedullary myeloid sarcoma without bone marrow involvement at the time of diagnosis and excluded patients with leukemia cutis or lymph nodes as their sole site of disease. Seventy -one patients were included in the analysis and the primary outcome was overall survival.
Results: There was no significant difference in overall survival (p=0.41) whether patients were initially treated with radiation therapy (n=14, 11.2 months, 10- NR), chemotherapy (n=40, 19 months, 13- 36) or the combination of chemotherapy and radiation (n=17, 24 months, 12- NR) (Figure 1). At two years, the cumulative incidence of progression of disease to involve the bone marrow is overall 0.70 (0.56- 0.81) with no difference seen amongst the three treatment groups (RT 0.79; 0.42- 0.93, CT 0.70; 0.48- 0.85, combination 0.66; 0.29- 0.87). In terms of overall survival, the median overall survival of patients presenting with isolated myeloid sarcoma is 16 months (n=71). For comparison, AML patients treated on the ECOG1900 trial who received standard therapy had a median overall survival of 15.7 months, suggesting that the survival of patients with isolated myeloid sarcoma is likely no worse than those with *de novo* AML who undergo standard induction therapy.

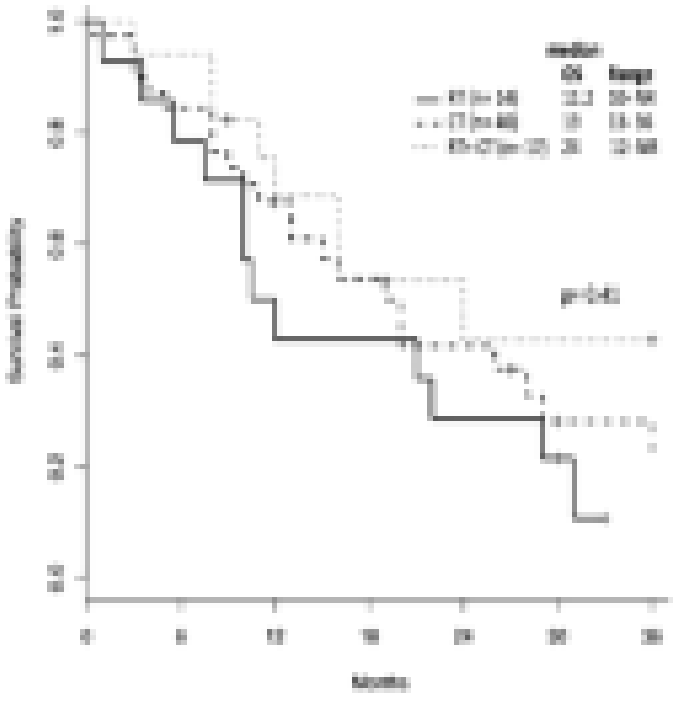

Figure 1.

Summary and Conclusions: In summary, this study is unique in its focus solely on adult isolated myeloid sarcoma as the initial presentation of AML with the exclusion of skin and lymph node sites of disease, acknowledging the relatively small number of patients included in some cohorts. Our data support the use of chemotherapy alone or radiation therapy alone as initial treatment for isolated myeloid sarcoma, and suggest that combined modality therapy would not have an additive effect. Notably, the rate of disease progression to involve the bone marrow was similar in both the chemotherapy and radiation therapy treated patients. This raises questions about possible unique aspects of the biology of myeloid sarcoma, which may allow it to evade the effects of systemic chemotherapy. Further evaluation involving larger numbers of patients and inclusion of primary patient data from Memorial Sloan- Kettering Cancer Center, as well as investigation of the role for allogeneic stem cell transplantation as part of first line therapy are required and currently underway.

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ACUTE MYELOID LEUKEMIA IN PATIENTS 70 AND ABOVE. AZACITIDINE *VERSUS* INTENSIVE CHEMOTHERAPY

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Background: Outcome with intensive chemotherapy in AML patients above the age of 70 is disappointing (median OS 4.6 months). The MD Anderson CC has identified as OS risk factors: age >80 yrs, ECOG ≥2, creatinine >1.3mg/dL and adverse cytogenetic (0 as opposed to 4 risk factors OS: 11.3 and 0.5 months respectively*; Kantarjian et al. Blood 2010)*. Azacitidine (AZA) has been explored in bone marrow blast <30% AML patients (median OS 24.5 months; *Fenaux et al. JCO 2010)*. The *ALMA study* has suggested as OS risk factors for first line AZA treated AML patients: ECOG≥2, pretreatment WBC >10.000/µL, bone marrow blast >30% and adverse cytogenetic (*Ramos et al., ASH 2012)*.
Aims: The aim of this study is to analyze the effectiveness and toxicity of AZA in 70 yr and above AML patients and to validate both, *MDACC* and *ALMA* scales.
Methods: We carried out the analysis of the 70 yr old and above patients included in the *ALMA study*, which consisted of AML patients from 22 Spanish sites, treated with AZA as first line. We evaluated effectiveness as *ELN-2010* criteria, toxicity as *CTCAE v3.0 scale*, *OS* and the mortality within the first 8 weeks (*M8wks*).
Results: Eighty eight (65 male/23female) out of 110 pt included in ALMA study were 70 or older, median age 76 (70-89). Risk factors as follows, age ≥80y: 28pt, ECOG≥2: 26pt, Creatinine >1.3 mg/dL: 17pt, adverse cytogenetic: 22pt, pretreatment WBC >10.000/µL: 25pt, BM blast >30%: 55pt. Seventy four patients were evaluated for efficacy, ORR 17% (CR+CRi 11pt, PR2pt). Median follow up 8.5 months (0.5-52), 49pt progressed and 74pt died. The *M8wks* was 9% and median OS 10 months. (1 year OS 40%). In this study age >80, ECOG≥2 and pretreatment WBC >10.000/µL predicted *M8wks* (p 0.05, 0.05, 0.04) and ECOG≥2, adverse cytogenetic, bone marrow blast >30% predicted OS (p<0.01). The *ALMA scale* (0 *versus* 1-2 *versus* 3-4 risk factors) predicted *M8wks* (p<0.01) as well as OS (16.5 *vs* 10 *vs* 3 months; p<0.01), while *MDACC scale* (0 *versus* 1-2 *versus* 3-4 risk factors) predicted OS (15 *vs* 7.7 *vs* 4.5 months; p<0.01) but didn't predicted *M8wks* (p 0.57). We analyzed a total of 540 AZA cycles (median 4, range 1-29) for toxicity, only in 3 cases treatment was discontinued because of toxicity.
Summary and Conclusions: These results suggest AZA as an effective and well-tolerated first line treatments in 70 and above AML patients. The OS seems longer than the previous reported with intensive chemotherapy, even in those patients with no MDACC RF. The *ALMA scale* predicts OS and *M8wks* in these elderly AML pt treated with AZA as first line.

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LOW DOSE GEMTUZUMAB OZOGAMICIN PLUS FLAI AS INDUCTION THERAPY IN CD33-POSITIVE AML. DEFINITIVE RESULTS AND LONG TERM OUTCOME OF A PHASE III MULTICENTER PROSPECTIVE CLINICAL TRIAL (NCT.00909168).

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Background: The role and safety of Gemtuzumab-ozogamicin (GO) containing combinations in previously untreated AML patients younger than 65 remains to be defined. Here we report the definitive results from a phase III multicenter clinical trial combining low dose of GO with FLAI (Fludarabine, Cytarabine, Idarubicin) as Induction regimen (eudract: 2007-005248-26; ClinicalTrials.gov NCT00909168).
Aims: Here we report the definitive results from a phase III multicenter clinical trial combining low dose of GO with FLAI (Fludarabine, Cytarabine, Idarubicin) as Induction regimen (eudract: 2007-005248-26; ClinicalTrials.gov NCT00909168).
Methods: Primary endpoints: Feasibility, Efficacy (CR+PR rate) and Toxicity of FLAI+GO; DFS and OS. Secondary endpoints: Evaluation of Minimal Residual Disease by WT1 (and other biologic markers) expression and monitoring. Feasibility and outcome of consolidation with Hematopoietic stem cell transplant (HSCT). One hundred thirty consecutive and untreated AML patients were included. All patients were younger than 65 with a median age of 52 years (range, 18-65) and CD33 expression exceeded 20% in all cases. The M/F ratio was 65/65, and 92/130 (71%) of patients were poor-risk at diagnosis. The induction regimen (GO-FLAI) included fludarabine (30 mg/sqm) and Ara-C (2 g/sqm) on days 1-5, idarubicin (10 mg/sqm) on days 1, 3, and 5 and GO (3 mg/sqm) on day 6. HSCT was planned for all high risk AML patients in first complete remission (CR), after consolidation with intermediate doses of Ara-C and idarubicin (ID-AC and IDA). Cytogenetic, multidrug-resistance phenotype, FLT3 and NPM mutation status, WT1 quantitative expression analyses, were performed at diagnosis in all patients. Quantitative WT1 gene expression (RQ-PCR technique validated by Leukemia Net), cytogenetic (in positive cases) and specific molecular marker analyses were performed after induction to detect and follow Minimal Residual Disease.
Results: Patients were evaluated for response rate, treatment-related adverse events, overall survival (OS) and disease free survival (DFS), feasibility and outcome of intensification with HSCT. After induction with GO-FLAI, CR rate was 83% (104 of 126 evaluable pts); five patients achieved partial remission and 17/130 (13%) were resistant (Overall response rate 87%). There were only 4 cases of death during induction (DDI 3%). The haematological and extra haematological toxicity of GO-FLAI was manageable and will be reported in detail; 45% of patients experienced transient and reversible GO infusion-related adverse events (especially fever and chills), but no cases of veno-occlusive disease occurred during chemotherapy or after allogeneic HSCT. In the setting of patients who achieved a cytological CR after GO-FLAI, the mean of WT1 copies dropped from 8178±10040/10^4ABL (at diagnosis) to 165±227 copies/10^4ABL after induction therapy [P<0.05]. After a median follow-up of 48 months, 67/130 (52%) patients are alive. The probability of 1, 2, 5-year OS was 80%, 63% and 52%. The probability of 1, 2, 5-year DFS was 71%, 54% and 47%. Allogeneic and autologus HSCT was performed in 58 (44%) and 24 (18%) patients, respectively.
Summary and Conclusions: The definitive results of this trial confirm that GO (3 mg/sqm) combined with FLAI is an effective and well tolerated induction regimen for CD33 positive AML patients younger than 65 years, with a high complete response rate, good disease debulking, favourable safety profile and low DDI, allowing consolidation therapy with HSCT early and in a high proportion of cases (82/130, 62%). Moreover, these results support the reassessment of GO-containing combinations as front-line AML therapy.

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PHASE IB STUDY OF PF-04449913, A HEDGEHOG (HH) INHIBITOR, IN COMBINATION WITH LOW-DOSE CYTARABINE OR INTENSIVE CHEMOTHERAPY, IN ACUTE MYELOID LEUKEMIA (AML) OR HIGH-RISK MYELODYSPLASTIC SYNDROME (MDS)

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Background: Aberrant activation of the Hh pathway has been mechanistically linked to both hematologic malignancies and solid tumors through direct cell-cycle interactions, cancer stem cell inhibition, indirect stromal interactions, and anti-angiogenic effects. PF-04449913 (PF) is a potent, selective, oral inhibitor of the Hh pathway, which acts through inhibition of Smoothened, with demonstrated activity in pre-clinical models. Single-agent treatment of patients (pts) with hematologic diseases in a phase I study provided preliminary evidence of clinical activity.
Aims: Primary objectives of the phase IB dose-finding portion were to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of PF in combination with low-dose cytarabine (LDAC; Arm A for unfit pts, ie, those not fit for high-dose chemotherapy) or intensive chemotherapy (cytarabine and daunorubicin; Arm C for fit pts) in pts with AML or high-risk MDS.
Methods: The study followed a standard 3+3 design, and the primary endpoint was occurrence of first-cycle dose-limiting toxicities (DLTs). In Arm A pts received oral PF administered continuously once daily (starting dose 100 or 200 mg) in combination with 20 mg LDAC administered subcutaneously twice daily on days 1-10 of each 28-day cycle. In Arm C, pts received PF continuously once daily (starting dose 100 or 200 mg) beginning on day -3, in combination with IV daunorubicin (60 mg/m^2 on days 1-3 of induction) and cytarabine (100 mg/m^2, on days 1-7 of induction) followed by consolidation (2-4 cycles of cytarabine 1 g/m^2 twice daily on days 1, 3, and 5 of each cycle); thereafter, single-agent PF was administered as maintenance therapy in 28-day cycles.
Results: Eighteen unfit pts were enrolled in Arm A (11 males/7 females; 100 mg PF, N=12; 200 mg PF, N=6). Median age was 75 years (data undergoing validation). The most common PF-related adverse events (AEs) included dysgeusia (22%) and nausea (22%). The most frequently reported non-hematologic PF-related ≥G3 AEs included G3 dehydration, hyperuricemia, fatigue, muscle spasms (all 6%) and G4 asthenia (6%). Two on-study deaths were reported, one due to acute respiratory distress syndrome and one to acute myocardial infarction. Twenty-two fit pts were enrolled in Arm C (12 males/10 females; 100 mg PF, N=16; 200 mg PF, N=6). Median age was 59 years (data undergoing validation). The most common PF-related AEs included nausea (64%), diarrhea (32%) and muscle spasms (32%). The most frequently reported non-hematologic PF-related ≥G3 AEs included G3 pyrexia, myalgia, and rash (all 5%). A DLT of G4 polyneuropathy, later attributed to concomitant medication, was reported at the 100-mg dose level; treatment was permanently discontinued. No ≥G3 QTcF prolongation was observed in either arm. A 100-mg daily dose was selected as the RP2D for fit and unfit pts, based on the observed tolerability profile and the increase in PF exposures observed with strong CYP3A4 inhibitors such as azoles. Preliminary efficacy signals were observed, with complete responses reported in both fit and unfit pts.
Summary and Conclusions: Oral administration of PF in combination with LDAC or intensive chemotherapy was clinically manageable and relatively well tolerated in pts with AML or high-risk MDS, and showed favorable pharmacokinetic characteristics. Based on the observed safety profile and increased exposure from drug-drug interactions with strong CYP3A4 inhibitors, the RP2D was established at 100-mg daily for both combinations. The trial is registered at clinicaltrials.gov; NCT01546038.

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RESULTS OF A PHASE 1 STUDY OF BMN 673, A POTENT AND SPECIFIC PARP-1/2 INHIBITOR, IN PATIENTS WITH ADVANCED HEMATOLOGICAL MALIGNANCIES

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Background: BMN 673 is the most potent inhibitor of poly(ADP-ribose) polymerase (PARP)-1/2 in clinical development (IC50<1nM, *in vitro*), inducing synthetic lethality in tumors deficient in homologous recombination. Non-clinically, hematological malignancies, including Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS), Chronic Lymphocytic Leukemia (CLL) and Mantle Cell Leukemia (MCL), have exhibited sensitivity to PARP inhibition, as a consequence of defects in DNA repair that could lead to deficient homologous recombination.
Aims: The main objective of this study was to identify the maximum tolerated dose (MTD) independently in the 2 study arms (Arm 1: AML and MDS; Arm 2: CLL and MCL).
Methods: Safety, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary efficacy of BMN 673 were evaluated in a 2-arm study. Oral BMN 673 was administered on a continuous daily schedule (21-day cycle) at escalating dose levels.
Results: 33 pts (Arm 1: 21 AML, 4 MDS; Arm 2: 4 CLL, 4 MCL), 10F/23M, median age 71 (range 22-86), ECOG PS 0-1 were enrolled. Median number of previous regimens was 3 (range 1-7) in Arm 1 and 6 (range 1-13) in Arm 2. BMN 673 dose levels were: 100, 200, 300, 450, 900, 1350 and 2000 µg/day. Dose-limiting toxicities (DLTs) included febrile neutropenia and neu-

tropenic sepsis in 2/4 pts at 2000 µg/day in Arm 1 and severe neutropenia in 2/5 pts at 900 µg/day in Arm 2. Overall, the most frequent drug-related adverse events were fatigue (all grades=27%), neutropenia (27%), nausea (24%), infections (21%), and thrombocytopenia (12%). No alopecia was reported. No responses were seen. Stable disease was seen in 13/25 pts in Arm 1 and 5/8 pts in Arm 2. 1 MDS pt received 24 cycles of BMN 673 (484 days) and became RBC transfusion independent.
Summary and Conclusions: The MTD of BMN 673 appears to be different in pts with AML, MDS, CLL and MCL. As a single-agent, BMN 673 has demonstrated limited activity in an unselected group of pts with advanced hematological malignancies.

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PHASE 2, OPEN-LABEL STUDY OF E7070, IDARUBICIN AND CYTARABINE IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMA AND HIGH-RISK MYELODYSPLASTIC SYNDROME

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Background: E7070 (Indisulam) is a novel sulfonamide drug that impacts energy metabolism and cell cycle progression. E7070 suppresses cell cycle progression partly by reducing cyclin H expression and thereby inhibiting phosphorylation of Rb protein. One of its potential metabolic targets is malate dehydrogenase. Pre-clinical studies have established synergy of E7070 with nucleoside analogs as well as topoisomerase inhibitors.
Aims: We designed a phase 2 study of E7070 (E) in combination with idarubicin (I) and cytarabine (A) in patients with relapsed-refractory acute myelogenous leukemia (AML) and high-risk myelodysplastic syndromes (MDS).
Methods: In Stage 1 patients receive E7070 at 400 mg/m^2 intravenously on day 1 and day 8 in a 21 day cycle (E). Patients with clinical benefit after single agent E7070 can continue for another 5 cycles. In the absence of benefit with E7070 alone, patients can proceed to stage 2to receive E7070 at the same dose on day 1 and day 8 followed by Idarubicin 8 mg/m^2 IVx3 and Cytarabine 1.0 g/m^2 IV over 24 hours daily on day 9-12 (age <60 years) or days 9-11 (age >60 years) in a 28 day cycle (E+IA). Patients with response can receive up to 2 additional cycles of E+IA. The efficacy of the regimen is assessed according to the Simon's two-stage Minmax design. The investigational therapy will be considered promising if the response rate is 25% or higher. The study will stop if the toxicity is likely to be greater than 33%. The planned accrual is 40 patients. Patients are considered evaluable they received E and achieved response or received at least one treatment with E+IA.
Results: This report is a planned interim analysis for futility. Twenty-one patients with relapsed/refractory AML (N=20) or MDS (N=1) and median age of 65 years (range, 45-76 yrs) have been enrolled. Eight patients have poor risk cytogenetics, median number of prior treatments are 2 (range, 1-4), 13 (62%) received prior cytarabine based regimens (including stem cell transplant =2) and 16 (76%) received hypomethylating agent. No response was seen after stage 1 (E). Of the 15 patients who received at least one cycle of E and E+IA (stage 2) each, 8 (53%) achieved complete remission (CR) or CR with incomplete recovery of counts (CRi). Four of the responders have diploid cytogenetics while it is poor-risk in the other four. All received prior cytarabine based regimen or several cycles of hypomethylating agent. Infectious events were encountered in 16/21 patients and 2 patients had prolonged marrow hypoplasia (>42 days). Grade 3 hyperbilirubinemia has been the only ≥grade 3 non-hematological toxicity.
Summary and Conclusions: Interim analysis shows promising responses with a regimen of E7070 in combination with Idarubicin and cytarabine among patients with relapsed/refractory myeloid malignancies. E7070 appears to lack single agent activity and the treatment cycle with E7070 alone will be omitted in future patients. Based on study design accrual is continuing.

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AZACITIDINE AND INTENSIVE CHEMOTHERAPY IN THE TREATMENT OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A META-ANALYSIS

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Background: The prognosis of elderly patients with acute myeloid leukemia (AML) is poor and the optimal therapeutic regimen is still investigated. In recent years, increasing studies focused on azacitidine in the treatment of elderly patients with AML. Azacitidine treatment was linked to lower CR rates, but long-term survival was comparable with intensive chemotherapy. However, there are some controversies. Hence, the attempt on evaluating the efficacy between azacitidine and intensive chemotherapy was performed with a meta-analysis.
Aims: To compare the efficacy and safety of azacitidine and intensive chemotherapy in elderly patients with AML.
Methods: A literature search was undertaken through September 2013 looking for the cohort studies evaluating the efficacy and safety of azacitidine and intensive chemotherapy in the treatment of elderly patients with AML. From 287 full-text articles and 207 meeting abstracts, six were included in this study. Heterogeneity was assessed by using the I^2 index, and quality assessment was performed with the Newcastle-Ottawa Scale.
Results: Compared with intensive chemotherapy, azacitidine treatment was associated with a lower complete remission (CR) rate (OR=0.40; 95% IC=0.19-0.84; *P*=0.02), but a higher partial remission (PR) rate (OR=3.39; 95% IC=1.35-8.49; *P*=0.009). However, early mortality (OR=0.22; 95% IC=0.03-1.99; *P*=0.18), one year (OR=0.93; 95% IC=0.53-1.64; *P*=0.80), and two year (OR=0.51; 95% IC=0.25-1.06; *P*=0.07) survival were not significantly different between patients treatment with azacitidine and intensive chemotherapy.
Summary and Conclusions: Both this and previous studies indicate that azacitidine treatment might be a valuable alternative to intensive chemotherapy in elderly patients with AML.

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THE ADDITION OF CLADRIBINE TO THE STANDARD 7+3 (CYTARABINE/DAUNORUBICIN) DID NOT IMPROVE OUTCOMES OF THE FIT SECONDARY ACUTE MYELOID LEUKEMIA TREATMENT – RETROSPECTIVE MULTICENTER PALG STUDY.

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Background: Recognition of the secondary acute myeloid leukemia (sAML) is increasing because of more effective treatment of cancers in last years. Outcomes in this group of patients' treatment are particularly bad. Previous studies proved that addition of cladribine to the standard 3+7 induction in AML patientsbelow 60 improved complete remission rate (CR) and overall survival (OS) (Holowiecki *et al.* JCO 2012).
Aims: The goal of this retrospective, multicenter PALG study was assessment of the results of intensive chemotherapy of sAML treatment in Poland.
Methods: Forty three patients, 59% female with median of age 56 (21-73) diagnosed on 2004-2013 in 6 PALG centers were entered to the study. Median time from diagnosis of primary neoplasm (do czego do rozpoznania AML?) was 5 years. Median WBC at diagnosis of sAML was 12.35 G/l. Cytogenetic risk was low in 9%, intermediate 37%, high in 22%, not known in 32% of patients. According to FAB classification they were M0 in 9%, M1 in 26%, M2 in 26%, M4 in 27%, M5 in 9%, M6 in 3%. In 80% of cases diagnosis of sAML was preceded by solid cancers (the most frequent was breast cancer in 44%), in 20% hematologic neoplasms. In 76% chemotherapy, in 57% radiotherapy, in 46% both before diagnosis of sAML were given. In 46,5% (n=20) sAML patients DAC induction regimen (daunorubicin 60 mg/m^2/d 1-3, cytarabine 200 mg/m^2/d 1-7, cladribine 5 mg/m^2/d 1-5) was given. Reminders receiving as induction DA 7+3 regimen (the same regimen as DAC without cladribine). In DAC and DA arm after CR assessment allogeneic hematopoietic stem cell transplantation (alloHSCT) was carried out in 6 and 2 cases respectively.
Results: After whole induction CR was obtained in 55% (n=11) *vs.* 52% (n=12) in DAC and DA arm respectively, p=0.90. Toxicity was similar in both groups. Progression free survival (PFS) was 23% *vs.* 41% patients after 4 years in DAC and DA arm respectively, p=0.21. Median OS was 20% *vs.* 26% patients were alive in group DAC *vs.* DA after 4 years respectively, p=0.35.
Summary and Conclusions: Secondary AML remains a challenge because of increasing frequency and poor prognosis. In our study we do not observed improvement of the treatment outcomes of fit sAML by addition of cladribine to standard induction as it was proved in primary AML. This observation requires further prospective studies.

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ARTESUNATE, AN ANTIMALARIAL DRUG DISPLAYS PRECLINICAL ACTIVITY IN FLT3 MUTATED LEUKEMIA AND SYNERGISES WITH ARAC

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Background: Acute myeloid leukemias (AML) are a group of heterogeneous hematological clonal disorders, marked by an abnormal accumulation of undif-

ferentiated myeloblasts in the peripheral blood and bone marrow and is one of the leading causes of blood cancer related deaths worldwide. Novel targeted agents in combination with standard chemotherapy or combination therapy to target multiple pathogenic signaling pathways are needed to effectively cure or overcome therapy resistance in the disease. Studies have found that bone marrow is hypoxic and leukemia develops in a hypoxic bone marrow environment and hypoxia promotes chemoresistance. Approximately 20-25% of *de-novo* AML patients have mutations in the FLT-3 gene that is associated with poor prognosis. Despite many efforts in the past to develop a therapeutic target for FLT3, none of the agents tested have yet to be FDA approved for clinical use.
Aims: To identify a drug that can selectively target FLT3 mutated leukemia, which is effective under hypoxic conditions as well and will have limited toxicity to patients.
Results: Artesunate, derived from a plant *Artemisia annua* L. is an FDA approved drug for treating malaria and has been reported to have anti-cancer activity. We evaluated the *in vitro* toxicity of Artesunate in a panel of human AML cell lines representative of a number of molecular subtypes, with or without FLT3 mutation. Artesunate induced cytotoxicity in all tested AML cell lines under both normoxic and hypoxic conditions, but cells expressing FLT3/ITD showed greater sensitivity to the cytotoxic effects of Artesunate as compared to other AML cell lines. The anti-proliferative effect of Artesunate in FLT3/ITD expressing cell lines MV4-11 ($IC_{50:}$ 0.05μM) and MOLM-13 ($IC_{50:}$ 0.06μM) was 1100 and 900 fold greater respectively compared to the wild type FLT3 HL-60 cells ($IC_{50:}$56.15μM) under hypoxic conditions. We compared Artesunate with other FLT3 inhibitors that are under investigation, CEP-701 and PKC-412, and found a similar antiproliferative activity with similar $IC_{50.}$ In MV4-11 and MOLM-13 cells, artesunate exposure led to a decrease in S and G2/M phase, down-regulation in the downstream phospho-signaling proteins such as STAT-5, AKT and ERK and also a disruption in the mitochondrial membrane potential. The *in vivo* anti leukemic activity of Artesunate was evaluated using a NOD.Cg-Prkdc^{scid} IL2rg^{tmWjl} /Sz (NSG) mouse bone marrow engraftment model. MV4-11 cells ($2.5x10^4$, cells) transduced with GFP luciferase were injected intravenously into the tail veins of 9 mice. Vehicle (DMSO) or Artesunate (100mg/kg/day once daily) were given intraperitoneally for 14 days, after the engraftment of tumor cells was confirmed using bioluminescent imaging. The control animals had a median survival time of 43 days; whereas the Artesunate treated mice had a median survival of 66 days. After establishing the *in vitro* and *in vivo* cytotoxicity of Artesunate in FLT3/ITD expressing cells, we looked at the combination of Artesunate with AraC, a standard chemotherapeutic agent for AML. We found synergism in our *in vitro* study.
Summary and Conclusions: The encouraging results reported in this study indicate that a clinical study combining Artesunate with AraC in AML patients should be considered.

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PHASE I STUDY OF OPB-51602, AN INHIBITOR OF STAT3 PHOSPHORYLATION, IN PATIENTS WITH RELAPSED/REFRACTORY HEMATOLOGICAL MALIGNANCIES

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Background: STAT3 is constitutively activated in growth signaling pathways of various hematological malignancies, and inhibition of STAT3 in cell lines such as KG-1 and U937 can lead to cell death *in vitro* and *in vivo*. OPB-51602 is an oral STAT3 inhibitor of small molecular compound.
Aims: A multicenter phase I study was conducted to evaluate the safety, pharmacokinetics (PK), pharmacodynamics (PD), and antitumor activity of OPB-51602 in patients (pts) with hematological malignancies.
Methods: OPB-51602 was administered orally once daily, continuously for 4 weeks per cycle until disease progression or unacceptable toxicity to assess the PD effects on STAT3 phosphorylation (pSTAT) in lymph nodes (LNs) and bone marrows (BMs) were conducted. The starting dose was 1 mg/day, and dose escalations to 2, 3, 4, and 6 mg/day were planned. In the first treatment cycle, single and repeated dose PK was assessed on Days 1 to 4 and 28 to 31, respectively. Dose escalation was based on the "3+3" design, maximum tolerated dose (MTD) was defined as the dose with at least 2/6 dose-limiting toxicities (DLTs) in the 1st cycle, and toxicities were graded by NCI-CTC v4.0.
Results: Twenty pts including AML (n=7), FL (n=3), DLBCL (n=5), MM (n=4), and AITL (n=1) were treated at doses of 1 mg/day (n=4), 2 mg/day (n=3), 3 mg/day (n=4), 4 mg/day (n=6), and 6 mg/day (n=3). Median number of treatment cycles for 1, 2, 3, 4, and 6 mg/day was 3.5 (range: 1-8), 2 (1-17), 1 (0-1), 1 (0-7), and 1 (1-2), respectively. Fourteen pts were assessed for pSTAT in LNs and BMs at screening, and 4 pts tested positive for pSTAT. The main toxicities of all grades were nausea (73%), peripheral sensory neuropathy (45%), fatigue/malaise (45%), vomiting (36%) and diarrhea (35%). These toxicities were predominantly of grade 1 or 2. Six mg/day was judged to be the MTD, where DLT of grade 3 lactic acidosis occurred in 1 pts. The recommended dose for OPB-51602 for hematological malignancies was determined to be 4 mg/day. OPB-51602 was rapidly absorbed after both single and repeated doses, with maximum plasma concentrations at a median Tmax between 4.0 and 4.5 hours after the single dosing on Day 1, and between 2.0 and 4.0 hours after repeated dosing. The mean elimination half-lives of repeated doses were between 50.2 and 78.4 hours. AUC24h and Cmax increased dose-dependently following single and multiple dosing across the 1 to 6 mg dose range. No clear response, including pSTAT3 positive pts, was observed, while stable disease was observed in 3 ts.
Summary and Conclusions: The MTD of OPB-51602 was determined to be 6 mg/day. Long-term administration of OPB-51602 at higher doses was difficult with this daily dosing schedule and there was no responder. Therefore, further clinical development of OPB-51602 in hematological malignancies with this schedule was to be terminated.

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TREATMENT WITH QUIZARTINIB (AC220) ENABLES A HIGH RATE OF PATIENTS WITH RELAPSED OR REFRACTORY FLT3-ITD(+) ACUTE MYELOID LEUKEMIA TO BE BRIDGED TO HSCT

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Background: For FLT3-ITD(+) AML patients (pts) who are relapsed or refractory to chemotherapy, allogeneic hematopoietic stem cell transplant (HSCT) offers the best prospect for long-term survival. Pts are unlikely to undergo HSCT unless their blast count can be reduced to an acceptable minimum, ideally below 5% blasts, which defines composite complete response (CRc: CR+CRp+CRi). Quizartinib is an orally active inhibitor of the FLT3 receptor tyrosine kinase being developed for the treatment of AML. We present a new analysis from 212 FLT3-ITD(+) pts who were relapsed or refractory to previous salvage therapy or HSCT and who were then treated with quizartinib across two Phase 2 studies.
Aims: The aims of both studies were to determine the composite complete remission rate (CRc) defined as CR+CRi+CRp and the impact of treatment with quizartinib to enable pts to become eligible for HSCT.
Methods: All pts gave informed consent. In Study A, 136 pts received 90-200 mg/day quizartinib; in Study B, 76 pts were randomized to either 30 mg/day or 60 mg/day. In both studies quizartinib was given orally during continuous 28 day cycles, until relapse, intolerance or proceeding to HSCT.
Results: Median baseline blast count in Study A was 81% and 67% in Study B. Median age in Study A was 50 yrs and 55 yrs in Study B. In Study A, 47 of 136 (35%) pts proceeded to HSCT; of these 26/47 (55%) had achieved a CRc and 18/47 (38%) achieved a PR prior to HSCT. In Study B 28/76 (37%) pts proceeded to HSCT and of these 23/28 (82%) had achieved a CRc and 4/28 (14%) achieved a PR prior to HSCT. In Study A, pts proceeding to HSCT had a median overall survival (OS) of 34.1 weeks and a 1 yr survival rate of 36% compared to an OS of 18.4 weeks and 1 yr survival of 12% for pts not undergoing HSCT. In Study B, pts randomized to 30 mg/day quizartinib and who underwent HSCT had an OS of 31 weeks compared to 19 weeks for pts without HSCT; pts treated with 60 mg/day and who underwent HSCT had an OS of 28.1 weeks compared to 16.3 weeks for pts without HSCT. When both treatment groups in Study B are combined, for all pts who received a HSCT the OS was 28.1 weeks and the OS for pts who did not receive a HSCT the OS was 16.4 weeks. The current follow-up period in Study B is considerably shorter than the follow-up period for Study A and there remain 35 (46%) subjects in Study B alive and censored for overall survival.
Summary and Conclusions: Pts able to receive a HSCT after response to quizartinib have an improved outcome compared to those who did not have a HSCT. The ability of quizartinib to lower the blast count in a high percentage of pts (46% pts in both studies combined achieved a CRc) and bridge these pts to a potentially curative HSCT, together with an acceptable safety profile, represents an important clinical benefit from quizartinib. Updated results with additional follow up from Study B will be available for presentation.

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QUIZARTINIB (AC220) IN PATIENTS WITH FLT3-ITD(+) RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA: FINAL RESULTS OF A RANDOMIZED PHASE 2 STUDY

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Background: The presence of FLT3-internal tandem duplication (ITD) in patients (pts) with acute myeloid leukemia (AML) is associated with early relapse and poor survival. Quizartinib (AC220) is an orally active inhibitor of the FLT3 receptor tyrosine kinase being developed for the treatment of pts with FLT3-ITD(+) AML. A previous Phase 2 study showed a high level of activity at doses of 90 to 200 mg daily. This randomized, open-label, study was conducted to assess the safety and efficacy of two lower doses of quizartinib in FLT3-ITD+ pts, aged 18 years or older, with relapsed or refractory AML after either one-second line therapy or HSCT.
Aims: The aim of the study was to evaluate the composite complete remission rate (CRc) at different doses of quizartinib and the rate of Grade 2 or higher prolongation of QTcF. CRc was defined as CR+CRi+CRp.
Methods: All pts gave informed consent and were randomized to quizartinib 30 mg/day (Group A) or 60 mg/day (Group B) given orally during 28-day continuous treatment cycles, until relapse, intolerance or proceeding to HSCT.
Results: Seventy six pts were randomized equally to the two arms. Demographics and baseline characteristics were balanced between the two arms except for age over 60 years (42% Group A, 26% Group B) and the percentage with secondary AML (8% Group A, 18% Group B). The analysis included a minimum of 8 weeks of follow-up. The median baseline blast count was 67%. The median duration of treatment in Group A was 10.9 weeks (range 2.1 to 24.9+ weeks) and 11.0 weeks (range 2.6 to 26 weeks) in Group B. The CRc (CR+CRp+CRi) rate in both groups was 47% and the overall response rate (CRc+partial response (PR)) was 61% in Group A and 71% in Group B. Median time to CRc was 4.4 weeks (95%CI: 4.1 – 7.7 weeks) in Group A and 4.6 weeks (95% CI: 4.1 – 8.0 weeks) in Group B. The median duration of CRc in Group A was 4.1 weeks with 28% of subjects who achieved a CRc censored for undergoing HSCT and 20.0 weeks in Group B with 50% of subjects who achieved a CRc censored for undergoing HSCT. 32% of pts in Group A and 42% in Group B were able to receive a HSCT, mostly after achieving CRc (82%) or PR (14%). The median overall survival was 20.7 weeks in Group A; and 25.4 weeks in Group B with 35 of the 76 patients alive at last follow-up (range: 7.4 – 40.4+ weeks). Of the 35 (46%) subjects who remain alive, 24 subjects were alive >24 weeks and 2 subjects were alive >36 weeks. Grade 2 or greater QTcF prolongation occurred in 11% Group A pts and in 17% Group B pts; overall, 4% of subjects had Grade 3 QTcF prolongation. The most common treatment related adverse events occurring in 15% or more pts were diarrhea (18%), febrile neutropenia (16%), and QT prolongation (15%).
Summary and Conclusions: Quizartinib in second salvage or post HSCT FLT3-ITD+ AML demonstrated a high degree of efficacy (CRc 47%) with an acceptable safety profile, specifically decreased QTcF prolongation rates compared to higher doses used previously. Further analysis with additional follow-up will be available for presentation.

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BUPARLISIB, A PI3K INHIBITOR, DEMONSTRATES ACCEPTABLE TOLERABILITY AND PRELIMINARY ACTIVITY IN A PHASE I/II TRIAL OF PATIENTS WITH ADVANCED LEUKEMIAS

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Background: Phosphatidylinositol-3-kinase (PI3K) signaling plays a crucial role in oncogene-mediated tumor growth and proliferation. In many tumors, the PI3K signaling pathway is constitutively activated. Buparlisib (BKM120) is an oral pan-class I PI3K inhibitor belonging to the 2,6-dimorpholino pyrimidine derivative family.
Aims: This phase I/II study was conducted to determine the dose limiting toxicity (DLT) and maximum tolerated dose (MTD) of buparlisib in patients (pts) with acute leukemias.
Methods: Pts with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL) or mixed phenotype acute leukemia (MPAL); relapsed/refractory to standard chemotherapy or unsuitable for standard chemotherapy were eligible. Pts with ECOG performance, status 0–2 with adequate organ function and no active,, uncontrolled intercurrent illness or infection, received oral buparlisib continuously daily in 28-day cycles. The starting dose was 80 mg/day. Western blot analysis was performed to evaluate the depth of PI3K/Akt/mTOR inhibition.
Results: Eleven pts received at least 1 dose of buparlisib (10 AML, 1 MPAL) and were included in this analysis. The median (med) age was 70 years (range, 32-85). All pts had received prior therapy. The med number of prior therapies was 4 (range 1–6): hypomethylating agents (n=13), fludarabine (n=6), clofarabine (n=5), cladribine (n=2), and allogeneic stem cell transplant (n=2); either alone or in combination. Cytogenetics analysis showed diploid karyotype in 4 (36%), complex cytogenetics including chromosome 5 and/or 7 abnormalities in 3 (27%), and other cytogenetic aberrations in 4 (36%) pts. Molecular analysis showed *IDH 1/2* mutations in 2, *K-RAS, NRAS, DNMT3A, NPM1, KIT* and *FGFR1* mutation in 1 pt each, respectively. Nine pts received buparlisib 80 mg/day; six of these pts were evaluable for toxicity and DLT (Gr 2 confusion) was seen in 1 pt. Per protocol the dose was escalated to 100 mg/day. Two pts received 100 mg/day: both pts experienced DLTs in the form of Gr 2 and Gr 3 confusion, respectively. The MTD was established as 80mg/day. Med number of cycles received was 1 (range 1–3) and the most common cause of discontinuation was progressive disease (n=7). Of the 11 pts treated, 1 had stable disease at the 80mg/day dosage. The response lasted 80 days. Med overall survival was 51 days (range 11–260). Other drug-related toxicities ≥grade 2 included Gr 3 mucositis (n=1), Gr 2 dysphagia (n=1), Gr 2 fatigue (n=2), Gr 2 elevated serum bilirubin (n=1) and Gr 2 nausea (n=1). On Western blot profiling, a decrease in p-pS6K/total pS6K was observed in 5/7 (71%) samples with a mean quantitative inhibition of 65% (range 32–100%). Similarly, a decrease in p-FOXO3/total FOXO3 was observed in 4/6 (67%) samples with a mean quantitative inhibition of 93% (range 89 –100%. The pt with stable disease had decreases in both p-pS6K/total PS6K (45%) and p-FOXO3/total FOXO3 (100%).
Summary and Conclusions: Buparlisib administered at 80 mg/day showed modest efficacy and was tolerable in pts with previously treated acute leukemias. Additional studies of buparlisib; either alone or in combination are warranted.

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CHARACTERIZATION OF GLOBAL HYDROXYMETHYLATION LEVELS IN AML

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Background: Patients with acute myeloid leukemia (AML) frequently harbor mutations in genes involved in the DNA (hydroxy)methylation pathway, namely *DNMT3A*, *TET2*, *IDH1* and *IDH2*. DNMT3A is responsible for *de novo* DNA methylation, whereas TET proteins are able to initiate DNA demethylation by converting 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC). One of the essential co-substrates for the TET proteins, α-ketoglutarate, is produced by IDH proteins. Altered DNA methylation is frequently observed in leukemia, however less is known about hydroxymethylation levels.
Aims: Measure global (hydroxy)methylation levels in AML patients, and correlate these levels with mutational status and overall survival.
Methods: DNA samples from 206 clinically and molecularly well-characterized younger adult AML patients (≤60 years) included in the EORTC/GIMEMA AML-12 06991 clinical trial were analyzed for mutations in *DNMT3A*, *TET2*, *IDH1*, *IDH2*, *FLT3* and *NPM1*, and for *CBFB-MYH11*, *BCR-ABL1*, *AML1-ETO* and *MLL* translocations. Global 5-(hydroxy)methylcytosine levels were measured using HPLC-MS/MS.
Results: A wide range of 5hmC levels was detected in AML patients (15 fold difference), whereas 5hmC levels in healthy control cells were confined to a narrow range (1.5 fold difference). In remission, 5hmC values were normalized to levels comparable to healthy bone marrow and peripheral blood, indicating that the aberrant 5hmC levels at diagnosis are intrinsic to the leukemic cells. Patients with mutations in *TET2* or *IDH1/2* had significantly lower levels of 5hmC compared to patients without mutated *TET2* and *IDH1/2* (both $P<.001$), whereas mutations in *DNMT3A* did not influence global 5hmC levels. Interestingly, high 5hmC correlated with poor overall survival (P=.047, HR=1.81). *AML1-ETO* and *MLL* translocations were frequently present in patients with high 5hmC levels.

Summary and Conclusions: High levels of 5hmC independently correlated with inferior overall survival in AML.

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FRONTLINE TREATMENT WITH INTENSIVE CHEMOTHERAPY, AZACITIDINE OR BEST SUPPORTIVE CARE IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: A POPULATION-BASED ANALYSIS FROM A REGIONAL HEALTHCARE NETWORK

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Background: The efficacy of conventional treatments in older patients (pts) with acute myeloid leukemia (AML) remains unsatisfactory, with low remission rates and poor overall survival. Few pts above 60 years (yrs) really benefit from intensive chemotherapy (ICT). Azacitidine (AZA) has been recently approved for AML pts with 20-30% bone marrow (BM) blasts but little is known in pts with more than 30% BM blasts.
Aims: Taking advantage of our regional healthcare network, the first aim of this study was to describe the distribution of treatments, prognostic factors and outcome of all newly diagnosed non-M3 AML pts aged 60 years or older, selected in daily practice for ICT, AZA or best supportive care (BSC) over a 4-year period of time (2007-2010). The second aim was to compare the survival of pts treated with AZA *vs* respectively ICT and BSC using time-dependent analysis (Royston and Parmar model) and propensity score matching.
Results: Among the 385 AML pts aged 60 y or more, diagnosed between 2007 and 2010, 51 were excluded from the analysis because of early death (ED) occurring before therapeutic decision in 20 pts and missing information in 31 pts. Thus, 334 pts were evaluable (86.7%), including 115 pts treated with ICT (34.4%), 95 pts treated with AZA (28.4%), and 124 pts treated with BSC (37.1%). Median follow-up was 35 months. Overall median age was 75 yrs (ICT, 68 yrs; AZA, 76 yrs and BSC, 80.5 yrs; p<0.001). Twenty percent of the entire population had prior history of myelodysplastic syndrome (ICT 6.7%, AZA 29.5%, and BSC 25%; p<0.01 for AZA *vs.* ICT and p=0.017 for AZA *vs.* BSC), median WBC was 4.25 G/L (ICT 9.5 G/L, AZA 2.3 G/L and BSC 5.3 G/L, p<0.001) and cytogenetics (CG) were adverse in 28.4% (ICT 17.4%, AZA 45.3% and BSC 25.8%, p<0.001 for AZA *vs.* ICT and p=1 for AZA *vs.* BSC). ED (occurring within two months after diagnosis) was observed in 92 pts (27.5%) including 18 pts treated with ICT (15.7%), 11 pts in AZA group (11.6%) and 60 pts in BSC group (48.4%) (CTI *vs* AZA p=0,39; AZA *vs* BSC p<0.0001). We identified 56 pts in the AZA arm (59%) requiring hospitalization for infection (median number of hospitalization per patient, 1; range 1 to 7). Overall response (CR+CRi) was documented in 78 pts treated with ICT (67.8%), and in 18 pts treated with AZA (18.9%). Additionaly 6 pts treated with AZA have obtained PR and 24 pts (25.3%) with stable disease were classified as major hematological improvement on at least one lineage according to MDS response criteria. Median overall survival (OS) in ICT, AZA and BSC were of 18.9, 11.3 and 1.8 months, respectively. In pts treated with AZA, OS was negatively impacted in multivariate analysis by higher age (95% Confidence Interval, 1.01-1.10, HR 1.05 for one year increase, p=0.010), adverse CG (95% CI, 1.44-4.49, HR 2.55, p=0.001), lymphocyte count <0.5 G/L at diagnosis (95% CI, 1.19-5.26, HR 2.5, p=0.015) and higher LDH level (95% CI, 1.0002-1.0012, HR 1.0007 for one unit increase, p=0.005). We compared the survival of pts treated by AZA *vs* ICT and BSC using time-dependent analysis and propensity score matching (calculated on age, AML *de novo* or secondary status, BM blasts, platelets count and CG for ICT *vs* AZA and on age and WBC for AZA *vs* BSC comparison). Pts treated by ICT had a better overall survival compared to those treated with AZA from 6 months after diagnosis, whereas pts treated with AZA had a better overall survival compared to those treated with BSC from 1 day after diagnosis.
Summary and Conclusions: This study of "real life" practice shows that there is a room for low intensive therapies such as AZA in selected elderly AML patients.

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INCIDENCE AND MANAGEMENT OF VENOUS THROMBOSIS IN ACUTE LEUKEMIA: A MULTICENTER STUDY

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Background: Venous thrombosis (VT) frequently complicates the clinical course of cancer. Available data on the incidence and management of VT in Acute Leukemia (AL) are scanty and quite discordant.
Aims: We have performed a multicenter study with the primary objective to evaluate the incidence of venous thrombotic complications in a population of patients with AL. Secondary objective was to evaluate the management of these complications in patients with AL.
Methods: Available clinical records of out and in-patients diagnosed with AL from January 2008 to June 2013 in 7 Regional Reference Hospitals were analyzed. Cases of venous thrombosis (VT), including thrombosis in atypical sites [Retinal occlusion (RO) and Cerebral Sinus Thrombosis (CST)], were reported. Available laboratory tests at diagnosis of VT included complete blood cells count (CBC), basal coagulation tests (PT, aPTT, fibrinogen), Antithrombin, anticoagulant Protein S and C and D-dimer. Instrumental diagnosis of deep vein thrombosis (DVT), pulmonary embolism (PE) and RO and CST was performed according to ACCP guidelines. In the statistical analysis, logistic regression model was applied. Fisher's exact test was used to determine relationships between categorical variables.
Results: Over a population of 1487 patients with AL, 97 cases of VT were recorded: 71 cases were associated with Acute Myeloid Leukemia (AML) and 26 with Acute Lymphoblastic Leukemia (ALL). Fifty-four patients were males and 43 females (4 with AML, 10 with ALL), with a mean age of 52±15,5 years; Twenty-four patients presented at least a concomitant chronic disease,12 were receiving anticoagulant prophylactic treatment with low molecular weight heparin (LMWH). There were 72 cases of DVT of upper arms, 16 cases of proximal DVT of limbs (two complicated with PE), 4 cases of PE, 2 cases of RO, 2 of CST and 1 intracardiac clot. In 76/97 (78,6%) cases of VT, a central venous catheter (CVC) was placed; moreover, 68/72 DVT of upper arms were significantly associated with a CVC insertion (p<0.01).VT occurred during chemotherapy (CHT) in 88/97 (90.7%) cases,9 cases were diagnosed in concomitance with AL. In both subgroups with VT,there was no statistical significant difference between time at diagnosis of VT and time at diagnosis of AL. At CBC, thrombocytopenia was the most frequent abnormality. Severe thrombocytopenia (PLT<30000/mmc) was recorded in 25 cases. Coagulation tests were normal in all cases. Prothrombotic mutations were available only for 19/97 cases, 1 case was heterozygous for Factor V Leiden and 1 was homozygous for Factor II (G20210A) mutation. Most VT (83/97) were treated with LMWH at therapeutic doses for the first month witha dose reduction in the following months, for severe thrombocytopenia after CHT; 1 case was treated with unfractioned heparin, 1 case with warfarin; 6 cases did not receive treatment due to severe thrombocytopenia. No cases of VT–related deaths nor fatal complications during treatment were recorded. Three cases of mild bleeding were reported.Treatments with LMWH lasted from 3 to 6 months. All patients clinically recovered from VT, only 2 late recurrences (PEs) were observed.
Summary and Conclusions: The incidence (6.6%) of VT in the analyzed cohort of patients with AL is almost similar to previous reports.. Atypical sites VT must be suspected to be correctly diagnosed and treated. Anticoagulant treatment schedules and duration in patients with VT and AL seems safe and effective, even if it is influenced by many factors, mainly related to CHT and severe thrombocytopenia. The optimal management of VT in patients with AL requires further, prospective studies

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HIGH CORRELATION BETWEEN CLINICAL RESPONSES TO 1ST LINE AML PATIENTS TREATED WITH CYTARABINE AND IDARUBICIN AND THEIR PHARMACOLOGICAL PROFILES IN PATIENT SAMPLES MEASURED BY EXVITECH

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Background: Complete remission (CR) after induction therapy is the first treatment goal in acute myeloid leukemia (AML) patients.
Aims: To determine the ability of the Vivia's novel *ex vivo* drug sensitivity platform Exvitech to predict the CR rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in 1st line AML.
Methods: This non-interventional and prospective study included samples from adult patients diagnosed with *de novo* AML in Spanish centers from the PETHEMA group. Marrow samples were sent to Vivia laboratories and incubated for 48h in whole samples in well plates containing Ara-C, Ida, or their combination, each at 8 different concentrations. Depletion of leukemic cells was quantified by subtracting live cells in each well with drugs from the control wells without drugs. Pharmacological responses are calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant. Patients dying during induction response assessment were non-evaluable. The correlation was modeled using a generalized additive model with a logit link and a binomial distribution for residuals. Kernel density estimates were then used to plot empirical probability density functions for both groups. Their separation was quantified as the area under the ROC curve and a cut point was selected using the Youden's criteria to optimize the classification probabilities (sensitivity, specificity). 95% confidence intervals for sampling errors were calculated for all these quantifiers.
Results: 180 patient samples were used to calculate the dose response (DR) curves for Ara-C alone, Ida alone, and their synergism. For clinical correlation we used 77 patients with a median age of 55 years (range 31 to 73). DR for Ara-C alone are shown in Figure 1A; note that for many samples there is a significant number (>20%) of resistant cells to Ara-C (bracket). This is a strong clinical predictor of resistance because in the patient the drug will never be present at these high doses for 48h. The second variable that is a good predictor of response is the synergism between these 2 drugs. The generalized additive model identified an algebraic combination of these 2 variables that yielded the best marker to separate both groups of patients. The probability density functions had minimal overlap. The area under the corresponding ROC curve was 0.935 (0.872, 0.997), and the classification probabilities for the optimal cut point, were 87% (68% to 95%) and 91% (80% to 96%) for sensitivity and specificity, respectively. Results are shown in Figure 1B; 54 patients (70.1%) achieved CR after Ida+Ara-C, and the remaining 23 (29.9%) were resistant. Correlations of the PM test are shown in Figure 1B. 20 of the 23 (86.9%) patients who fail to achieve CR were predicted as resistance in the *ex vivo* test. 49 of the 54 patients (90.74%) who achieved CR showed good *ex vivo* sensitivity to Ida+Ara-C predicting for CR. When the *ex vivo* test predicted a patient as sensitive it was correct in 49/52 cases (94.23%), and when it predicted resistant it was correct 20/25 cases (80%). Overall, 69/77 patients (89.61%) had an accurate prediction of their response to treatment.

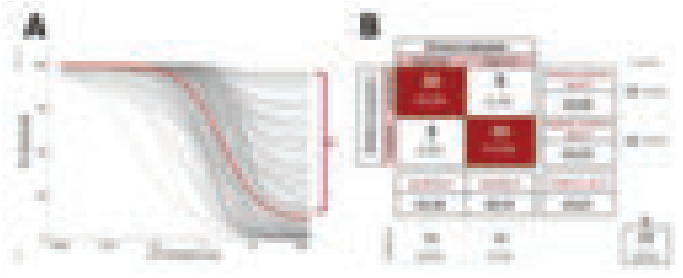

Figure 1. A) Dose response curves for Ara-C on 180 samples from AML patients in terms of the % survival of leukemic cells show the pharmacological profile of this drug. Note the 40% of samples with submaximal efficacy (bracket). B) Correlation with clinical outcome of the *ex vivo* pharmacological profiles in terms of sensitive *vs* resistant patients.

Summary and Conclusions: This study shows that this novel *ex vivo* pharmacological profile test is able to predict the clinical response to Ida+Ara-C induction. We are increasing the number of patients in this ongoing study, and we are planning a PM Test-adapted Clinical Trial.

Chronic lymphocytic leukemia and related disorders - Biology 1

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RUXOLITINIB INDUCES APOPTOSIS OF B-CELL RECEPTOR STIMULATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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Background: Stimulation of the B-cell receptor (BCR) protects chronic lymphocytic leukemia (CLL) cells from apoptosis and induces their proliferation. It is assumed that when CLL cells reside in the lymph-nodes extracellular signals activates the BCR and provides them with survival advantage. Whether the JAK-2/STAT3 pathway, known to elicit similar effects in various hematological and solid tumors is engaged in this process is unknown.
Aims: 1) Determine whether stimulation of the BCR induces JAK-2/STAT3 mediated tyrosine pSTAT3 and protects CLL cells from apoptosis. 2) Determine whether the JAK1/2 inhibitor ruxolitinib inhibits this pathway in CLL cells
Methods: CLL cells, fractionated from the peripheral blood of 19 previously untreated patients were used in the different experiments performed in this study. Anti-IgM antibodies were used to stimulate the BCR, and stimulated or unstimulated cells were treated with the JAK1/2 inhibitor, ruxolitinib, the bcr-abl/lyn inhibitor, dasatinib or with U0126, a MAPK signaling pathway inhibitor. The protein levels of STAT3 and tyrosine pSTAT3 were assessed by western immunobloting, and for immunopercipitation studies we used JAK2, pJAK2, STAT3 and pSTAT3 antibodies. Nuclear and cytoplasmatic extracts were prepared and the purity was confirmed by the absence of lamin B in the cytoplasmic extract and the absence of ribosomal S6 from the nuclear extracts. Localization of pSTAT3 to the nuclear and cytoplasmic extracts was confirmed by confocal microscopy. Apoptosis was assessed by Annexin V/PI, and levels of STAT3 target genes by real time PCR (RT-PCR) and quantitative reverse-transcription PCR (qRT-PCR).
Results: Tyrosine pSTAT3 was not detected in unstimulated CLL cells. Stimulation of the CLL-BCR using anti-IgM antibodies induced tyrosine phosphorylation of STAT3, which was transient. STAT3 remained phosphorylated for 48 h in the presence of anti-IgM antibodies. However, 2 h after anti-IgM antibodies were washed out of the culture media we could no longer detect tyrosine pSTAT3. When the BCR was stimulated, tyrosine pSTAT3 was found in the cytoplasmic and nuclear extracts. Confocal microscopy confirmed that following BCR stimulation tyrosine pSTAT3 was localized to the nucleus. By RT-PCR and qRT-PCR we show that following stimulation of the BCR and localization of tyrosine pSTAT3 in the nucleus, STAT3-regulated genes are upregulated, suggesting that BCR mediated induction of tyrosine pSTAT3 is biologically active and induce transcription. Immunopercipitation studies revealed that pJAK2 and tyrosine pSTAT3 co-immunopercipitated suggesting that STAT3 phosphorylation occurs through the JAK-2/STAT3 pathway. Because tyrosine pSTAT3 protects CLL from apoptosis we hypothesized that ruxolitinib, a JAK1/2 inhibitor, would prevent phosphorylation of STAT3 and induce apoptosis of BCR-stimulated CLL cells. Ruxolitinib, but not dasatinib or U0126 inhibited tyrosine phosphorylation of STAT3 in a time and dose dependent manner. Likewise, ruxolitinib, but not dasatinib or U0126 induced apoptosis of BCR-stimulated CLL cells.
Summary and Conclusions: Our findings suggest that stimulation of the BCR activates the JAK-2/STAT3 pathway and induces transient phosphorylation of STAT3. Ruxolitinib inhibited the phosphorylation of STAT3 and induced apoptosis of CLL cells. Whether treatment with ruxolitinib would benefit patients with CLL remains to be determined.

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VARIABLE REACTIVE OXYGEN SPECIES LEVELS ARE ASSOCIATED WITH ANERGY IN CLL

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Background: Reactive oxygen species (ROS) are important regulators of cell signalling, replication and survival, and are essential for normal cell physiology. If uncontrolled however, ROS are associated with cell damage and implicated in therapy resistance. Previous studies have demonstrated that chronic lymphocytic leukaemia (CLL) cells contain variable levels of ROS which are, on average, higher than normal B cells. However, the relationship between ROS levels and disease subsets has not been examined.
Aims: We have sougt to investigate potential associations between ROS levels, *IGHV* mutation status and the extent of B-cell receptor (BCR) anergy, an important determinant of clinical behaviour.
Methods: Examine intracellular ROS levels in primary CLL cells stained with CM-H_2DCFDA using FACS.
Protein expression analysis of pErk-1/2 and p-Akt in cells treated with N-acetyl

cysteine (NAC) or left untreated prior to stimulation with immoblised anti-IgM and IgD. Examine surface expression of the migration molecule CXCR4 using FACS. Transwell migration assays to dertermine effect of NAC on CLL migration to the chemokines CXCL12 and CXCL13. Protein expression analysis of p-Erk-1/2 and p-Akt in cells pre-treated with NAC or left untreated prior to stimulation with CXCL12 or CXCL13.
Results: Flow cytometry analysis of unstimulated primary CLL cells demonstrated that ROS levels differed substantially between samples. Overall, ROS levels were higher in M-CLL compared to U-CLL (*P*=0.003). ROS levels were also higher in samples that had strong features of anergy, including down-modulation of surface IgM (sIgM) expression (*P*=0.003) and signalling capacity (*P*=0.001). Some patients also demonstrated intraclonal variation in ROS production. This was observed in both M-CLL and U-CLL, but somewhat more frequent in U-CLL. Analysis of these 'sub-populations' found that ROS levels were inversely proportional to CXCR4 expression, which is down-modulated in the tissues and therefore marks the most recent emigrees into the blood. Further studies demonstrated that the antioxidant N-acetyl cysteine decreased anti-IgM signalling responses, and CXCL12 or CXCL13-induced migration, implying functional roles for ROS in CLL signal transduction.
Summary and Conclusions: Overall, these results indicate that increased ROS is a consequence of antigen engagement in CLL, potentially linked to the extent of anergy.

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HIGH LEVEL OF COBLL1, A NOVEL ROR1 BINDING PARTNER, IDENTIFIES CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS WITH MUTATED IGHV

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Background: Chronic lymphocytic leukemia (CLL) is a disorder with a variable clinical course and up-regulated expression of a transmembrane tyrosine-protein kinase (ROR1), a member of Wnt – planar cell polarity (PCP) pathway. This pathway has an important role in the early embryonic development and controls pathogenesis of CLL via regulation of cell migration and chemotaxis.
Aims: The main aim of the study was (i) to identify ROR1 downstream signalling by the proteomic analysis of ROR1 binding proteins in CLL and (ii) to analyse the role and clinical value of selected binding partners.
Methods: The search for ROR1 binding partners was performed using mass spectrometry. For expression analysis, a cohort of 186 unselected CLL patients was tested. B-lymphocytes were separated from peripheral blood using gradient centrifugation with non-B-cell depletion. mRNA expression was examined by qRT-PCR. The protein expression was further tested using Western Blot. B-cells from healthy tonsills (n=4) were fasc-sorted via IgD and CD38 staining into naïve, germinal centre and memory B-cells, which were tested by qRT-PCR. Expression vectors containing COBLL1 and ROR1 were transfected into HEK293 cells to visualize their localization.
Results: We identified a novel Ror1 binding partner in CLL cells – cordon blue protein like 1 (COBLL1). In HEK293 cells, COBLL1 was localized mainly in cytoplasm but following ROR1 overexpression both proteins were strongly co-localized in the membrane filopodia. In CLL patients COBLL1 showed variable expression with significantly higher expression in the patients with mutated IGHV (n=70) compared to the patients with unmutated IGHV (n=116; p<0.0001, Mann-Whitney U test). Accordingly, the patients with COBLL1 expression above defined threshold had longer treatment-free survival (48 months) compared to the patients with expression bellow this threshold (32 months; p=0.0004, Mantel-Cox test). Also, the expression of COBLL1 decreased with disease severity according to the cytogenetic hierarchical model (del 13q, normal karyotype *vs.* del 17p, del 11q; p<0.03, Mann-Whitney U test). The difference in COBLL1 expression between the patients with mutated and unmutated IGHV was also confirmed at a protein level (n=10). Median expression in the patients with mutated IGHV was similar to the expression in healthy germinal centre B-cells, whereas median expression in the patients with unmutated IGHV corresponded to the expression in healthy naïve and memory B-cells.
Summary and Conclusions: We identified COBLL1 as a novel ROR1 interaction partner. COBLL1 expression in CLL cells correlates with patient's prognosis. Different expression in healthy tonsillar subpopulations suggests its physiological role in germinal centre transition.
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MONOCYTE/MACROPHAGE LINEAGE CELLS SIGNIFICANTLY INFLUENCE THE SURVIVAL AND PROLIFERATION OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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Background: The development and progression of Chronic Lymphocytic Leukemia (CLL) is dependent upon a complex microenvironmental network of cellular and molecular signals. As an example, the *in vitro* survival of CLL cells is supported by nurse-like cells, which have been identified as a CLL-specific tumor-associated macrophage (TAM) population. However little is known regarding the role of TAMs in CLL development and progression.
Aims: Aim of this study is to investigate to what extent and through which molecular mechanisms the interaction between CLL cells and TAMs influence the leukemic growth *in vivo* and, finally, to explore this cellular interaction for therapeutic purposes.
Methods: For *in vivo* studies we took advantage of different CLL mouse systems: i) the Eμ-TCL1 mouse model; the xenograft model we developed, based on the engraftment of MEC1 CLL human cell line into Rag2$^{-/-}$γc$^{-/-}$ mice which recapitulates the distribution of aggressive human CLL. To characterize the molecular repertoire of CLL infiltrating TAMs, a whole genome transcriptional profile analysis was performed, by using the Illumina direct hybridization system, directly on TAMs and leukemic cells purified from the bone marrow (BM) of Rag2$^{-/-}$γc$^{-/-}$, xeno-transplanted mice. We exploited a liposome-mediated macrophage killing approach, based on the liposome mediated internalization of clodronate (clodrolip) in macrophages for both *in vivo* and *in vitro* cytotoxicity studies performed in CLL animal systems and primary samples from CLL patients.
Results: We analyzed the whole-genome transcriptome of TAMs and leukemic B cells isolated from the bone marrow of MEC1-bearing mice and found an enrichment of genes involved in inflammation and interaction between TAMs and B cells. To unravel the functional role of TAMs in CLL development and progression, we transplanted Rag2$^{-/-}$γc$^{-/-}$, mice intravenously (i.v.) with MEC1 cells and depleted macrophages by i.v. clodrolip delivery. Macrophage depletion resulted in a drastically reduced accumulation of CLL cells in all tissues. Moreover, when C57BL/6 mice were transplanted with leukemic cells from Eμ-TCL1 transgenic mice and macrophage-depleted, significantly fewer leukemic B cells were detected in the lymphoid tissues analyzed. Interestingly, *in vitro* cytotoxicity studies performed on MEC1 cells as well as on human primary CLL cells proved that clodrolip have no direct toxic effect on leukemic B cells. However when unseparated PBMC of CLL patients were treated with different doses of clodrolip *in vitro*, we observed a marked depletion of both monocytes/macrophages and leukemic cells. Finally, to explore the possibility that manipulating leukemic cell/macrophage interactions might be exploited as a potential novel therapeutic strategy, Rag2$^{-/-}$γc$^{-/-}$ animals transplanted subcutaneously with MEC1 cells and carrying visible leukemic lymph nodes (LN) were injected with clodrolip in the proximity of the LNs. This treatment drastically reduced LN size and favourably impacted on the overall mouse survival.
Summary and Conclusions: In summary all these *in vitro* and *in vivo* observations clearly suggest that the survival and proliferation of CLL cells strongly depend on the support of monocyte/macrophage lineage cells. Our data open up novel therapeutic venues for CLL based on interfering with leukemic cell-macrophage interactions.

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HYPOXIA - A CENTRAL MICROENVIRONMENTAL DETERMINANT OF PROLIFERATION AND DRUG-RESISTANCE OF PRIMARY CLL CELLS

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Background: Although common *in vitro* set-ups tried to address the most relevant pro-survival stimuli (*e.g.* CD40L, BCR, IL-4) in CLL in the past, it remains still unknown *e.g.* why ABT-199 eradicates CLL cells from lymph nodes much more efficiently compared to fludarabine. Hypoxia as a general environmental variable is poorly understood so far. It is known that the physiologic oxygen tension in malignant lymph nodes and bone marrow is around 1% O_2. Moreover, we know from solid malignancies that oxygen tension tremendously affect proliferation and drug-response.
Aims: Therefore our aim was to elucidate the impact of hypoxia on physiology and drug-resistance of primary CLL cells.

Methods: We have established an *in vitro* model, which mimics hypoxic conditions in concert with established pro-survival stimuli (CD40L, BCR, CpGs), in order to understand the molecular basis of biology of CLL cells resident in the microenvironment. CLL cells were cultured up to seven days in hypoxia (1% O_2) or normoxia (21% O_2). Hypoxic conditions were validated by overexpression of HIF1α and miRNA-210.
Results: Hypoxia is known to protect malignant cells in solid cancers from chemotherapy. Surprisingly, we made similar observations, since classical DNA-targeting drugs like fludarabine were inefficient to kill CLL cells under hypoxic conditions. However, we identified ABT-737 and ABT-199, which affect mitochondrial integrity, to be even more efficient under hypoxic conditions compared to normoxia. In order to explain this discrepancy we investigated the expression of mitochondrial localized anti-/proapoptotic genes on mRNA and protein level. We show that the de-regulation Mcl-1 under hypoxic conditions is essential for ABT sensitivity. Our data reveal that Mcl-1 is regulated under hypoxic conditions by un-coupled phospho-proteomics. Importantly, genetic and pharmacologic approaches, which target phospho-kinases reverts ABT-sensitivity towards resistance and sensitizes CLL cells towards fludarabine in hypoxia. Moreover, we investigated the impact of hypoxia on proliferation of CLL cells. Surprisingly, our data uncover, that proliferation of primary CLL cells is controlled by BCR signaling, while survival is enhanced by CD40:CD40L interaction under hypoxia.
Summary and Conclusions: Our findings give answers on fundamental clinical observations regarding drug-response, proliferation and survival of CLL cells within the microenvironment. Hypoxic *in vitro* settings seem to reflect existing *in vivo* settings. Further improvement of novel *in vitro* models like ours will help us understand the complex *in vivo* situation.

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LENALIDOMIDE INDUCES A PRO-INFLAMMATORY PHENOTYPE IN NURSE-LIKE CELLS DERIVED FROM CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Lenalidomide is an immunomodulatory agent clinically active in CLL patients. The specific mechanism of action is still undefined, but includes the modulation of microenvironment. In CLL patients, nurse-like cells (NLCs) differentiate from CD14+ mononuclear cells and nurture/protect CLL cells from apoptosis. NLCs resemble M2 macrophages with potent immunosuppressive functions.
Aims: We investigated whether lenalidomide may interfere with the nursing and protective phenotype of NLCs.
Methods: Upon lenalidomide stimulation *in vitro*, actin polymerization, adhesion to HUVEC and migration were evaluated on CD14+ circulating monocytes isolated from CLL patients. NLCs were generated in presence or absence of lenalidomide: cell surface markers, phagocytosis and induction of T cell proliferation were analyzed after 10 days. In some experiments, we used Rho GTPases inhibitors.
Results: CLL-derived CD14+ monocytes showed higher actin polymerization levels after treatment with lenalidomide compared to unstimulated control. F-actin formation increased to 120%, 225% and 268% upon stimulation with lenalidomide 0.5μM, 1μM, 10μM compared to control (100%). The addition of specific GTPases inhibitors (RhoA, Rap1 and Rac1 inhibitors) strongly affected F-actin formation induced by lenalidomide treatment. When CD14+ monocytes were cultured on HUVEC endothelial layer, treatment with lenalidomide strongly stimulated the adhesion from 100% to 238% (±37%) ($p<0.05$). In accordance, lenalidomide increased Akt phosphorylation and ILK (integrin linked kinase) expression in CD14+ monocytes. Lenalidomide-induced adhesion was mediated through Rap1 and Rac1. Moreover, we investigated the ability of CD14+ monocytes to migrate in response to CCL2, CCL3, CXCL12 during stimulation with lenalidomide. Lenalidomide determined a reduction of 24%, 32% and 20% in monocytes migration in response to CCL2, CCL3 and CXCL12 respectively. These impairments were not determined by a reduction in monocytes viability or down-regulation of chemokine receptors, but we found an increased expression of CORO1B and a down-regulation of RhoH ($p<0.05$) as documented by real-time PCR and Western blot. Then, we focused our attention on CLL tissue monocytes, also known as NLCs, which showed a different and particular morphology *in vitro* after treatment with lenalidomide with higher FSC and SSC compared to untreated control. First, treatment with lenalidomide 0.5μM and 1μM increased the number of NLCs to 268% and 309% compared to untreated control respectively ($p<0.05$). At the same time, lenalidomide strongly increased CLL adhesion to NLCs to 227% and 212% with the addition of 0.5μM and 1μM doses (Figure 1A) inducing a strong expression of CD11b and MAC1 on NLCs surface. In this scenario, NLCs did not exert a pro-survival effect on CLL cells, in fact lenalidomide treatment reduced CLL viability in contact with NLCs from 54.2% to 44.5% ($p<0.05$). Of interest, we found that lenalidomide is able to interfere with leukemia-promoting properties of NLCs by increasing their phagocytic activity and improving NLCs-mediated T cell proliferation. In fact stimulation with lenalidomide increased the phagocytic activity of NLCs to 141% and 155% with 0.5μM and 1μM compared to control (100%) ($p<0.05$), and strongly improved theability of T cells to undergo proliferation from 19.5% to 35% (%of dividing cells) ($p<0.05$, Figure.1B).

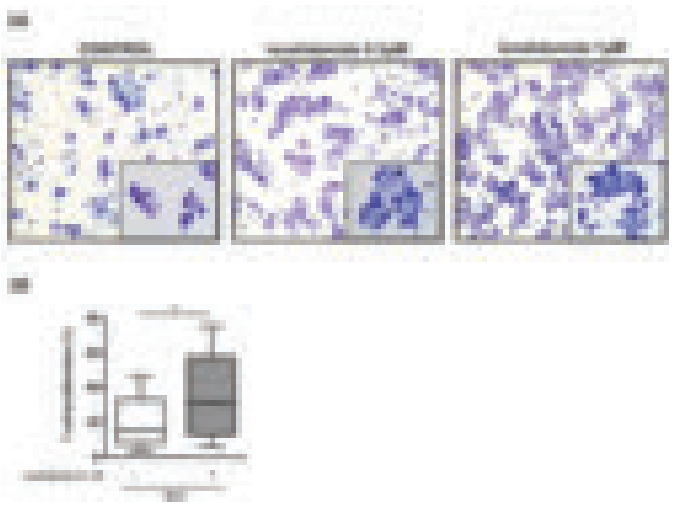

Figure 1.

Summary and Conclusions: Collectively, our data provide new insights into the mechanism of action of lenalidomide that interferes with the supporting and protective microenvironment generated by CLL into tissues.

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CLL DERIVED EXOSOMES ENCAPSULATE SPECIFIC MICRORNAS AND INFLUENCE STROMAL CELL BEHAVIOUR

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Background: Exosomes, nanometre-sized (40–100 nm) vesicles of endocytic origin, are vehicles of communication between tumour cells and their microenvironment. Exosomes are discrete lipid-bound vesicles that encapsulate cytoplasmic proteins and RNA (including microRNA). Uptake of exosomes results in altered expression of mRNA and proteins in recipient cells. Previous reports have highlighted the importance, and tumour-specific differences in exosomal cargo, in the pathophysiology and behaviour of individual cancers. Given the critical role of microRNAs in Chronic lymphocytic leukemia (CLL), the importance of CLL derived exosomes and their microRNA content in disease biology is not yet established.
Aims: In this study, we investigated secreted exosomes and cells from primary CLL samples for their microRNA content and sought to elucidate their effects on stromal cells.
Results: Having optimised a protocol for purifying exosomes derived from cultured CLL cells and patient plasma, validation of their physical characteristics was performed by Electron microscopy (EM) and Atomic force microscopy (AFM). Verification of the purity and integrity of the exosomes was achieved by flow cytometry and western blotting and further confirmed their derivation from CLL cells. MicroRNA expression profiling was performed on patient cellular RNA and exosomal RNA using Exiqon LNA arrays. qRT-PCR was used to further validate expression of differentially expressed selected microRNAs.. We show that, amongst other microRNAs, miR-202 is specifically enriched in CLL derived exosomes and down regulated in paired CLL cells. Importantly, miR-202 is down regulated in a larger cohort of primary CLL cells, compared to normal B cells, implying a tumour suppressive role. In co-culture experiments, using the normal human stromal cells (HS-5 cell line) and exosomes, and subsequent confocal microscopy we demonstrate uptake and co-localisation of CLL derived exosomes with Lamp-1 (a marker for late endosomes), verifying that the endocytosed exosomes are trafficked to endosomal organelles. Moreover, uptake of CLL exosomes by the stromal cells resulted in increased expression of genes such as c-fos and ATM, while the expression of 'suppressor of fused' (Sufu) (one of the validated targets for miR-202) was decreased. Finally, we show that CLL exosomes derived *in vitro* (culture medium) or *in vivo* (patient plasma) significantly increases cell proliferation in HS-5 compared with exosomes derived from normal plasma.
Summary and Conclusions: Our findings show that CLL derived exosomes indeed harbour unique molecular profiles and are enriched in miR-202. CLL exosomes modulate mRNA and protein expression in recipient stromal cells and alter cellular behaviour. Our study suggests an important role for exo-

somes, and their content, in CLL-microenvironment interactions to influence disease behaviour and outcomes.

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DIFFERENT CLL STEREOTYPED SUBSETS, VARYING TELOMERE LENGTHS: A MEASURE OF DISTINCT PROLIFERATIVE HISTORIES LINKED WITH UNIQUE CLINICOBIOLOGICAL FEATURES

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Background: Telomeres play a critical role in maintaining genomic integrity and are also implicated in the regulation of cell proliferation and senescence. Indeed, telomere length (TL) reflects the replicative history of individual cells with repetitive rounds of cell division and proliferation leading to telomere erosion at the ends of DNA. In CLL, short telomeres have been associated with unmutated IGHV genes, complex karyotype and inferior outcome, linking extensive replication histories with clinical aggressiveness.
Aims: Given the extreme heterogeneity of CLL, analysis of more homogeneous subgroups would provide an accurate view of TL function and hence its relevance for clonal behavior. Here we investigated TL in a series of 446 patients with CLL, particularly focusing on subsets of cases with stereotyped B-cell receptor immunoglobulins (BcR IG); members of which are increasingly shown to display similarities extending beyond IG sequences to clinicobiological profiles and outcomes.
Methods: TL measurements were performed on DNA isolated from diagnostic peripheral blood samples (>70% $CD19^+$ cells) by quantitative real-time PCR. Overall, TL was found to be highly variable. Using a telomere/single copy gene (T/S) threshold value of 1086 units (cut-off at the median) we divided the cohort into two subgroups based on long or short telomeres.
Results: In keeping with the literature, shorter telomeres were significantly ($p<0.001$) more frequent among cases with Binet stages B-C at diagnosis, unmutated IGHV genes and TP53 aberration (TP53ab) due to del(17p) and/or TP53 mutation. Within our cohort, 129/447 (29%) cases belonged to major stereotyped subsets, including both IGHV-unmutated (U-CLL) subsets #1 (n=49), 6 (n=6), 7 (n=5), 8 (n=8) and IGHV-mutated (M-CLL) subsets #4 (n=30), 16 (n=7), 77 (n=5) as well as subset #2 (IGHV3-21/IGLV3-21) with variable IGHV mutational status (n=19, of whom 14 concerned M-CLL). Overall, significant ($p<0.05$) differences were identified between subsets, however, average TLs for different subsets largely followed the general association with IGHV mutational status. In detail, among M-CLL, subset #16 cases had the longest telomeres (T/S=2464.8) followed by subset #4 (T/S=1858.3) and subset #77 (T/S=1840.6), thus differing from all U-CLL subsets that carried considerably shorter telomeres (T/S values: 718.3, 817.5, 703.9, 901.8, for subsets #1, 6, 7 and 8, respectively). Subset #2 exhibited intermediate TL between M-CLL and U-CLL (average T/S=1044.5), significantly ($p<0.001$) differing from the former. Remarkably, within subset #2, M-CLL cases had significantly shorter telomeres than U-CLL (average T/S values: 947.2 *vs* 1316.2; $p<0.0001$). Concerning possible clinical implications, significantly shorter time-to-first-treatment ($p\leq0.007$) was found for patients with Binet stages B-C, TP53ab, del(11q), U-CLL, subsets #1 and #2, as well as shorter TLs. However, TL did not retain prognostic significance for TTFT on multivariate analysis, where only clinical stage, TP53ab and U-CLL status emerged as independent negative prognostic factors ($p<0.001$).
Summary and Conclusions: In conclusion, we document different TLs for different CLL stereotyped subsets, indicating distinct replication histories shaped by subset-biased profiles of cell activation and apoptosis resistance due to both cell-extrinsic (*i.e.* antigenic activation) and cell intrinsic mechanisms (*i.e.* genetic defects). The finding of short telomeres in subset #2, even in IGHV-mutated cases, strongly alludes to intense proliferation in this highly aggressive CLL subset.

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SKEWING OF THE T-CELL RECEPTOR REPERTOIRE IN CHRONIC LYMPHOCYTIC LEUKEMIA: WHAT LIES BENEATH?

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a remarkably restricted immunoglobulin (IG) gene repertoire, mainly attributable to the existence of subsets of patients with stereotyped B-cell receptor (BcR) IGs, strongly implying clonal selection by a restricted set of antigens. Little information exists regarding the role of antigenic stimulation in the selection and activation of cognate T cells, yet this information would be highly relevant in light of the interactions of the malignant B cells with T cells.
Aims: Our preliminary studies of the T-cell receptor β chain (TRB) gene repertoire in 12 cases from CLL subset #4 revealed TRBV repertoire skewing in all cases and shared T-cell clonotypes among different patients. In the present study, we expanded our sample series so as to include cases belonging to other major subsets as well as cases with heterogeneous (non-stereotyped) BcR IGs.
Methods: In total, we analyzed 71 peripheral blood samples from 56 CLL patients, belonging to subset #1 (n=15 cases), subset #2 (n=15 cases) or to no known subset (n=6 cases), together with 20 subset #4 cases; samples from different time points were analyzed for 6 cases (5 subset #4 and 1 subset #1 case). No case had evidence of infection at sampling. PCR amplicons for TRBV-TRBD-TRBJ gene rearrangements (BIOMED2 protocol) were subcloned by transformation into E. coli/TOP10F bacteria and individual colonies were chosen randomly and subjected to Sanger sequencing. Only productive rearrangements (n=1310, range 5-77 per case) were included in the analysis.
Results: Forty-seven of 56 (84%) cases were found to carry clusters of identical rearrangements (≥2) corresponding to distinct clonotypes; the number of expanded clonotypes/case ranged from 1-15 (median 3). The frequency of the most expanded (immunodominant) clonotype/case ranged from 4-80% (median 17.4%). Collectively, the frequency of all expanded clonotypes/case ranged from 10.7-88.8% (median 43.5%). In 2/6 cases where samples from different time points were analyzed, at least one clonotype was found to persist (both cases belonged to subset #4). The TRBV gene repertoire exhibited remarkable restrictions, with 6 genes (namely TRBV12-3, TRBV19, TRBV20-1, TRBV27, TRBV6-5 and TRBV7-9) accounting for almost half of the cohort (46.5%). The exact same genes dominated the TRBV repertoire of clonotypes (45.6%). Similar results were obtained at the individual subset level since these genes were found among the most frequent within each subset, as well as within heterogeneous rearrangements (only unique rearrangements were included in repertoire analyses). In terms of TRB CDR3 sequence analysis, we identified 4 "public" clonotypes shared by 4 pairs of different patients; interestingly, 2/4 pairs of patients with identical clonotypes did not belong to the same subset. In addition, 4 highly similar clonotypes (>85% sequence identity) were identified between different patients.
Summary and Conclusions: In conclusion, the present study, by far the largest to-date, provides clear molecular evidence of TRBV repertoire skewing in CLL, strongly supporting antigen selection of T cells. The finding of "public" clonotypes together with the similar distribution of TRBV genes among CLL patients, regardless of whether they belong to stereotyped subsets or not, may suggest that the T cell driving antigens could include epitopes implicated in the selection of the malignant B cells and/or CLL-cell (tumor-) derived epitopes. The exact functional role of clonally expanded T-cells remains to be elucidated.

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BIOLOGICAL AND CLINICAL RELEVANCE OF MIRNAS INDUCED IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS DURING ADMINISTRATION OF THERAPY *IN VIVO* AND *IN VITRO*

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Background: MiRNAs are known to be involved in the pathogenesis of CLL and affect its clinical course. However, their function in the apoptotic pathways in CLL B-cells, and their roles in primary and/or acquired resistance to therapy are unclear.
Aims: The aim of this study was to reveal miRNAs changed with apoptotic response of CLL cells and to test their role in response to FCR treatment *in vivo*.
Results: To screen for DNA damage-regulated miRNAs we used RNAs isolated from patients (pts.) B-cells (purity>95%, CD5+19+) treated *in vitro* with fludarabine dose of ~LC50 (3.5 μg/ml; 48hrs). Five paired samples (n=10) were analyzed for miRNAs expression each using 2 next-generation sequencing (NGS) approaches (SOLiD; HiSeq). Obtained sequences were mapped to miRBase and miRNA changes were analyzed by a pair-wise comparison with edgeR and baySeq packages (Bioconductor). The overlap of 2 NGS sequencing platforms analyzed by the 2 mentioned statistical approaches identified 6 miRNAs significantly changed with DNA damage (FDR<0.1). HiSeq validation on 5 additional independent paired samples (n=10) confirmed changed expression of all 6 miRNAs (3 down-, 3 up-regulated). We further screened the expression of the two most constantly up-regulated miRNAs, namely miR-34a and miR-1246, in a

cohort of pts. (n=40) treated with fludarabine, cyclophosphamide and rituximab (FCR) regimen *in vivo*. We observed significant induction of miR-34a and miR-1246 at 48 hrs post FCR administration (fold induction [FI]=1.8; p<0.001; FI=2.2; p=0.01, respectively). Interestingly, the basal miR-34a levels before the therapeutic intervention were able to distinguish pts. with more aggressive disease. The pts. with low miR-34a expression (lowest quartile) had a shorter time to treatment failure (1.04 yrs. *vs.* 2.16 years; p=0.002; HR=4.3; 95% CI=1.68-10.98) and a remarkably shorter time to relapse after FCR-achieved remission (1.28 yrs. *vs.* not reached; p=0.05; HR=3.07; 95% CI=0.97-9.65). We further screened the expression of miR-34a in 158 CLL pts. using an in-house designed assay for its absolute quantification. We defined a cut-point (copies of miR-34a) that segregates pts. with extremely unfavorable prognosis (overall survival [OS] 1.37 yrs. *vs.* not reached; p=0.0001; HR=3.89; CI=2.05-7.39) and this was independent of other prognostic markers (FISH, IgHV, age, sex) in a multivariate analysis. We have previously described that low miR-34a levels associate with p53 mutations, and low-miR-34a identified such CLL cases with a sensitivity and specificity of 0.75 and 0.91, respectively. In an OS multivariate analysis limited to wt-*TP53* samples (n=116) miR-34a was the strongest predictor of OS (1.29 yrs. *vs.* not reached; p=0.002; HR=9.82; CI=2.30-42.05).

Summary and Conclusions: We have defined that FCR-induced miR-34a can be used as a strong predictive and prognostic marker. We have newly identified other 5 miRNAs involved in DNA damage response in CLL (including miR-1246). The investigation of the biological and prognostic role of miR-1246 and other miRNAs is ongoing. These miRNAs likely have convergent functions since the DNA damage induced miRNAs share >50 predicted targets with miR-34a, and thus could affect therapy response and aggressiveness of CLL. This is currently being investigated using integrated analysis of miRNA and mRNA profiling.

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P203

INTRA-PATIENT CLONAL AND SUBCLONAL HETEROGENEITY OF CHRONIC LYMPHOCYTIC LEUKEMIA: EVIDENCES FROM CIRCULATING AND LYMPH NODAL COMPARTMENTS

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Background: In lymph nodes (LN), the crosstalk with accessory cells prevents chronic lymphocytic leukemia (CLL) cells from apoptosis and enhances their proliferation. A better knowledge of the biology and trafficking of CLL cells derived from different compartments, *i.e.* peripheral blood (PB) and LN, could help to elucidate the effects of tumor-host interactions *in vivo*, critical for the maintenance and expansion of the neoplastic B-cell clone.

Aims: To evaluate if CLL cells resident in the LN compartment and those simultaneously circulating in the PB show different genomic features according to the site of origin.

Methods: We performed Whole-Exome Sequencing (WES, Agilent SureSelect Human All Exon 50Mb, Illumina HiSeq 2000) and Copy Number Aberration (CNA) detection (CytoScan HD array, Affymetrix) on genomic DNA extracted from paired PB, LN and saliva samples of 9 untreated CLL patients. Cases were selected on the basis of: *i)* availability of CLL cells simultaneously extracted from PB and LN biopsies; *ii)* $CD5^+/CD19^+$ cells in PB and LN >70%; *iii*) absence of Richter syndrome and *iv)* availability of germline material (saliva). Candidate mutations were verified by Sanger sequencing, that was also used to determine their distribution in the two compartments. The Roche GSJ 454 platform was utilized to determine the allele variant frequency (VF) in each compartment for all the identified mutations.

Results: Sanger validation of WES data identified 64 nonsynonymous somatic mutations in 60 genes, with the following distribution: 53 (82.9%) present in both compartments, 4 (6.2%) specific of PB, 7 (10.9%) specific of LN. Six of the 7 mutations specific of the LN compartment (*ZNF215, KIF26B, POLR2A, DAPK1, WLS, SF3B1*) were identified as circulating subclones (VF 0.5-5.3%) in the corresponding PB compartment by an ultra-deep sequencing approach (10,000X coverage); similarly, 2 (*ABCC9, MUC5B)* of the 4 PB specific mutations were found in the corresponding LN (VF 1.3-9.6%). CNAs (82.4% losses and 17.6% gains) consisted of 41 lesions: 27 (65.8%) present in both compartments, 7 (17.1%) specific of PB and 7 (17.1%) specific of LN. An inter-patients and intra-patient heterogeneity in the load and distribution of mutations and CNAs was evident. Two were the most informative cases: case #10 (2 mutations and 4 CNAs specific of LN) and case #100 (2 mutations and 7 CNAs specific of PB; 5 mutations and 1 CNAs specific of LN). They were further characterized in the PB of a subsequent relapse. In case #10, the LN specific *ZNF215* mutation expanded from 3.6% to 27.15%, and the previously LN specific CNAs - del8(p23.3-p11.1), del9(p24.3-p13.1), gain 2(p25.3-p14) - appeared in the PB at disease relapse. In case #100, the LN specific *SF3B1* mutation expanded from 5.93% to 44.46% and the PB specific *ABCC9* and *MUC5B* mutations disappeared in the PB at disease relapse. In addition, del11q was larger (19.4 Mb) than what previously detected in the PB (0.3 Mb, including *ATM*) and included *BIRC3*, similarly to the lesion documented in the LN (36 Mb including both *ATM* and *BIRC3*).

Summary and Conclusions: A subset of untreated CLL patients with LN involvement shows a specific pattern of mutations and CNAs in LN-derived tumor cells compared with the corresponding circulating counterpart, providing evidence of an intra-patient clonal heterogeneity according to the disease compartment. The appearance in the PB at relapse of LN-derived mutations and CNAs supports the notion that the LN microenvironment contributes to CLL cell proliferation and selection of clones carrying unfavorable lesions.

P204

OVERCOMING RESISTANCE TO THE NOVEL BH3-MIMETIC ABT-199 IN A CLL LYMPH NODE MODEL; THE ROLE OF ABL AND BTK

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Background: Chronic lymphocytic leukemia (CLL) cells are highly sensitive to the Bcl-2-selective BH3-mimetic ABT-199. In patients, ABT-199 induces clear reductions in peripheral blood lymphocyte counts and lymph nodes (LN) size. Yet in LNs, pro-survival signals can upregulate Bcl-2 members that are not targeted by ABT-199, and this may induce (partial) resistance.

Aims: Here, we analysed sensitivity to ABT-199 in a simplified CLL LN model, using *in* vitro CD40 stimulation.

Results: Prolonged CD40 stimulation resulted in full resistance to 10μM ABT-199, due to strong induction of Bcl-XL, Mcl-1 and Bfl-1. These CD40-mediated effects could blocked by the broad spectrum kinase inhibitor dasatinib. Using interaction proteomics, Abl and Btk were identified as dominant targets of dasatinib in primary CLL. Like dasatinib, the Abl inhibitor imatinib, but not Btk inhibitor ibrutinib, could overcome resistance for BH3-mimetics. Conversely, BCR- and chemokine-controlled adhesion could be abolished by dasatinib and ibrutinib, but not by imatinib.

Summary and Conclusions: Our *in vitro* data suggest that long-term clinical application of ABT-199 in CLL might select for resistant clones at protective niches. This may be overcome by combination treatment with kinase inhibitors that prevent emergence of resistance (Figure 1).

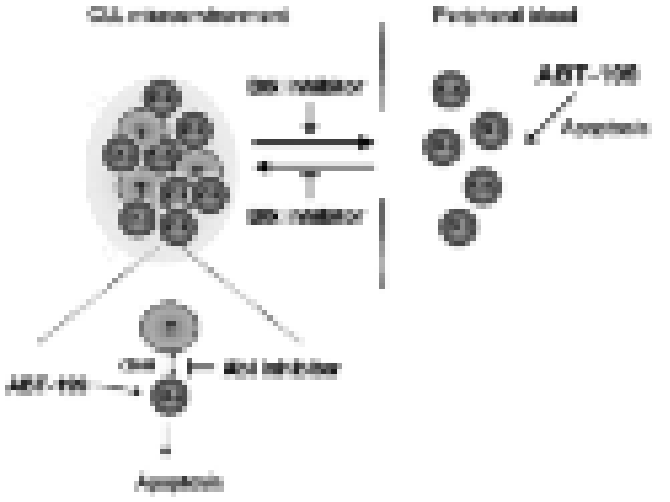

Figure 1.

P205

TARGETING NOTCH2 SIGNALING BY GLIOTOXIN INDUCES APOPTOSIS IN CLL CELLS BY A MECHANISM INVOLVING NOTCH3 AND NR4A1

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Background: Chronic lymphocytic leukemia (CLL) cells express constitutively activated NOTCH2 in a protein kinase C (PKC) dependent manner linking NOTCH2 to the activated state of the leukemic cells. The transcriptional activity of NOTCH2 is associated with the expression of the B-cell differentiation/acti-

vation marker CD23 and CLL cell viability. Moreover, *NOTCH1* gain of function mutations are found in a subset of CLL cases and have an adverse prognostic impact. We have recently shown that the NOTCH2 transactivation inhibitor gliotoxin (WO 2006/135949) induces apoptosis in CLL cells.
Aims: The aim of this study was to clarify the regulation and possible functions of the individual NOTCH family members (NOTCH1-4) in the apoptotic response of CLL cells to gliotoxin.
Methods: Twenty CLL patients, including all prognostic subtypes and four cases with *NOTCH1* gain of function mutations were enrolled in this study. Dose and time dependent effects of gliotoxin on CLL cell viability and on the induction of apoptosis was determined by MTT-assays and by FACS analysis, respectively. The expression of NOTCH and apoptosis related genes was determined by RT-PCR.
Results: Gliotoxin inhibited cell viability and effectively induced apoptosis (IC_{50} between 0.2μM and 0.5μM) in CLL cells after 3 days of incubation. NOTCH1 mutational status had no significant impact on the sensitivity of CLL cells to gliotoxin. At the molecular level, short term (4 hours) exposure of CLL cells revealed that *NOTCH1* was equally transcribed in unstimulated and in PMA activated CLL cells. *NOTCH2* was upregulated in PMA activated CLL lymphocytes whereas *NOTCH4* was only weakly detectable in unstimulated CLL cells. Gliotoxin treatment resulted in the downregulation of *NOTCH1, NOTCH2* and *NOTCH4* mRNA expression. Interestingly, the inhibition of NOTCH2 activity by gliotoxin was associated with the concomitant induction of NOTCH3 signaling. This was indicated by the induced mRNA expression of *NOTCH3,* the NOTCH ligands *JAG2* (in unstimulated CLL cells) and *DLL1* (in PMA activated CLL cells), and the NOTCH3 preferred target gene *HEY1*. Moreover, the induced transcription of *HEY1* correlated with enhanced *NR4A1* gene activity, a key regulator of activation induced cell death (AICD) in lymphocytes. These data may thus point to a pro-apoptotic role for NOTCH3 mediated *NR4A1* regulation in CLL cells. In line with this hypothesis, the inhibition of all NOTCH receptors with the the γ-secretase inhibitor (GSI) DAPT led to downregulation of *NR4A1* and counteracted the pro-apoptotic effect of gliotoxin.
Summary and Conclusions: In summary, the data suggest that gliotoxin may selectively target the anti-apoptotic arm of NOTCH signaling irrespective of the NOTCH1 mutational status in CLL cells. Therefore, the individual NOTCH receptors may have opposite effects on CLL cell viability which should be considered in therapeutic approaches aimed to target NOTCH signaling in CLL.

P206

IMMUNOHISTOCHEMICAL ANALYSIS OF IL-6, IL-8/CXCR2 AXIS, TYROSINE P-STAT–3 AND SOCS-3 IN CLL LYMPH NODES: CORRELATION WITH MICROVASCULAR CHARACTERISTICS, CLINICAL FEATURES AND PROGNOSIS

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Background: A number of studies pursued during the last few years have looked into the potential pathophysiological role of angiogenesis in CLL but the results have often been inconsistent.
Aims: To: evaluate simultaneously the immunohistochemical expression of pro-angiogenic factor VEGF, cytokine IL-6, chemokine IL-8 and its receptor CXCR2 along with tyrosine p-STAT–3 and SOCS-3 within and outside the proliferation centres (PCs) of lymph nodes, examine the relationships of these molecules with microvascular characteristics of lymph nodes and determine their prognostic significancy.
Methods: Immunostaining for IL-8, IL-6, CXCR2, SOCS-3, tyrosine p-STAT-3 and VEGF was performed on paraffin-embedded 4μm sections inlymph nodes of 62 CLL patients. The microvascular characteristics were evaluated using CD34. A Histo-score (H-score) based on the percentage of stained neoplastic cells multiplied by staining intensity was calculated.
Results: Microvessel density, major axis length, minor axis length, area, perimeter, shape factor and Total vascular area were higher in the PCs when compared to the non-PC areas.IL-8 and CXCR2 expression were detected only in 5/51 (9.80%) 7/51 (13.21%) of the examined cases respectively, whereas IL-6 expression was detected in 49/51 (96.08%). Tyrosine p-STAT-3 expression was nuclear and was detected in 36/51 (66.67%) of the examined cases. In 21/35 (66.67%) positive cases immunoreactivity was mainly observed in the PCs. SOCS-3 expression was cytoplasmic and was detected in 34/35 (97.1%) of the examined cases. VEGF expression was cytoplasmic and was observed in all the examined cases (41/41, 100%) cases. Further on,VEGF H-score was negatively correlated with MVD and shape factor, whereas VEGF H-score within the PCs was unrelated to the microvessels characteristics in the same area (p>0.10) and it was negatively correlated with Binet stage Tyrosine p-STAT-3 positivity (p=0.0303) and increased VEGF H-score (p=0.0205) were correlated with increased TTP (median TTP for p-STAT-3 positivity 137.766 months *vs* 58.26 and median TTP for increased VEGF H-score 100,3 months *vs* 42,36). The former relationship remained significant in multivariate survival analysis (HR=0.202, p=0,024).
Summary and Conclusions: The present study constitutes the first attempt to elucidate the significance of angiogenesis and proangiogenic cytokines, IL-6, IL-8 and VEGF along with tyrosine p-STAT-3 and SOCS-3 in lymph nodes involved by CLL. Both the extent of microvasculature and microvessel caliber were significantly lower within the PCs as compared to the non-PC area.Tyrosine p-STAT-3 positivity emerged as an independent favourable prognosticator in terms of time to progression.

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EXPRESSION AND FUNCTIONAL ACTIVITY OF THE TNFR-SUPERFAMILY MEMBER DEATH RECEPTOR 3 IN CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS ACTIVATED BY THE BCR

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Background: Growing evidences suggest a dynamic balance of B-cell chronic lymphocytic leukemia (B-CLL) cells between the blood and lymphoid tissues, which represent permissive niches for cell proliferation and survival. Within lymphoid tissue sites, molecules of the tumor necrosis factor (TNF) superfamily have been shown to play a supportive role and contribute to the pathogenesis of B-CLL. Thereby, investigating expression and functions of novel TNFR-superfamily members in B-CLL would allow us to gain deeper insights into molecular crosstalk within leukemic microenvironments. Death receptor (DR) 3 is a TNFR-superfamily member expressed in lymphocyte-enriched tissues that binds to the TNF-like ligand 1A (TL1A), a TNF superfamily member. The TL1A/DR3 axis is implicated in regulatory mechanisms of adaptive immune response under physiological and pathological settings. Further evidence for the implication of TL1A in regulatory mechanisms arises from our recent data showing an inhibitory function of TL1A on B-cell proliferation. In leukemia cells, DR3 expression and functions have not been explored so far.
Aims: Our aims were to investigate the expression of DR3 in B cells from B-CLL patients and to explore the possible role of TL1A/DR3 axis in the disease.
Methods: B cells were purified from PBMC of 37 B-CLL patients by negative selection with magnetic beads. DR3 surface expression was measured by flow cytometry at baseline and following stimulation with F(ab')2 anti-human IgM conjugated to latex microspheres. DR3 expression was confirmed by western blot and immunofluorescence analysis on B-CLL lymph nodes. DR3 function was studied by MTT assay and Annexin V assay. TL1A serum levels were measured by ELISA.
Results: Under basal conditions CLL B cells *in vitro* expressed low levels of DR3. Stimulation of the B cell receptor (BCR) with anti-IgM antibodies induced a statistically significant increase of DR3 expression in CLL B cells ($p<0.001$). Induced DR3 expression showed great variability amongst B-CLL cells (variance (σ^2)=6.38). Flow cytometry data were confirmed by Western blot analysis. The relevance of these findings was further confirmed by immuofluorescence analysis of B-CLL lymph-node specimens showing that i*n vivo* high levels of DR3 were expressed by a number of B-CLL cells. To assess whether the anti-IgM-induced DR3 molecule was functionally active in CLL B cells, we examined the ability of DR3 to modulate their metabolic activity. Stimulation of DR3 with TL1A in the presence of BCR engagement showed that TL1A induced an equal or greater than 25% decrease of metabolic activity in 37.5% of B-CLL cell samples. In these samples the modulation is not due to reduced survival, as assessed by Annexin V assay. No change in metabolic activity was observed following TL1A treatment, in the absence of anti-IgM. Higher levels of DR3 expression were significantly associated with early-stage (Rai 0) disease (p=0.019) and higher serum levels of TL1A were significantly associated with early-stage (Rai 0) disease (p=0.023) and absence of CD38 expressions (p=0.035).
Summary and Conclusions: In this study, we describe for the first time that B-CLL cells activated by the BCR stimulation express DR3 and TL1A reduces metabolic activity in some B-CLL cells. Herein, our data show a novel regulatory role for TL1A that, in the presence of antigen stimulation, may modulate leukemic cell metabolism through DR3 in early-stage B-CLL and suggest that homeostatic functions of TL1A may influence the clinical course of B-CLL disease.

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VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) INCREASES CELL MIGRATION THROUGH INTERACTION WITH THE CXCR4/SDF1 AXIS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Background: Vascular endothelial cell growth factor (VEGF) is a multifunction-

al cytokine involved in angiogenesis and development. In B-CLL, it is known that malignant cells possess VEGF receptors and secrete VEGF. Patients with advanced stage have higher levels of VEGF, so it is probably involved in tumor progression. In other cell lines, VEGF is a major stimulating factor for migration, survival and proliferation and its action is mediated through another G-coupled protein system, CXCR4/SDF-1.
Aims: In the present study, we analyzed the role of exogenous VEGF on B-CLL migration as well its relationship with the G-coupled proteins CXCR4, CXCR7 and CD49d.
Methods: We obtained peripheral blood mononuclear cells from 33 patients diagnosed of CLL according to established clinical and laboratory protocols. We also used the CLL cell line Mec-1 and human umbilical vein endothelial cells (HUVEC). B-lymphocytes were purified by Ficoll-Hypaque density gradient centrifugation anti-CD19 conjugated Dynabeads. Then we analysed by flow cytometry the expression of CXCR4, CXCR7 and CD49d before and after exogenous VEGF exposure, VEGF-R2 inhibitor or both. In order to evaluate the role of VEGF in the motility of these cells, we performed an *in vitro* 6h transmigration assay towards a media containing (or not) SDF-1. B-CLL ($5x10^5$ cells) cells were incubated on the upper chamber of transwell filters coated with HUVEC in the presence or absence of VEGF [50 ng/ml] 24h, VEGFR2/KDR inhibitor [70nM] or both simultaneously. Migration ratio was given as mean±SD between B-CLL cells treated with VEGF, VEGF-R2 inhibitor *versus* control. Statistical analysis was performed by non-parametric Wilcoxon test using SPSS statistical software (version 19.0). We also studied the expression CXCR4 and p-CXCR4 using confocal laser scanning microscopy. Mec-1 cells were cultured in the presence of VEGF [50 ng/ml] for 24h or absence. The cells were fixed in 3.7% paraformaldehyde and permeabilized by 0.2% triton X-100. The slides were blocked in 5% serum and incubated with primary antibodies CXCR4 and p-CXCR4 overnight at 4ºC and stained with corresponding secondary antibodies.
Results: Basal CXCR4, CXCR7 and CD49d expression levels of B-CLL cells were highly variable among the 33 patients analyzed. Mean fluorescent intensity (MFI) of CXCR4 expression was significantly higher on cells treated with VEGF *versus* untreated cells (9.64±95, p=0.028). However, we did not detect a significant difference in the percentage of cells expressing this receptor. On the other hand, VEGF treatment did not influence either MFI or the number of CXCR7 and CD49d expressing cells. Exposure to a VEGFR2 inhibitor reduced the percentage of cells expressing CXCR4 and CXCR7, suggesting a potential regulatory role of this receptor in the expression of these chemokines. Concerning cell migration, we observed a significant increase of Mec-1 and B-CLL cells treated with VEGF *versus* the control (27.66±69.97 p=0.03). Furthermore, the treatment with VEGFR2 inhibitor reduced significantly the migration index (-23.18±33.5, p=0.001) and the motility was restored by the addition both, VEGF and R2-inhibitor (36.04±39.33, p=0.001). Finally, we observed and increased fluorescent signals of both, CXCR4 and p-CXCR4 in Mec1 treated with VEGF *versus* controls (Figure 1).

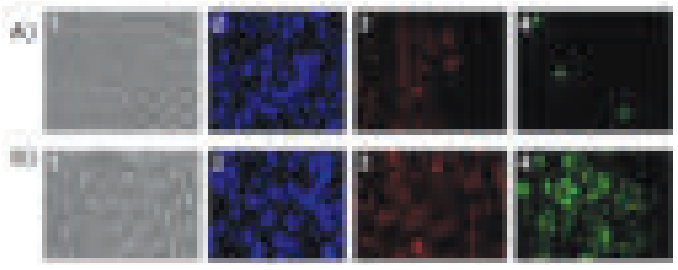

Figure 1. Fluorescent signals from CXCR4- Alexa fluor 647 (3) as well as from Phospho-CXCR4- Fluorescein goat anti rabbit IgGa (4) detected by immunofluorescence were analyzed by confocal microscopy. Nuclei were counter strained with Hoescht 33258 (5mg/100ul). A) Cell line MEC-1 control. B) Cell line MEC-1 treated with VEGF (50 ng/ml) for 24h.

Summary and Conclusions: These preliminary data suggest that VEGF seems to be involved in B-CLL migration through the up-regulation of CXCR4 levels and it also interacts with other g-protein receptor coupled sinaling pathways. New therapeutic strategies focussed on blocking both the SDF1-CXCR4 axis and/or VEGF pathway could have a potential therapeutic role in the treatment of this entity. Detailed molecular mechanisms implicated in this process should be further studied.
This work has been supported by grants FIS PI06/1637 and PI10/02095

P209

ANALYSIS OF IMMUNOGLOBULIN GENE REARRANGEMENTS IN CHINESE AND ITALIAN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: In Asia, chronic lymphocytic leukemia (CLL) incidence is 5-10 times lower than in the Western world and does not change in Asian residents in the US and in their descendants. The underlying environmental and genetic bases remain obscure. Previous data seem to suggest a different distribution of immunoglobulin heavy chain variable (IGHV) gene families between Asian and Caucasian CLL.
Aims: To characterize IGHV-D-J rearrangements and stereotype of the HCDR3 region of a series of Chinese CLL and compare them to those present in Italian CLL.
Methods: CLL cells from 399 Chinese patients at presentation (319 from China, 80 from Hong Kong) and from two Italian cohorts have been analyzed. IGHV-D-J gene rearrangements were analyzed by the IMGT database (http://imgt.cines.fr). We evaluated: IGHV-D-J gene usage and IGHV mutational status; HCDR3 region by the ClustalW2 software (http://www.ebi.ac.uk). Stereotyped HCDR3s were defined according to published criteria.
Results: Of the 399 Chinese patients, 38.6% were IGHV unmutated (UM) and 61.4% mutated (M). The IGHV family distribution was as follows: VH3 (49.9%), VH4 (27.6%), VH1 (14.3%), VH2 (3%), VH5 (2.2%), VH6 and VH7 (1.5% each). The most representative IGHV genes were: V4-34 (10.5%), V3-23 (9.8%), V3-7 (9.8%), V4-39 (6%), V1-69 (5.3%), V4-59 (5%), V3-30 (4.3%), V3-74 (3.7%), V3-33 (3.2%), V3-48 (3%), V3-21 (3%), V5-51 (2%), V1-2 (1.7%). We compared the Chinese series with two Italian CLL cohorts: i) 579 unselected patients (49.4% UM, 50.6% M), comparable with those reported by Agathangelidis *et al.*, 2012; ii) 329 patients at diagnosis (38% UM, 62% M, similar to the Chinese series). From the first comparison, the frequency of VH1 in Chinese CLL was lower (p=0.0001) and that of VH4 was higher (p=0.01). V1-69 (p=0.0001), V1-2 (p=0.002) and V3-30 (p=0.04) were underrepresented in Chinese CLL, whilst V4-59 (p=0.003), V3-7 (p=0.03) and V4-39 (p=0.06) were more recurrent in Chinese than Italian CLL. Regarding IGHD, in Chinese CLL, the DH3 (40.8%) was the most frequent followed by DH6 (20.3%) and DH2 (13.5%), with a higher representation of DH6 (p=0.0034). D3-3 (p=0.0009) was lower, whilst D6-13 (p=0.0028) and D3-10 (p=0.006) were higher in Chinese CLL. We then examined the 329 Italian CLL at diagnosis, in order to avoid selection biases. VH1 was again lower in Chinese CLL (p=0.01); V1-69 (p=0.003), V1-2 (p=0.01), V3-30 (p=0.05) and D3-3 (p=0.005) were underrepresented in Chinese CLL, whilst V4-39 (p=0.04), DH6 (p=0.01), D6-13 (p=0.003) and D3-10 (p=0.046) were more recurrent in Chinese than Italian CLL. No difference was found in the IGHJ families. The proportion of known stereotyped receptors was significantly lower in Chinese (14.4%) than in Italian CLL (31.4%) (p=0.0001), although a significantly higher frequency of subset #8 was observed in Chinese (10.4% of stereotyped cases) compared to Italian CLL (1.4%) (p=0.0095). Moreover, 6 new paired clusters were identified among Chinese cases.
Summary and Conclusions: Our data show a different IGHV and IGHD gene usage between Chinese and Italian CLL. Of interest is the low frequency of V1-69, V1-2, V3-30, D3-3 and the high frequency of V4-39, DH6, D6-13, D3-10 in the Chinese series. In addition, a low representation of stereotyped receptors was identified among Chinese CLL, although with a high frequency of subset #8. By extending this study to other Chinese collaborators, it will be possible to corroborate these results and identify potential new subsets of Chinese CLL. These data could ultimately translate into pathogenetic hypotheses for Chinese CLL.

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BASAL CA2+ SIGNALING IS PARTICULARLY INCREASED IN MUTATED CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) patients can be divided on the basis of the somatic hypermutation (SHM) status of their B-cell antigen receptor (BCR) genes into unmutated CLL (U-CLL) or mutated CLL (M-CLL) subgroups. Furthermore, approximately 30% of CLL patients express a stereotypic BCR, while the other 70% express a heterogeneous receptor. The existence of these stereotyped B cell receptors (BCR) in different CLL patients is interpreted as an indication for specific antigenic stimulation driving CLL pathogenesis. However, it was recently reported that CLL is driven by (higher basal) autonomous signaling based on Ca^{2+}, influx assays instead of antigen-dependent signals. This autonomous signal is induced by the immunoglobulin heavy chain complementarity determining region 3 (HCDR3) of the CLL BCRs, which are supposed to recognize an internal epitope within framework 2 (FR2) of the BCR. We hypothesized that these seemingly contrasting observations may not be mutually exclusive, and that the level of cell-autonomous signaling may differ between CLL subgroups.
Aims: Our aim is to determine whether basal signaling is different between CLL subgroups.

Methods: From our cohort of well-defined CLL samples consisting of cases belonging to specific stereotypic CLL subsets and heterogeneous CLL, we selected representative samples of stereotypic or heterogenous U-CLL and M-CLL cases (n=68). From these MNC samples, CLL B cells were untouched isolated by MACS. As controls we used MNCs from healthy individuals over 50 years of age (n=14). After loading the cells with labeled Ca^{2+} indicators Fluo-3 AM and Fura Red AM, we investigated BCR signaling by use of Ca^{2+}, influx assays on a LSR II flow cytometer.
Results: Our results show variation in basal BCR signaling between B cells of healthy individuals and CLL cells from stereotypic and heterogeneous U-CLL and M-CLL cases. We confirmed that basal Ca^{2+} signaling in CLL cells is higher than in normal B cells, but we also found that basal signaling is particularly high in M-CLL subgroups and to a lesser extent in U-CLL subgroups. The degree of basal signaling was not correlated with BCR characteristics like membrane Ig levels, HCDR3 length, or HCDR3 charge. Furthermore, the expression levels of CD5 (negative BCR-regulator) did not influence basal Ca^{2+} levels. Additionally, within our cohort we could not detect mutations leading to amino acid change within the earlier found FR2 epitope known to induce autonomous BCR signaling in CLL, which could influence our observed variation in basal Ca^{2+} levels.
Summary and Conclusions: Basal Ca^{2+}, signaling in CLL samples is higher compared to healthy controls. The Ca^{2+} levels were not uniformly high in all samples, but correlated to the IGHV SHM status. U-CLL showed marginally increased Ca^{2+} levels, whereas M-CLL samples had the highest basal Ca^{2+} levels. We therefore conclude that the level of basal Ca^{2+} signaling is not uniformly enhanced in CLL B cells, and speculate that this may depend on the degree of anergy of these cells.

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SRC INHIBITORS DOWNREGULATE CD20 AND MODULATE THE ACTIVITY OF THE CD20 PROMOTER

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Background: The monoclonal antibodies against CD20 antigen (rituximab and ofatumumab) have been developed and used in clinic as a therapeutic strategy in B-cell malignancies. These antibodies eliminate B cells by triggering indirect effector mechanisms of the immune system, namely complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), or immunophagocytosis. Although for many years CD20 has been described as a stable antigen, accumulating evidence indicates that CD20 can be modulated at both transcriptional and posttranscriptional levels. It seems that down-regulation of CD20 levels is one of the reasons of tumor resistance to rituximab. We have previously discovered that CD20 expression is strictly dependent on the activity of BCR signaling pathways. However, to the best of our knowledge, the role of SRC family kinases (SFKs) in the regulation of CD20 expression has not been studied so far.
Aims: The aim of this study was to explore the molecular mechanisms of SFK-dependent CD20 regulation in B-cell neoplasms.
Methods: CD20 surface level, rituximab-mediated CDC (R-CDC) and ADCC in CD20-positive lymphoma cell lines and primary cells from patients were determined with flow cytometry. Total CD20 protein levels were assayed with Western blotting, the expression of CD20 gene was determined with qRT-PCR. The CD20 promoter activity was measured with reporter *Firefly* luciferase assay.
Results: Initial experiments showed upon treatment with SFKs inhibitors a significantly reduced binding of anti-CD20 mAb to lymphoma cell lines as well as primary cells isolated from patients. All tested SFKs inhibitors, as well as shRNA targeting Lyn, Fyn or Lck kinase impaired CDC and ADCC over a dose range of rituximab concentrations (1-100 ug/ml) in Raji cells. Interestingly, in Raji cells incubated for 48h with dasatinib we also observed a dose-dependent reduction of total CD20 protein levels, when assayed by Western blotting. Moreover, quantitative PCR analysis revealed that the transcriptional regulation is the major mechanism responsible for the reduction of CD20 level upon dasatinib treatment. Consistently, the exogenously expressed CD20 under the control of CMV promoter was not sensitive to dasatinib treatment. To further elucidate the mechanism of transcriptional regulation of CD20 we performed the luciferase assays to estimate the activity of CD20 promoter and its truncated forms. Dasatinib or AKT kinase inhibitor (MK-2206) strongly decreased the activity of CD20 promoter, while the overexpression of CA-AKT partially blocked the inhibition caused by dasatinib. Using the truncated versions of the CD20 promoter we found that lack of the region (-313/-198) made the promoter unsensitive to dasatinib treatment. Since this particular region is known to contain a putative Octamer transcription factor binding site, we introduced mutations in the BAT-box sequence. Although basal promoter activity was indeed decreased, dasatinib was equally effective in reducing the activity of both wild-type and mutated CD20 promoter.
Summary and Conclusions: The most important finding of this study is that SFKs inhibitors strongly down-regulate in the transcriptional mechanism CD20 levels in tumor cells, leading to decreased binding of anti-CD20 mAbs to the surface CD20 and to impaired activation of antitumor effector mechanisms of the immune system. While impaired CDC is a direct result of decreased CD20 levels, inhibition of ADCC is caused by impairment of cytolytic activity of NK cells.

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COMPLEX ACTIVATION OF ANGIOGENIC SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA: EVIDENCE FROM CIRCULATING ANGIOGENIC CYTOKINES

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Background: Chronic lymphocytic leukemia (CLL) has a remarkably heterogeneous clinical course. Assessment of angiogenesis may potentially improve individual patient´s prognostic stratification.
Aims: To investigate prognostic relevance of circulating basic fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF), soluble endoglin (sCD105), endostatin, and angiopoietin-2 (Ang-2) in patients with CLL.
Methods: Plasma levels of angiogenic factors were quantified using enzyme-linked immunosorbent assay in patients with untreated CLL (median age, 63 years [range, 31-88]; males, 69%) (FGF-2, VEGF, n=181; sCD105, n=175; endostatin, n=137; Ang-2, n=130). Eighty healthy donors served as control group.
Results: All circulating cytokines except endostatin were significantly elevated in CLL patients *vs.* controls (FGF-2 and sCD105: p<0.0001; VEGF, p=0.0004; Ang-2: p=0.001). sCD105 progressively increased with advancing Rai stages (Rai low *vs.* intermediate *vs.* high, p=0.024 and p=0.033). Patients with progressive disease had significantly higher levels of sCD105, endostatin and Ang-2 in comparison to patients with stable course (sCD105: p=0.0008; endostatin: p=0.019; Ang-2: p=0.015). Time to treatment was significantly shorter in patients with high sCD105 levels (median 15 months *vs.* not reached, p=0.0045). In patients who achieved at least partial response after fludarabine-based chemo(immuno)therapy, levels of FGF-2, VEGF, and sCD105 decreased significantly (p<0.0001, p=0.0027 and p=0.0098).
Summary and Conclusions: Our results indicate that a complex network of angiogenic signaling is active in CLL and has an impact on clinical course; sCD105 appears to have the best prognostic value among these cytokines. Updated results will be presented. Supported by grant NT/13412-4 from the Internal Grant Agency, Ministry of Health, Czech Republic, by DRO (Univ Hospital Hradec Králové, 00179906) from Ministry of Health, Czech Republic, and by programme PRVOUK P37/08.

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CHRONIC LYMPHOCYTIC LEUKEMIA CELL CAPACITY OF EXPRESSING AND RESPONDING TO SURFACE IGM (S-IGM) OR S-IGD PREDICTS DISEASE PROGRESSION AND IS ASSOCIATED WITH SPECIFIC MIRNA/MRNA SIGNATURES

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Background: Signaling via BcR is thought to activate proliferation and survival pathways in CLL cells and has also been linked to poor outcome.
Aims: We investigated i) the intensity of sIgM and sIgD expression by flow cytometry, ii) their ability to predict progression free survival (PFS) and iii) specific miRNA and GEP signatures associated with sIgM and sIgG expression.
Methods: 372 newly diagnosed patients were prospectively enrolled from diagnosis (clinicaltrial.gov identifier NCT00917540). The intensity of sIgM and sIgD expression was calculated as Relative Fluorescence Intensity (RFI). Highly-purified B-cells were employed for GEP (GeneChip® Gene 1.0ST Array, Affymetrix Inc.) and miRNA (Agilent's Human V2 platform) analyses.
Results: The mean sIgM density measured as RFI value±sem was 56.9±5.6 in the entire cohort, with a median value of 34 (range 6.7-1430). ROC analysis showed that 10 was the most suitable RFI threshold for sIgM to distinguish those cases operationally defined as sIgM-low group (234/372, 62.9%) from sIgM-high group (AUC=0.629, *P*<0.0001). The PFS of the sIgM-low group was significantly longer than sIgM-high cases (H.R. 2.2, 95%CI 1.4-3.5, *P*<0.0001). Similarly, using a RFI threshold value for sIgD of 12, the 86 cases with sIgD-high had a significantly higher risk to progress (HR=2.4, 95%CI 1.5-3.8, *P*<.0001). Among the 234 sIgM-low cases there was a concordantly low sIgD density in 194 cases, while 46/138 of sIgM-high cases exhibited high sIgD RFI levels (*P*=0.001). Cases were subdivided in 3 groups: sIgM-low sIgD-low (n=194), sIgM or sIgD-high (n=132), and sIgM and sIgD-high (n=46). Differences in clinical outcome were observed between sIgM-low sIgD-low, sIgM-or IgD-high, and sIgM-and sIgD-high cases with 4.5 times higher risk of progression (95%C.I. 2.5-8.1, P<0.0001) for the latter group. A significantly higher number of CD38 and ZAP-70 positive as well as *IGHV*-unmutated (*IGHV*-UM) cases were detected in sIgD- and sIgM-high group, while a significantly higher percentage of patients with trisomy 12, del(11)(q22.3) and del(17)(p13) was noticed. In a Cox multivariate model, CD38-positive [HR=2.2, 95%CI 1.2-3.8, P=.006], *IGHV*-UM [HR=3.0, 95%CI 1.6-5.6, P=.001] and sIgM- and sIgD-high cases [HR=2.1, 95%CI 1.2-4.0, P=.016] maintained an independent association with PFS. A GEP multiclass supervised analyses according to the three sIgD/sIgM density categories identified 23 differently modulated genes, 18 of which showed a prognostic relevance in predicting PFS. Finally, a miRNA multiclass supervised analysis identified 4 differently modulated miRNAs. Specifically, miR-146b-5p showed an up-regulation gradient from sIgDlow/sIgM low to sIgD high/sIgM high groups, while an opposite gradient was documented for miR-575, miR-1225-5p and miR-373*: miR-146b-5p (*P*=.011) was significantly associated with PFS. *In vitro* stimulation of CLL cells by autologous T cells activated and expanded by CD3/CD28 MACSiBead™(Miltenyi) particles and IL2, causes up-regulation of both sIgD and sIgM expression in a subset of B-cells also showing CD38bight, CXCR4dim and CD5bright, indicating that microenvironment signals have a role in BcR regulation.
Summary and Conclusions: A high cell surface density of IgM and IgD correlates with shorter PFS, independently from other known prognostic markers. Moreover, some genes and miRNAs are differently regulated in those cases highly expressing sIgD and sIgM and predict a poor clinical outcome. Finally, we highlight the relevance of sIgD and sIgM regulation in the natural history of early unfavorable CLL patients.

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B-CELL RECEPTOR SIGNALLING ENHANCES GLYCOLYSIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Signaling via the B-cell receptor (BCR) plays a key role in chronic lymphocytic leukaemia (CLL) and is an effective target for therapeutic attack. We have previously demonstrated that stimulation of surface IgM of CLL cells induces expression of the growth promoting oncoprotein, MYC. In addition to its roles in driving cell division, MYC promotes a switch in metabolism, promoting glycolysis, even in the presence of adequate levels of oxygen. This is called aerobic glycolysis, or the Warburg effect, and is important to provide biosynthetic metabolites.
Aims: Here, we have investigated the effects of BCR stimulation on the expression of glycolytic enzymes in primary CLL samples.
Methods: Analysis of the protein expression of MYC, pERK1/2 and pAKT in CLL samples after stimulation with soluble or immobilised anti-IgM (Western blot), and the change in expression after ibrutinib and tamatinib treatment. Analysis of the gene expression of CLL samples stimulated with sIgM (afimetrix array). Validation by Q-PCR analysis of MYC and metabolic target genes: HK2, LDHA and ODC1, after stimulation of CLL cells with both immobilised anti-IgM and CpG-ODN and the change in expression after ibrutinib and tamatinib treatment.
Results: Analysis of gene expression array data, revealed that anti-IgM stimulation of CLL cells induced expression of a wide range of enzymes involved in glycolysis, as well as glutaminolysis which is also controlled by MYC. Q-PCR analysis confirmed that anti-IgM expression increased expression of hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), as well as ornithine decarboxylase (ODC) which catalyses the rate-limiting step in polyamine biosynthesis. These MYC target genes were more strongly induced in cells stimulated with immobilized anti-IgM, compared to soluble antibody. Anti-IgM-induced expression of MYC, HK2, LDHA and ODC was inhibited by the BTK inhibitor ibrutinib and the SYK inhibitor tamatinib. Similar results were obtained following treatment of CLL cells with the toll-like receptor 9 ligand CpG-ODN.
Summary and Conclusions: These results demonstrate that BCR signaling can enhance growth-promoting glycolysis in CLL cells. Inhibition of glycolysis-promoting signaling may contribute to the therapeutic effects of novel kinase inhibitors targeted towards the BCR in this disease.

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NICHEFORMING STROMAL ELEMENTS OF BONE MARROW AND LYMPH NODES IN CLL

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Background: The niche microenvironment of bone marrow controls development HSC and lineage commitent of T and B lymphocytis. Niche lymph nodes regulate development B-precursors that travel from bone marrow. However, the state niche hemopoietic and lymphoid microenvironment when lymphocytis proliferation is impaired continues to be studied insufficiently.
Aims: The aim of the study were investigation of the morphofunctional features of stromal elements of hemopoietic and lymphoid microenvironment that form niche lymphoid precursors in CLL.
Methods: Endosteal cells and microvessels of bone marrow 79 patients with CLL has been studied in trephine biopsies. Follicular dendritic cells and microvessels lymph nodes of 57 patients were studied. Histological, histochemical, morphometric and immunohistochemical methods were used. The statistical significance was considered with p≤0,05 (Student criterion).
Results: Nodular, interstitial and diffuse lymphocytic infiltration of bone marrow are detected. Disorders of microenvironment at all types of infiltration was established. Loose network of reticulin fibers in nodular infiltration. Silver impregnation showed the increase number reticulin fibers endosteal areas bone marrow in interstitial and diffuse types. Density microvessels increase in the progression of bone marrow infiltration. In diffuse infiltration size of vessels increased by almost 2 times in comparison with the control group (17,9±of 4.7% instead of 9.1±1,2%), and, what is especially important, the increase of number of vessels in subendosteal spaces was noted. Analysis of endosteal cells showed an increase of number of cells per unit of area at interstitial (1,8±0,4 against 1.4±0.2 in the control group) and diffuse (2,3±0,7) infiltration. The change of morphology endosteal cell was established too. In lymph node biopsies of CLL patients restructuring of the stroma was noted. Identified destructive changes of reticulin frame. Disintegration of follicular dendritic cells and reduction of their number almost 3 times (to 7.2±1.3% in CLL and 23±3.1% in the control group) was noted. Density microvessels lymph nodes was increased - 12,8±0.3% in CLL (6,5 approximately 0.5% in the control group). Thus, the reduction of the number of follicular dendritic cells and increase of angiogenesis are the key factors of stroma damage lymphoid microenvironment in CLL.
Summary and Conclusions: Changes of stromal nicheforming structures of bone marrow and lymph nodes indicate of their involvement in the genesis of neoplastic transformation of lymphoid precursors and can identify the realignment of the microenvironment function directed on maintenance of leukemic clone.

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DISTRIBUTION OF CIRCULATING RESIDUAL NORMAL B-CELLS AND OTHER IMMUNE CELLS IN ADVANCED-STAGE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ACCORDING TO THE NUMBER OF PRIOR TREATMENT LINES RECEIVED

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Background: It is well-known that B-cell chronic lymphocytic leukemia (CLL) patients have an impaired immune function -particularly in advanced disease, which significantly contributes to a higher risk of infections. The introduction of new therapeutic agents, such as the purine analogues *plus* rituximab, has significantly improved the clinical responses in CLL, but so far their impact on the overall immune function in CLL patients after therapy, remains to be fully understood.
Aims: To evaluate the effect of the number of prior treatment lines received on the different normal circulating leucocyte cell populations, including normal B-cell subsets, in advanced-stage CLL patients (B/C Binet stages).
Methods: The distribution of peripheral blood (PB) leukocytes was analyzed in 128 untreated CLL patients, and compared to that of 83 patients who had previously been treated with 1 line of treatment (n=53) or >1 line of treatment (n=30), and who failed to respond. Analysis was performed by 8-color flow cytometry with monoclonal antibodies against CD3, CD4, CD5, CD8, TCRgd, CD19, CD20, CD27, CD38, CD45, CD56, sIgM, sIgA, sIgG, sIgLambda and sIgKappa.
Results: The absolute count of circulating malignant B cells was not significantly different (p>0.05) in the untreated *vs.* previously treated patients who received 1 or >1 treatment lines (83,081±87,956 *vs* 72,533±89,258 *vs* 63,764±85,153 cells/uL; respectively). In contrast, other prognostic parameters (proportion of cases in Binet C stage and degree of thrombocytopenia) were significantly lower in untreated *vs.* 2-lines treated patients. Also, as compared to untreated patients, PB normal B cells were reduced in patients who had received either 1 line or >1 lines of treatment (81±123 *vs* 38±61 and 56±154 cells/uL, p<0.001). When dissecting the normal B-cell subsets, therapy-related decreased B-cell numbers were mostly due to a reduced number of circulating memory B cells (62±86 *vs* 21±41 and 19±29 cells/uL; p<0.001), including all memory isotypes (p≤0.001): IgM (21±29 *vs* 6±12 and 6±10 cells/uL), IgG (23±51 *vs* 10±29 and 8±15 cells/uL) and IgA (17±27 *vs* 5±9 and 5±11 cells/uL). No differences (p>0.1) were found as regards the absolute count of immature (5±10 *vs* 7±24 and 7±20 cells/uL) and naïve B cells (13±46 *vs* 9±30 *vs* 30±120 cells/uL), nor for circulating plasma cells (5±29 *vs* 4±18 and 2±5 cells/uL), regardless of the therapy status. As compared to untreated patients, the absolute count of CD4+ T cells, CD4/CD8 double negative TCRαβ cells and neutrophils were significantly (p≤0.02) lower in patients previously treated with 1 or more lines of treatment (1,801±1,291 *vs* 1,446±1,308 and 1254±1014 CD4+ T cells/ul; 122±145 *vs* 159±649 and 63±94 CD4-CD8- T cells/uL and 5,432±3,337 *vs* 4,362±2,943 and 3,679±3,453 neutrophils/uL). In contrast, therapy did not show a significant impact on the absolute count of PB T CD8+ and TCRgd cells. No statistically significant differences (excluding neutrophils) were observed in the number of PB eosinophils, basophils, monocytes, NK cells and dendritic cells.
Summary and Conclusions: While there are no differences regarding the number of leukemic cells, previously treated patients have significantly reduced counts of total and memory (all isotypes) normal B-cell subsets when compared to untreated patients. Together with this, CD4+ helper and double negative T cells, as well as neutrophils, could also be compromised after treatment. Monitoring of these therapy-related immune defects could contribute to a better management of infectious complications in advanced-stage CLL patients.

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THE INFLUENCE OF BENDAMUSTINE USED ALONE OR IN COMBINATION WITH RITUXIMAB ON APOPTOSIS AND THE EXPRESSION OF SOME APOPTOSIS-REGULATING PROTEINS DEPENDING ON THE IGVH MUTATIONAL STATUS OF CLL CELLS

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Background: Bendamustine (BENDA) used either alone or in combination with rituximab (RIT) is effective in the treatment of chronic lymphocytic leukemia (CLL). However, the influence of these drugs on the expression of apoptosis-regulating markers depending on IGVH mutational status has not been studied yet.
Aims: The aim of our study was to evaluate the influence of the above mentioned drugs on the expression of apoptosis-involved proteins depending on the IGVH mutational status of CLL cells *in vitro*.
Methods: The study was performed in 26 untreated CLL patients (11F, 15 M), aged 49-80 (mean 65.6) yrs who gave written consent for collection of the peripheral blood. The IGVH gene was mutated (IGVH+) in 13 patients, and unmutated (IGVH-) in 13 patients. Peripheral blood CD19+ cells at the concentration of 1.0x106 cells/ml were incubated in RPMI1640 medium containing 10% fetal calf serum and 10% autologous serum with addition of BENDA (40mg/ml), RIT (10mg/ml) or BENDA (40mg/ml)+RIT (10mg/ml), for 48 hours. Control cultures were incubated without the drugs. The influence of these drugs on the mitochondrial potential decrease ($\Delta\Psi_m^{low}$), the active forms of caspases-3,-9 and -8, the expression of BAX, PUMA, P53, APAF-1, BCL-2 and FADD proteins were evaluated by the flow cytometry.
Results: BENDA used alone and in combination with RIT statistically significantly increased the percentage of $\Delta\Psi_m^{low}$ cells (52.4% and 60.0%; respectively for IGVH+, and 60.7% and 62.8%; respectively for IGVH- group) as compared to the control culture (31.6%). However, an increase of the percentage of $\Delta\Psi_{m-}^{low}$ cells after the incubation with BENDA or BENDA+RIT was similar in both groups. The percentage of cells with expression of the active forms of caspases-3,-9,-8 in both groups was also significantly higher under the influence of BENDA or BENDA+RIT than after incubation with RIT or in the control culture. There were also no significant differences with regard to the mutational status. BENDA used alone and BENDA+RIT significantly increased the percentage of BAX+ cells as compared to the control culture and RIT alone. The percentage of PUMA+ cells increased significantly under the influence BENDA as compared to the control culture in IGVH+ group, whereas an increase of the percentage of these cells in IGVH- group was observed after BENDA+RIT as compared to either control culture or RIT used alone. Expression of P53 increased significantly under the influence of each of the drugs used alone as compared to the control culture, or BENDA and BENDA+RIT as compared to RIT. Additionally, BENDA increased the percentage of APAF-1 positive cells as compared to the control culture, but the differences were insignificant. There was no effect of these drugs on the BCL-2 and FADD expressing cells. There were also no differences in the influence of the drugs on the expression of the above mentioned apoptosis-involved proteins in both IGVH groups.
Summary and Conclusions: We demonstrated that BENDA used either alone or in combination with RIT induces apoptosis as well as expression of the apoptosis-involved proteins, regardless of the IGVH gene mutational status. However, the impact of IGVH mutational status on the BENDA-induced expression of apoptosis-regulating factors deserves further studies.
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DECREASED DKK-1 LEVELS IN CHRONIC LYMPHOCYTIC LEUKEMIA AT INITIAL DIAGNOSIS MIGHT BE PREDICTIVE OF A HIGHER MORTALITY

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Background: In addition to its various physiologic functions, the Wnt pathway also plays a role in tumorigenesis. Dickkopf-1 (DKK-1) binds with high-affinity to the Wnt coreceptor LRP6 domain. The clinical significance of Wnt pathway inhibition with DKK-1 was shown in various cancers; however, there is too few data in chronic lymphocytic leukemia (CLL) and nonHodgkin lymphoma (NHL). It was reported that the Wnt pathway had an antiapoptotic role; and that osteopontin (OPN) gene was directly targeted by Wnt.
Aims: In our study, we determined whether plasma levels of DKK-1 and OPN which have roles in the Wnt pathway were different in CLL and NHL patients than in healthy controls. In addition, we tested whether DKK-1 and OPN levels could be of clinical or prognostic importance in CLL and NHL.
Methods: We included 36 CLL patients (26 males, 10 females, mean age: 60.4±12.5), 24 NHL patients (17 males, 7 females, mean age: 54.3±16.4), and 21 healthy controls (13 males, 8 females, mean age: 55.7±8.3) into our study. Ethical committee approval and consent from all subjects were obtained. Patients' clinical and demographic features, treatment modalities, and response to treatment were obtained from hospital files. Blood was obtained from all patients at the time of initial diagnosis; DKK-1 and OPN levels in plasma were determined with ELISA.
Results: CLL patients (668.9±495.5 pg/mL) had significantly lower DKK-1 levels than NHL (978.9±263.7 pg/mL) and control groups (956.7±283.6) (p levels, respectively, 0.048 and 0.017). NHL and control groups had similar DKK-1 levels. OPN level was significantly higher in NHL group (5.7±9.4 ng/mL) than in CLL (1.37±1.15 ng/mL) and control groups (3.03±5.3 ng/mL) (p values, 0.017 and <0.001). CLL and control groups did not have significant difference in OPN

levels (p>0.05). CLL patients with early and late Rai stages of disease had similar DKK-1 and OPN levels. After a median follow-up of 48 months, 13 CLL patients died. When DKK-1 and OPN levels in initial plasma samples from CLL patients who have died were compared to those who were alive, it was observed that DKK-1 levels were significantly lower in the former group (449.3±365.5 pg/mL *vs.* 819.5±468 pg/mL, p=0.035). Among the NHL group, patients with extranodal involvement had significantly higher OPN levels than those with no involvement (9.1±13.3 ng/mL *vs.* 2.9±1.9 ng/mL, p=0.04).
Summary and Conclusions: Our results demonstrated that the Wnt pathway inhibitor DKK-1 was decreased in CLL; and lower levels were associated with higher mortality in CLL patients. OPN which is another protein related to Wnt pathway was increased in NHL and was associated with extranodal involvement. In order to reveal the pathogenic and clinical role of DKK-1 and OPN in CLL and NHL, larger studies need to be conducted.

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MRD ANALYSIS IN BONE MARROW ASPIRATE AT 3 MONTHS AFTER TREATMENT IS THE OPTIMAL RESPONSE ASSESSMENT FOR CLL AND CAN PREDICT SUSTAINED MRD-NEGATIVE REMISSION INDEPENDENT OF ADVERSE PROGNOSTIC MARKERS

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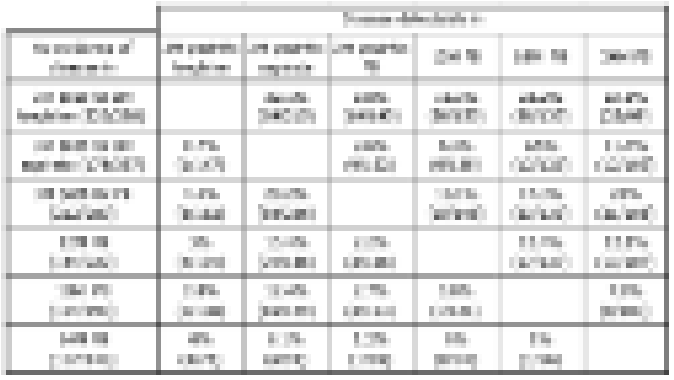
Background: The level of minimal residual disease (MRD) detected by sensitive quantitative assays is a strong independent predictor of progression-free and overall survival in CLL. Morphological assessment of trephine biopsies is a subjective analysis with significant patient discomfort and low sensitivity for detection of residual disease. There is usually a close correlation between the levels of CLL in the blood and bone marrow, raising the possibility that high sensitivity MRD analysis could be more informative in predicting outcome than morphological assessment of a bone marrow biopsy. However, therapeutic antibodies, including rituximab, preferentially deplete cells in the peripheral blood and are likely to compromise the prognostic value of peripheral blood MRD analysis during and soon after therapy.
Aims: To determine the optimal site and time point for response and residual disease assessment after chemo-immunotherapy.
Methods: Sensitive multi-parameter flow cytometry (1 CLL cell in 10^4 leucocytes) was performed in the peripheral blood (n=280) and bone marrow (n=337) at 3 months after treatment and at 12, 18 and 24 months after randomisation (evaluable in n=222, 190, and 148 respectively) in 415 participants in the UK FCR-based CLL Trials (ADMIRE and ARCTIC). Adverse prognostic features were defined as <2% IGHV mutation or deletion in 17p or 11q identified by FISH. Trephine biopsies were evaluated by two independent pathologists, with immunohistochemistry performed when lymphoid nodules were evident.
Results: Peripheral blood MRD levels at 3 months after treatment were >1% in 16/280 cases, of which 12/13 evaluable showed morphologically evident disease in the trephine biopsy. Blood MRD levels were <0.01% in 212/280 cases, of which 130/190 evaluable (68.4%) also had no detectable disease in the bone marrow by morphology or flow cytometry but disease was detected in the bone marrow in over a quarter of cases (Table 1). Bone marrow aspirate disease levels at 3 months after treatment were >1% in 49 cases of which 38/42 evaluable also showed morphologically evident disease. Aspirate MRD levels were <0.01% in 178/339 of which 147/178 had an assessable trephine biopsy and only 1/147 had morphologically evident disease. Sequential analysis of cases with <0.01% MRD in the bone marrow at 3 months after treatment demonstrated that 96% (132/138), 90% (114/126) and 89% (93/105) maintained <0.01% disease in the peripheral blood at 12, 18, and 24 months after randomisation respectively. Of all evaluable cases with adverse prognostic features, 62% (118/190) had detectable residual disease (>0.01%) within 12 months after randomisation compared to 34% (31/91) without adverse prognostic markers (P<0.0001). However, of the cases with adverse prognostic features who achieved <0.01% residual disease at 12 months after randomisation, CLL levels were persistently depleted (<0.01%) at the 2 year time-point in 85% (46/54) and this was not significantly different to the 91% (43/47) of cases without adverse prognostic markers who maintained <0.01% MRD at both time points (P=0.37).
Summary and Conclusions: The presence of >1% MRD in peripheral blood or bone marrow is equivalent to a partial response and assessment of a trephine biopsy provides little or no additional information. Peripheral blood evaluation within 6 months of treatment is less sensitive than bone marrow aspirate evaluation for the detection of residual disease. Approximately 80% of individuals achieving <0.01% MRD in the bone marrow at 3 months after treatment maintain an MRD-negative remission for the following 18 months, and this remains the most sensitive site and time-point for disease assessment after chemo-immunotherapy. Poor-risk prognostic features predict for a lower probability of achieving <0.01% MRD but the probability of maintaining a prolonged MRD-negative remission is similar in people with good-risk *vs.* adverse prognostic markers. This supports the concept that achieving deep depletion in frontline treatment can overcome adverse biological features.

Table 1.



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NOT ALL IGHV3-21 CLL ARE EQUAL: SUBSET #2 DISPLAYS A DISTINCTIVE CLINICOBIOLOGICAL PROFILE WITH REMARKABLE SIMILARITIES TO SUBSET #169, ITS CLOSE IMMUNOGENETIC RELATIVE

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Background: In CLL, subsets with stereotyped B-cell receptor immunoglobulins (BcR IG) represent a remarkable one-third of all cases. Intriguingly, CLL subset patients expressing certain stereotyped BcR IG have high intra-subset homogeneity regarding biological and clinical features as well as outcome. CLL subset #2, the largest subset overall, carries IGHV3-21/IGLV3-21 IG that virtually always bear somatic hypermutations (SHM), ranging from minimal or borderline (most frequently) to extensive (rarely).
Aims: Among 8593 CLL patients with available IG and clinicobiological information, we identified 254 (3%) cases classified as subset #2 that we characterized in detail.
Methods: Patient characteristics were as follows: 60% males; median age: 64; Binet stages B-C: 53%; SHM status: 61% IGHV-mutated (<98% germline identity, M-CLL), 39% IGHV-unmutated (U-CLL; only 2/254 cases with no SHM); FISH aberrations: isolated del(13q): 56% (47% in M-CLL *vs* 70% in U-CLL,

p=0.02) | trisomy 12: 4.7% | del(11q): 23% (23% in M-CLL *vs* 26% in U-CLL, p=0.8) | del(17p): 4.7%.
Results: No differences in time-to-first-treatment (TTFT) were found between U-CLL *vs* M-CLL subset #2 cases (19 *vs* 23 months, p=0.6). Interestingly, however, among the M-CLL, the presence of del(11q) was associated with significantly shorter TTFT (13 *vs* 29 months, p=0.03); no such differences were seen among U-CLL subset #2 cases, regarding the incidence of del(11q). We next compared subset #2 cases to 183 non-subset #2 IGHV3-21 (non#2/3-21) CLL cases and noted a significantly lower (p=0.002) SHM load with 53% of the non#2/3-21 cases (97/183) being U-CLL. Non#2/3-21 CLL also had significantly longer TTFT compared to subset #2 CLL (60 *vs* 19 months, p=0.001). This difference arose mainly from the superior outcome of non#2/3-21 cases with mutated IGs (median TTFT 153 months) since the TTFT of non#2/3-21 U-CLL, although longer, did not differ significantly from subset #2 (30 *vs* 19 months, p=0.4). No other major differences were found (genomic aberrations included). Within our cohort we also identified a minor group of 19 cases (0.2% of the series), termed subset #169, that express the IGHV3-48/IGLV3-21 gene combination, carry VH CDR3 identical in length and similar in composition to those of subset #2 IG and display an analogous SHM profile. Given the overall high identity (97%) between the IGHV3-21 and IGHV3-48 genes, we explored whether subsets #2 and #169, whose immunogenetic signatures are clearly related, share similar clinicobiological features and outcomes. Indeed, this turned out to be the case, since their FISH genomic profiles were similar, especially with regards to del(11q) (38% in subset #169) and del(17p) (absent in subset #169); as was their median TTFT (p=1). Of note, in an ongoing study from our group, subsets #2 and #169 display an almost identical (~45%) frequency of *SF3B1* mutations.
Summary and Conclusions: Altogether, we demonstrate that IGHV3-21 CLL should not be considered as homogeneous, with subset #2 emerging as uniformly aggressive, thus contasting non#2/3-21 patients whose prognosis clearly depends on SHM status like the rest CLL. We also argue that studying BcR stereotypy is relevant for improved understanding and eventual management of CLL through the identification of subsets with distinct features that can be expected to benefit from the implementation of specific treatments. Finally, we highlight for the first time the biological and clinical links between subsets with related immune signatures, raising the intriguing possibility that a higher-order organization of subsets based on their immunogenetic features will also be highly relevant.

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IMMUNOGLOBULIN HEAVY VARIABLE GENES AND ALLELES: NEW ENTITIES, NEW NAMES AND IMPLICATIONS FOR RESEARCH AND PROGNOSTICATION IN CLL

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Background: The development of novel sequencing technologies has led to a far more accurate view of the human genome. Indeed, recent findings have altered our notions regarding the configuration of immunoglobulin (IG) gene loci and the actual number of gene alleles, as described by the International ImMunoGeneTics information system (IMGT), the global reference in Immunogenetics and Immunoinformatics. Overall, 34 changes were incorporated in the latest version (3.3.0 reference directory release: 201408-4) of the IMGT/V-QUEST tool. Twenty of these changes concerned the identification and addition of new germline immunoglobulin heavy variable (IGHV) gene alleles (19 Functional and 1 Open Reading Frame), whereas the remaining 14 were due to the assignment of new allele names to the existing nomenclature system (13/14 Functional, 1 Open Reading Frame).
Aims: In CLL, the mutational status of the clonotypic rearranged IGHV gene is strongly associated with patient outcome. Correct determination of this parameter strictly depends on the comparison of the nucleotide sequence of the clonotypic rearranged IGHV gene with that of the closest germline counterpart. Consequently, changes in the reference directories could, in principle, affect the correct interpretation of the IGHV mutational status in CLL which is the focus of the present study.
Methods: In order to explore this issue, we analyzed 8337 IGHV-IGHD-IGHJ rearrangement sequences from CLL patients from our database with the IMGT/HighV-QUEST tool using two different versions of the IMGT/V-QUEST: version 3.2.32 (2 December 2013) *versus* version: 3.3.0 (20 February 2014) and compared the tool outputs.
Results: Differences due to change of the IGHV gene allele name were identified in 405 sequences (4.8%). In 291/405 (71.9%) of these cases, the germline identity (GI) remained the same, since the difference concerned only the renamed gene allele. In the remaining 114 (28.1%) cases, differences were also identified in% GI since a novel IGHV gene allele was recognized. Focusing on the IGHV mutational status and in keeping with previous reports, we subdivided our sequences into four different subgroups: truly unmutated (100% GI), minimally mutated (99-99.9% GI), borderline mutated (98-98.9% GI) and mutated (<98% GI). Overall, a change in the mutational status category was identified in 50 (0.6%) sequences, though never one that entailed crossing the 98% GI cut-off value used for prognostication in CLL. In more detail, 2 sequences moved from the mutated to the borderline mutated subgroup; 15 from the borderline mutated to either the truly unmutated (4/15) or the minimally mutated (11/15) subgroup; and 33 from the minimally mutated to the truly unmutated subgroup. Understandably, cases that were reported as truly unmutated from the previous IMGT/HighV-QUEST tool version were not affected. All 4 sequences that moved from the borderline mutated to the truly unmutated group were previously assigned to IGHV3-43*01 and are now assigned to the newly reported IGHV3-43D*01.
Summary and Conclusions: In conclusion, the recent addition of new germline IGHV gene alleles led to a number of changes concerning both IGHV gene nomenclature and germline sequence composition affecting the alignment output of the IMGT/HighV-QUEST tool. Given the prognostic value of somatic hypermutation status in CLL, both physicians and researchers should be alerted and re-evaluate sequence data, especially for those IGHV-IGHD-IGHJ gene rearrangements that up to date were considered as borderline mutated, where caution is warranted.

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AN ENTITY EVOLVING INTO A COMMUNITY: DEFINING THE COMMON ANCESTOR OF CLL SUBSET #4

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Background: CLL clones assigned to stereotyped subset #4 are characterized molecularly by B-cell receptor immunoglobulins (BcR IGs) that exhibit a series of distinctive immunogenetic features. More specifically, they are IgG-switched and composed of heavy chains encoded by the IGHV4-34 gene and light chains encoded by the IGKV2-30 gene. This distinctive BcR archetype expressed by subset #4 cases is itself significant owing to its absence from normal individuals or those with other lymphoproliferative disorders. The VH CDR3s of subset #4 BcR IGs are long and enriched in positively charged residues, reminiscent of pathogenic anti-DNA antibodies. Indeed, subset #4 is defined by a (K/R)RYY motif; a motif deemed to be not only 'CLL-biased' but also exclusive to subset #4. In addition, both the VH and VK domains of subset #4 demonstrate a high impact of somatic hypermutation (SHM) and are remarkable for carrying shared 'stereotyped' amino acid changes introduced by SHM. Subset #4 is also outstanding due to intense intraclonal diversification (ID) within the clonotypic BcR IG genes. By conducting a large-scale longitudinal study of subset #4 we previously: (i) established a hierarchical pattern of subclonal evolution, thus revealing which SHMs were negatively or positively selected; (ii) observed distinct clusters of subcloned sequences, with *e.g.* the minor ones often disappearing at later time-points and hence being selected against; and, (iii) noted that despite the high intensity of ID, certain residues remained essentially unaltered.
Aims: We here sought to revisit ID in subset #4 clones as a whole for the first time, and reconstruct their evolutionary history as a community of related clones profiled at different time-points.

Methods: We assessed both heavy and light chains (IGHV-IGHD-IGHJ rearrangements (n=511) and IGKV-IGKJ rearrangements (n=398) derived from 8 subset #4 cases. Sequence data was processed through application of the Damerau–Levenshtein distance, *i.e.* the number of changes required to transform one sequence to the other.
Results: Two important observations stemmed out of this analysis. Firstly, a number of clonal clusters from different patients were found to lie very close to one another due to a high degree of sequence relatedness, forming a core from which clusters that exhibited greater sequence variation stemmed from. This 'branching' could perhaps result from specific selective pressures that occurred in parallel in distinct subclones, thereby fine-tuning their BcR affinities. Secondly, minor clones from individual patients were found to have been mutated to such an extent that they now bore closer resemblance to the sequences of another patient. Viewing the entire subset #4 dataset as a single entity that has branched through diversification, facilitated reconstruction of its evolutionary history and enabled inference of a common ancestral sequence from which all subset #4 cases could have derived: ARGYADTAVVRRYYYGMDV, created by the association of the IGHV4-34 and IGHJ6 genes with the IGHD5-18 gene in reading frame 1. Of note, this task was hitherto unattainable due to the heavy SHM load within the antigen-binding sites, rendering the reliable identification of the IGHD gene particularly difficult if not altogether impossible.
Summary and Conclusions: These results have implications for improved understanding of the ontogeny of CLL subset #4, as well as the design of studies concerning the antigenic specificity of the clonotypic BcR IGs.

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MATCHED PATTERN DISCOVERY ACROSS PAIRED IMMUNOGLOBULIN HEAVY AND LIGHT CHAINS IN CLL REVEALS UNIQUE SUBSET-DEFINING AMINO ACID ASSOCIATIONS

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Background: Immunogenetic analyses have helped to understand the pathogenesis of CLL and also to define subgroups with strikingly different behavior and outcome on top of unprecedented B-cell receptor (BcR) restriction (stereotypy), cementing the idea that antigenic elements select the leukemic clones. Even though recognition of certain antigenic epitopes may be critically dependent on conformations achieved by the interaction of both the IG heavy chain (HC) and light chain (LC) proteins, the focus to date has been on the IG HC gene, with studies on CLL IG LCs lagging behind. That said, previous studies from our group have demonstrated that CLL LCs can be as important as HCs, and that even very slight sequence alterations in LCs can be selected for and likely confer a functional advantage to the clone.
Aims: We here aimed to gain an even deeper view of CLL IG primary structures by searching for pattern associations between paired HC and LC gene rearrangements of 1331 CLL cases, the largest series to date, focusing on the main antigen-binding sites *i.e.* the HC and LC CDR3s.
Methods: We applied exhaustive amino acid pattern discovery that initially detected patterns within the VH and VK/VL CDR3s before associating these patterns with each other, either within the same chain (intra-association) or across chains (heavy-light inter-association).
Results: Overall, 14,544 patterns were discovered, of which 10,050 concerned the VH CDR3s; the remaining involved the VK (n=2528) or VL (n=1966) CDR3s, respectively. The discovered patterns varied in length from 1 to 22 amino acids. A total of 248,626 patterns were discovered with at least 2 occurrences across VH-VK chains, and 161,940 such patterns across VH-VL chains, being detected in 84.2% and 67.6% of the respective datasets. Starting from VH CDR3 patterns characteristic of stereotyped subsets, we explored whether certain VH/VK or VH/VL associations could be unique to particular subsets. We identified subset-biased and, most interestingly, subset-specific associations, *i.e.*, matched patterns that were restricted to specific subsets. Of note, several of the subset-specific associations concerned dipeptides or even single amino acids within the VH and VK or VL CDR3, respectively. Examples include: (i) Q at VH CDR3 position 4 and S at VK CDR3 position 5 was detected only in subset #1 (clan I IGHV genes/IGKV1(D)-39), being present in 54/55 cases (98.1%); (ii) RY at VH CDR3 positions 12-13 and M at VK CDR3 position 1 was detected only in subset #4 (IGHV4-34/IGKV2-30), being present in all 26 (100%) subset #4 cases; (iii) DV at VH CDR3 positions 8-9 and D/P at VL CDR3 position 8 was detected only in subset #2 (IGHV3-21/IGLV3-21), being present in 48/51 (94%) subset #2 cases.
Summary and Conclusions: In conclusion, we herein demonstrate that CLL stereotyped subsets can now be more accurately defined based on IG gene usage, CDR3 length and pivotal short amino acid patterns or, remarkably, even single residues with a precise offset in both the HC and LC chain CDR3s. This finding draws further molecular analogies between stereotyped BcR IGs and the IGs expressed by mouse B-1 cells, supporting the notion that at least certain CLL clones may be derived from a B cell population intermediary to a true innate immune system and the conventional adaptive B cell immune system and hence functionally similar to what has previously been suggested for mouse B-1 cells.

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FCΓRIIB INHIBITS BCR SIGNALING IN HUMAN NORMAL B CELLS BUT IS DISRUPTED IN CLL CELLS

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Background: FcγRIIb is a low-affinity receptor that regulates B-cell activation by inhibiting signaling from the B-cell receptor (BCR) in murine normal B cells. BCR signaling is required for the survival and proliferation of human normal B cells and chronic lymphocytic leukemia (CLL) cells, but the role of FcγRIIb in these cells has been poorly studied.
Aims: To evaluate the expression and function of FcγRIIb in CLL and normal B cells.
Methods: We analyzed the expression of FcγRIIb on B cells from 85 patients with CLL and 14 healthy individuals by using a novel Alexa488-conjugated anti-human FcγRIIb mAb from Macrogenics, and evaluated the prognostic relevance of this receptor in patients with CLL. B cells were isolated from 18 CLL patients (9 *IgHV* mutated, 9 *IgHV* unmutated) and 10 healthy donors, co-cultured with HS-5 stromal cells, and stimulated with the following antibodies: $F(ab')_2$ fragments of polyclonal rabbit IgG anti-human IgM to induce BCR signaling, the undigested whole IgG rabbit anti-human IgM molecule to crosslink BCR and FcγRIIb, the unconjugated form of FcγRIIb mAb to stimulate FcγRIIb and two further combinations, FcγRIIb and $F(ab')_2$ and FcγRIIb and whole IgG antibody. Viability, activation and proliferation were analyzed by flow cytometry, and pSHIP and pAKT protein expression by Western Blot.
Results: The expression of FcγRIIb was significantly lower in leukemic cells than in CD19+CD5+ and CD19+CD5- normal cells (MFI 6901 *vs* 10180 and 12120, respectively; *p<0.01*). Patients with higher expression of FcγRIIb showed longer survival than those with lower FcγRIIb levels (7.8 *vs* 3.3 years, respectively; *p<0.01*). In normal B cells the co-ligation of BCR and FcγRIIb with the whole IgG form significantly decreased the activation and proliferation mediated by BCR. This inhibitory effect, however, was not observed when cells were stimulated either with the combination of FcγRIIb and $F(ab')_2$ or FcγRIIb and whole IgG antibody. With regard to CLL cells, the BCR-FcγRIIb coaggregation did not induce changes in cell activation and proliferation. However, this co-ligation was able to rescue the decrease of viability induced by BCR stimulation. We further observed that the effect of crosslinking BCR-FcγRIIb was significantly different in proliferation between unmutated and mutated CLL. A significant increase on proliferation was induced in unmutated cases while no effect was seen in mutated cases. At the molecular level, the co-ligation of BCR-FcγRIIb in normal B cells induced the upregulation of pSHIP and the downregulation of pAKT. Most CLL cells, however, showed high basal expression of pSHIP and no significant changes were observed in this protein after BCR-FcγRIIb co-ligation, although a slightly decrease of pAKT was observed. To date, no significant differences in pSHIP and pAKT expression were observed in mutated *vs.* unmutated cases.
Summary and Conclusions: Our results indicate that CLL cells show significantly lower expression of FcγRIIb than normal B cells. Furthermore, the function of FcγRIIb seems to be different when compared it in both populations. Thus, in normal B cells, FcγRIIb inhibits BCR signalling, an effect that clearly depends on its co-ligation with BCR, which triggers downstream signalling through SHIP and AKT. In contrast, in CLL cells, the co-ligation of FcγRIIb-BCR does not significantly inhibit BCR signalling, and particularly in unmutated CLL has a positive effect with an increase of BCR-mediated proliferation. All these findings support further investigation of FcγRIIb in the physiopathology of CLL.

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THE DUAL PI3K/MTOR INHIBITOR PF-04691502 INDUCES SUBSTANTIAL APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS INDEPENDENTLY OF PROGNOSTIC MARKERS

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Background: CLL is currently incurable by chemotherapy alone with drug resistance and treatment relapse a common occurrence. Pharmacological targeting of dysregulated signalling pathways within CLL cells however, offers the potential to provide improved therapeutic options with reduced toxicity. In this regard, the PI3K-mTOR pathway is particularly attractive because it is activated upon ligation of various chemokine and cytokine receptors expressed by CLL cells as well as following B cell receptor engagement. PI3K mediated signalling is known to be overactive in all CLL, although patients with un-mutated IGHV have increased PI3K expression compared to patients with mutated IGHV. The PI3Kδ selective inhibitor Idelalisib (GS-1101) is currently in Phase 3 clinical trials for CLL and in combination with rituximab significantly improved overall survival among patients with relapsed CLL compared to rituximab alone. In addition to PI3Kδ, there are three other PI3K isoforms, PI3Kα PI3Kβ and PI3Kγ, with PI3Kα known to have a role in CLL survival and chemotaxis. It has previously been shown in neutrophils that functional redundancy between PI3K isoforms is evident, with inhibition of any three PI3K isoforms required for maximal apoptosis. In addition, pharmacological inhibition of mTOR is known to induce cell cycle arrest and apoptosis in CLL cells, however prolonged selective inhibition of mTOR results in a positive feedback loop and PI3K\Akt reactivation. To overcome these caveats, pan-PI3K and dual mTOR inhibition may achieve superior toxicity against CLL cells compared to PI3Kδ inhibition alone.
Aims: To investigate the effect of a dual PI3K and mTOR inhibitor, PF-04691502, on CLL cells.
Methods: Twenty six primary CLL samples were treated with PF-04691502 in the presence or absence of stromal cells and apoptosis assessed using propidium iodide/ Annexin V staining and PARP cleavage. The effect of PF-04691502 on B cell receptor and chemokine receptor induced signalling, survival and chemotaxis were assessed by immunoblotting and flow cytometry.
Results: PI3Kδ inhibition using Idelalisib *in vitro* resulted in 20% apoptosis of CLL cells after 24 hours at 10μM concentration, in contrast, PF-04691502 induced 80% apoptosis of CLL cells at 10μM concentration after 24 hours. PF-04691502 had an IC_{50} value of 1μM as assessed by propidium iodide and Annexin V staining, with little toxicity to normal B or T cells evident. Induction of apoptosis by PF-04691502 occurred independently of IGHV mutational status, CD38 expression and ZAP-70 expression. PF-04691502 inhibited both soluble and immobilised anti-IgM induced signalling and overcame anti-IgM stimulated survival signals. PF-04691502 inhibited CXCL12 induced signalling and abrogated CLL migration towards CXCL12 in a Transwell chemotaxis assay. Finally, PF-04691502 was able to overcome protection following co-culture with stroma and induced significant apoptosis of CLL cells when added continuously or in wash out experiments.
Summary and Conclusions: PF-04691502 is a novel dual PI3K/mTOR inhibitor which induces substantial apoptosis of CLL cells independently of prognostic markers and may be of value for the treatment of CLL patients.

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INDIRECT INDUCTION OF REGULATORY T CELLS ACCOMPANIES IMMUNE RESPONSES DURING VACCINATION OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH SHORT, RHAMM-DERIVED, MHC-I-RESTRICTED PEPTIDE

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Background: The receptor for hyaluronic acid mediated motility (RHAMM) is a tumor-associated antigen in chronic lymphocytic leukemia (CLL). $CD8^+$ T cells primed with the RHAMM-derived epitope R3, which is restricted by HLA-A2, effectively lyse $RHAMM^+$ CLL cells. In phase I clinical trial of R3 peptide vaccination we proved safety and efficacy of specific induction of antitumor immune response against RHAMM antigen. In patients with clinical responses, we found increased frequencies of R3-specific $CD8^+$ T cells. Intriguingly, vaccination was associated with the induction of regulatory T cells (Treg) in four patients. This phenomena could be either interpreted as the result of effective induction of immune response that is followed by induction of suppressive mechanisms of immune system in an indirect manner or unexpected direct induction of Treg by short MHC I –restricted peptide. Both could result in reduced cytotoxic antitumor response limiting thereby clinical effectiveness of peptide immunotherapy. Recent findings also demonstrated specific Treg reduction after incubation with thalidomide both *in vivo* and *in vitro* providing a rationale to combine peptide vaccination with immunomodulatory drugs.
Aims: Define an influence of short MHC-restricted peptide R3 on Treg induction during vaccination in CLL patients.
Methods: Six HLA-$A2^+$ CLL patients were vaccinated four times at biweekly intervals with the R3 peptide emulsified in incomplete Freund's adjuvant and GM-CSF. Before and after each vaccine dose ELISA experiments were performed to assess IL-2 serum levels of 6 CLL patients and results were correlated with frequencies of Treg. Peripheral blood mononuclear cells were incubated with peptide R3 at concentration of 10 μg/mL and flow cytometry analyses of the frequency of Treg were evaluated after 1, 2 and 3 weeks of cell culture. To analyze suppressive potential of Treg mixed lymphocyte peptide cultures (MLPC) were performed. At day +21 of MLPC, cells were harvested and evaluated for their specific cytotoxicity in ELISpot assays. To characterize possible immunostimulatory effect of thalidomide able to restore immune response in this *in vitro* system, cell cultures were supplemented with thalidomide concentration of 10 μg/mL and specific release of interferon γ was assessed using ELISpot methodology.
Results: The IL-2 serum levels in CLL patients during vaccination strongly correlated with the frequency of Treg (r2=0.970, p<0.001). In *in vitro* experiments we observed no induction of Treg by R3 peptide both in CLL and HV after 7, 14 and 21 days of cell culture. However, we found statistically significant difference between Treg rates before cell culture in CLL patients and HV (13.88 *vs* 5.85, p=0.008). MLPC revealed suppressive Treg effect on R3-specific cytotoxic immune responses in 9 of 12 CLL patients. The addition of thalidomide to the MLPC resulted in the increase of specific immune responses against R3 epitope in only 2 of 8 patients.
Summary and Conclusions: There is no evidence on direct effect of short MHC class I epitopes on Treg induction during peptide vaccination. Increased frequencies of Treg in CLL could effectively inhibit immune responses against $R3^+$ target cells. In our *in vitro* system the addition of thalidomide to cell culture could effectively restore immune responses *in vitro* against $R3^+$ target cells only in limited numbers of patients. The induction of Treg during vaccination might result from activation of suppressive mechanisms of immune response; one of possible inducer could be IL-2 which increased concentrations after vaccination correlated with Treg. Future peptide vaccination schedules should be accompanied by drugs that limit Treg.

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ANTI-ROR1 MONOCLONAL ANTIBODIES INDUCED APOPTOSIS OF CLL CELLS AND ALTERED PHOSPHORYLATION OF SRC AND PI3-KINASES

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Background: Phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases playing an important role in the regulation of survival of cancer cells. In CLL cells, the PI3K pathway is constitutively activated. Activation of PI3Ks in cancer occurs via different mechanisms. Mutation, amplification or overexpression of upstream receptor tyrosine kinases (RTKs) is such a mechanism. The class IA PI3K p110 catalytic subunits are activated upon Src homology 2 domain-mediated binding of the p85 regulatory subunits to tyrosine phosphorylated motifs in RTKs. In CLL and AML the p110-δ subunit (p110δ) is shown to be the predominant isoform of PI3K. Increased p110δ activity has also been seen in colon and bladder cancers, as well as in glioblastomas. Src proteins are activated in various cancers such as colon, liver, lung, breast and pancreas. Phosphorylation of Src proteins induces survival, proliferation and invasion of cancer cells. ROR1 is a type I transmembrane RTK, overexpressed and constitutively phosphorylated in CLL cells. ROR1 mediated Src phosphorylation triggered activation of AKT in lung carcinoma.
Aims: To study the effect of an anti-ROR1 CRD mAb on apoptosis and phosphorylation of Src and PI3Kδ in CLL.
Methods: Cell lysates were prepared from untreated and anti-ROR1 CRD mAb treated CLL samples and subjected to Western blot analysis for identification of total and phosphorylated Src, PI3K p85 isoform and PI3Kδ. Apoptotic effect of anti-ROR1 mAb on CLL cells was assayed by MTT and Annexin, V/PI in 24 h. ROR1 expression was determined by flowcytometry and Western blot. Phosphoproteins were measured before incubation with the mAb and after 20 min-24 h.
Results: The percentage of $ROR1^+$/CLL cells was 77±4% (mean±SEM) (range: 70-89%). The frequency of apoptotic cells induced by the anti-CRD mAb was in the range of 40-62% (mean±SEM: 48±2%). The difference compared to CLL cells with isotype control was highly statistically significant (p<0.0001). Western blot analysis showed that co-culturing of CLL cells with the anti-ROR1 CRD mAb decreased the level of phosphorylated Src. PI3Kδ (p110δ) as well as PI3K p85 isoform but not the p55 isoform, were dephosphorylated in treated as compared to untreated samples
Summary and Conclusions: Incubation of CLL cells with an anti-ROR1 CRD mAb induced apoptosis of primary CLL cells. Apoptosis was preceded by dephosphorylation of Src, PI3K p85 isoform, and PI3Kδ proteins indicating deactivation of these signaling proteins by the anti-ROR1 mAb. p110 (PI3Kδ) phosphorylation is mediated by Src and p85 recruitment increases the catalytic activity of the p110 subunit. Our data may suggest that binding of anti-ROR1 CRD mAb to ROR1 RTK might induce an inhibitory signal, decreasing phosphorylation of the Src protein leading to dephosphorylation of the PI3K p85 isoform thus abrogating p85 recruitment. This might lead to inactivation of the PI3K catalytic subunit (p110) preventing the signal transmission of pathways

downstream of PI3K. Further studies are warranted to better understand the signaling pathways associated with ROR1 and downstream signaling effects of ROR1 targeting drugs.

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A B-CELL EPIGENETIC SIGNATURE DEFINES THREE BIOLOGICAL SUBGROUPS OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH MAJOR CLINICAL IMPACT

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Background: Prospective identification of patients with chronic lymphocytic leukemia (CLL) destined to progress would greatly facilitate their clinical management. Recently, whole-genome DNA methylation analyses identified three clinico-biological CLL subgroups with an epigenetic signature related to different normal B-cell counterparts.
Aims: To develop a clinically-applicable method to identify these three new subgroups and to study their clinical relevance.
Methods: Using a linear discriminant analysis, we built a prediction model using five epigenetic biomarkers that was able to accurately classify CLL patients into the three subgroups, namely naive B cell-like, intermediate and memory B cell-like CLL. DNA methylation was quantified by highly reproducible bisulfite pyrosequencing (BPS) assays in two independent CLL series.
Results: In the initial series (n=211), the three subgroups showed differential levels of *IGHV* mutation ($P<0.001$) and *VH* usage ($P<0.04$), as well as different clinical features and outcome in terms of time-to-first-treatment (TTT) and overall survival ($P<0.001$). A multivariate Cox model showed that epigenetic classification was the strongest predictor of TTT ($P<0.001$) along with Binet stage ($P<0.001$). These findings were corroborated in a validation series (n=97).
Summary and Conclusions: In this study, we developed a simple and robust method using epigenetic biomarkers to categorize CLLs into three subgroups with different clinico-biological features and outcome.

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF TUMOR SPECIFIC ANTIBODY RESPONSES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is characterized by extreme clinical and biological variability. Several phenomena of immune dysfunction and autoimmunity have been described in CLL but little is known about antibody (Ab) immune responses.
Aims: This study aims at understanding the functional role of humoral responses in CLL and specifically at 1) identifying circulating Ab in CLL pts' sera; 2) characterizing tumor antigens (Ag) recognized by serum Ab and 3) evaluating whether the identified immune responses have a cytotoxic effect toward leukemic cells.
Methods: Proteins from CLL cells were separated to obtain proteomic maps which were blotted with autologous sera to reveal Ab-based reactivity. To verify the CLL-specificity of recognition, 8 pts maps were also probed with sera of 8 healthy donors (HD). Ag spots were identified by Mass Spectrometry (MS). Ag surface expression on viable and apoptotic cells was evaluated by flow cytometry after 4 days of culture. C4 deposit was evaluated by flow cytometry. Complement-dependent cytotoxicity (CDC) was performed incubating CLL cells alone or in presence of alemtuzumab and subsequently with CLL pts' sera for 1h at 37°C. Cell viability was evaluated by Annexin-V/Propidium Iodide assay. Disease progression was evaluated according to IWCLL/NCI-WG 2008 guidelines for CLL. Statistical correlations were performed using t-test, Mann-Withney rank sum test and 2-test.
Results: Sera obtained from 35 untreated CLL pts were individually screened for the presence of IgG-based reactivity against autologous proteins extracted from leukemic cells. As a control, sera obtained from 8 HD were probed on as many CLL maps. Overall, the number of spots produced from the 35 CLL sera was significantly higher than the number produced by the 8 HD sera (145 *vs* 3, respectively; p=.007). MS analysis allowed the identification of 50 proteins that were recurrently recognized by pts sera. The 5 most frequently recognized proteins were ENOA, CAZA1, G3P, ODPB, GDIR2. Sera from 29 out of 35 CLL pts exhibited immunoreactivity. Among these, 69% were polyreactive and recognized up to 20 Ag. Pts with progressive CLL showed a significantly higher number of spots compared to pts with stable disease (p=.031). Sera from 54% of pts exhibited reactivity toward ENOA, whereas none of the HD sera was ENOA-reactive. Flow cytometry revealed that ENOA was not expressed on the surface of CLL B cells at baseline conditions. We observed that ENOA surface expression was significantly increased on the apoptotic fraction of CLL cells (p=.008) but not on viable fraction after 4 days of culture. ENOA Ab-reactivity significantly correlated with higher number of leucocytes, higher lymphocytosis and lower platelet count (always p<0.01). The presence of specific anti ENOA-, G3P-, GDIR2- Ab was significantly correlated with the status of progressive disease (p=.02). We therefore evaluated whether the humoral responses identified by SERPA were capable of trigger CDC. Results from CDC assay demonstrated that there was no deposit of the C4 component on CLL cells surface and that CLL sera had no cytotoxic effect toward leukemic cells.
Summary and Conclusions: In this study we identified tumor Ag capable of eliciting humoral responses in CLL. Circulating Ab identified by SERPA in most pts' sera were not able to induce CDC toward leukemic cells and the Ab-based reactivity correlated with parameters of disease progression. Our results suggest that the immunoreactivity against self-Ag may be the expression of a dysfunctional immune system in CLL.

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RAPID DETERMINATION OF SOMATIC HYPERMUTATION STATUS OF IMMUNOGLOBULIN HEAVY CHAIN GENES USING NEXT GENERATION SEQUENCING AND LYMPHOTRACK™ BIOINFORMATICS

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Background: During B-cell development, genes encoding the *IGH* molecules are assembled from V, D, and J gene segments to generate V-D-J combinations of unique length and sequence in each cell. Since leukemias and lymphomas originate from the malignant transformation of individual lymphoid cells, they generally share one or more of these cell specific or "clonal" gene rearrangements. After gene rearrangement, normal B cells undergo somatic hypermutation (SHM) in germinal centers in an antigen driven process designed to generate additional diversity within the antigen binding CDR3 regions. The cells then undergo further selection as part of a process termed affinity maturation. The presence of SHM is defined as ≥2% difference from the germline V gene sequence. SHM status provides important prognostic information for patients with chronic lymphocytic leukemia (CLL). Presence of SHM is associated with better prognosis. Sequencing of IGH provides information on variable segment usage, and, in specific instances, clinical outcomes can be further predicted based on V gene usage. Specifically, presence of V3-21 portends a worse prognosis independent of mutation status or other prognostic indicators. The gold standard method used to determine SHM status requires two steps: a PCR/capillary electrophoresis (CE) based method to detect clonality, followed by Sanger sequencing to establish SHM status. This approach is labor intensive and time consuming.
Aims: Develop a sensitive, robust and reliable NGS assay with bioinformatics tools for identifying clonal *IGH* gene rearrangements, specific clonal V-J sequences, and SHM status.
Methods: Invivoscribe's MiSeq formatted SHM Assay employs two master mixes. One amplifies the genomic DNA between the upstream leader (VHL) region and the downstream joining (J) region of the IGH gene. The other amplifies from the framework1 (FR1) to J region. Amplicon products from VHL/J primers span the entire variable (V) region, including the FR1, CDR1, FR2, CDR2, FR3, and CDR3 regions. Amplicon products from FR1/J primers encompass portions of the FR1 region to the downstream J region. Multiplexed PCR is followed by amplicon purification using the AMPureXP PCR system. The purified equimolar amounts of amplicons are pooled to form a library. A portion of the library is loaded onto the MiSeq sequencing system and sequenced using the MiSeq v3 Reagent kit (600 cycles). The MiSeq data is analyzed using Invivoscribe LymphoTrack™ bioinformatics software which generates frequency distributions, identifies V-J DNA sequences, V-J gene usage, and determines the SHM status.
Results: This assay demonstrated excellent linearity ($R^2>0.95$), sensitivity (<1%) and reproducibility using contrived samples. Both the SHM results obtained from this assay, as well as V3-21 status, were concordant with data from traditional methods using gel extraction and Sanger sequencing. CLL samples tested with this assay demonstrated excellent agreement with clinical outcomes.
Summary and Conclusions: A comprehensive NGS assay has been developed for the Illumina MiSeq platform that identifies clonal IGH V-J rearrangements and associated specific V-J region DNA sequences. The assay can be used to determine the SHM status in CLL specimens and provide frequency

distributions of V region and J region segments important in detecting IGH V3-21, an independent determinant of poor prognosis in CLL.

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CONCORDANCE IN IGH & TRG CLONALITY TESTING: COMPARISON OF DATA GENERATED USING THE MISEQ & PGM PLATFORMS.

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Background: Assays that identify clonal lymphocyte populations in clinical specimens are used on a routine basis to assist in the detection of lymphoproliferative disease. Sensitive assays that identify antigen receptor loci-specific V-J gene rearrangement DNA sequences in clinical samples are proving to be powerful tools for evaluating the efficacy of therapeutics, aiding in the detection of initial clonal populations, and identifying sequence information required to track those clones in subsequent samples. PCR-based methods with amplicon detection by capillary electrophoresis have long been the gold standard for clonality testing. However, these methods often do not provide sufficient sensitivity and, without separate sequencing steps, do not provide the data required to identify the specific V-J DNA sequences required to track clones in follow up testing. The emergence of massively parallel sequencing (MPS) platforms has facilitated development of powerful new approaches for detecting and monitoring clonality. Here we present data on the analytical performance of Invivoscribe's LymphoTrack™ assays and associated bioinformatics on two popular MPS platforms.
Aims: To develop NGS assays for T and B cell clonality detection utilizing TRG and IGH gene rearrangements respectively and to further demonstrate the concordance of their results on both the PGM and MiSeq platforms.
Methods: Genomic DNA from peripheral blood, tonsil and bone marrow aspirates were tested for *TRG* and *IGH* gene rearrangements. Optimized V and J consensus primers targeted all *IGH* and *TRG* V and J region gene segments that are rearranged in lymphoid malignancies. Multiplex PCR master mixes were used to amplify *IGH* or *TRG* V-J gene rearrangements separately. On the Illumina platform the libraries for IGH and TRG assays and products were routinely combined and run on the same MiSeq run. For PGM formatted assays separate PCRs were used to generate amplicon for reads in each direction. The amplicon libraries were combined prior to the emulsion PCR step. PGM amplicon products were purified, quantified, pooled and the harmonized libraries were loaded onto the OneTouch 2. The enriched emulsion PCR libraries were sequenced using the Ion 316 Chip Kit v2 and Ion PGM Sequencing 400 Kits. MiSeq libraries were quantified and harmonized then loaded directly onto the MiSeq. Data from both instruments were analyzed using Invivoscribe's LymphoTrack™ bioinformatics software run on a standard Windows computer.
Results: Cell line DNA serially diluted into polyclonal tonsil DNA confirmed the linearity and low run-to-run variance of the assay on both platforms. Both PGM and MiSeq formatted assays analyzed using the LymphoTrack, ^{TM}, bioinformatics software package reproducibly identified clonality and DNA sequences for each *TRG* and *IGH* V-J gene rearrangement. Automated data outputs from paired samples on both platforms were concordant demonstrating equivalent results between platforms, as well as platform-specific intra- and inter-assay reproducibility. When coupled with the LymphoTrack, ^{TM}, bioinformatics and visualization software these assays provide robust detection and enhanced data-rich outputs.
Summary and Conclusions: Data from both platforms are equivalent and can be used to identify clonal lymphocyte populations by their unique DNA sequence, which can be subsequently used to track clones in follow up testing. Having access to both platforms allows laboratories to adapt workflow according to testing needs and throughput.

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THE GENE EXPRESSION RESPONSE TO INTERLEUKIN-4 AND ITS DIFFERENTIAL MODULATION BY NFKB CORRELATE WITH ZAP70 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: In chronic lymphocytic leukemia (CLL), interactions of the malignant B lymphocytes with microenvironment play a crucial role in disease progression. Interleukin 4 (IL-4), an essential mediator of B cell development, induces signaling cascades that lead to activation of transcription factors STAT6 and NFkB, affecting proliferation, differentiation and survival.
Aims: The aims of this study were to compare the gene expression changes induced by IL-4 in CLL and in normal B cells (NBC), and to determine how these are affected in CLL by inhibitors of the IL-4 pathway.
Methods: The gene expression profiles (GEPs) of the B cells from 23 patients and 13 controls were performed by oligonucleotide microarrays including ~ 40 000 probe sets designed to cover the whole human genome. The basal GEPs, and the GEPs of cells cultured with nothing, were used as references for comparison with the GEPs of cells cultured with IL-4. An one-way ANOVA test with post hoc analysis was used to identify the IL-4 targets, defined as genes significantly up-regulated or down-regulated by IL-4 with respect to the two references ($p<0.05$, 2-fold cut-off). Real time RT-PCR was performed for target validation. The effect of the NF-kB activation inhibitor 6-Amino-4-(4-phenoxyphenylethylamino)quinazoline on specific IL-4 targets was studied in selected patients.
Results: Sets of 232 non-redundant entities in CLL and 146 in NBC (95 common, 283 altogether), of which 189 were well-defined genes in CLL and 123 in NBC (83 common, 229 altogether) were identified as IL-4 targets. To the best of our knowledge, most of them were novel IL-4 targets for CLL (98%), B cells of any source (83%), or any cell type (70%). The intensity of their responses was significantly higher for 54 genes in CLL compared to NBC, and for 11 genes in NBC compared to CLL ($p<0.05$). In CLL, the response of a substantial part of the genes correlated positively or negatively with expression of ZAP70, and these were designated $ZAP70^{Pos}$ genes or $ZAP70^{Neg}$ genes. Wnt signaling, regulation of epithelial to mesenchymal transition, and cell adhesion, were associated to $ZAP70^{Pos}$ genes, whereas oxidative stress regulation, and angiogenesis were associated to $ZAP70^{Neg}$ genes. The response of most $ZAP70^{Pos}$ genes to IL-4 was downregulated by an NFkB activation inhibitor, and the response of most $ZAP70^{Neg}$ genes to IL-4 was upregulated by the inhibitor, suggesting a novel mechanism by which NFkB sinergizes or antagonizes the response of specific genes to IL-4.
Summary and Conclusions: Sets of novel and specific IL-4 targets were identified in CLL and NBC. A relationship between IL-4, ZAP70 expression and NFkB inhibition suggests a novel mechanism for regulating gene expression in CLL. Our study support that strategies directed to the IL-4 pathway may be of therapeutic interest.

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ORAL FCR INDUCES HIGHER COMPLETE REMISSION RATES AND MRD NEGATIVITY IN UNTREATED CLL THAN PREVIOUS REPORTS OF INTRAVENOUS THERAPY: COMBINED RESULTS OF THE NCRI ADMIRE AND ARCTIC TRIALS

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Background: Fludarabine, cyclophosphamide and rituximab (FCR) is the gold standard treatment for patients with CLL who require therapy and are considered fit for fludarabine based treatment. FC has been delivered intravenously previously but in the UK the oral route of administration for F and C is standard. Here we report higher rates of complete remission and MRD negativity in 207 patients treated with oral FCR in two parallel phase IIB NCRI trials (ADMIRE and ARCTIC).

Aims: To assess the efficacy of oral FCR control arm of ARCTIC and ADMIRE trial.

Methods: ARCTIC and ADMIRE were two phase IIB randomised controlled trials in the same patient population of untreated CLL conducted between June 2009-September 2012. ADMIRE recruited 215 patients from 29 UK research centres; ARCTIC recruited 200 patients from 35 UK research centres. Both trials had the same FCR control arm (n=207) but different experimental arms. FCR treatment included IV rituximab given on day 1 (375mg/m^2 Cycle 1; 500mg/m^2 Cycles 2-6), oral fludarabine (24mg/m^2/day for 5 days) and oral cyclophosphamide (150mg/m^2/day) for 5 days repeated every 28 days. ADMIRE compared FCR to FCM-R (addition of mitoxantrone to FCR) while ARCTIC compared FCR to FCM-miniR (addition of low dose rituximab to FCM). Patients with neutropenia delaying therapy received G-CSF (lenograstim 263mcg/day; Days 7-13) on all remaining cycles. Prophylaxis with co-trimoxazole and acyclovir was used. For both trials, the primary endpoint was complete response rates (CR/CRi); short-term secondary endpoints included MRD eradication, overall response rates (ORR) and safety.

Results: A total of 207 patients were randomised to the oral FCR control arms in ADMIRE and ARCTIC. The median age was 62 (38-77) and 35.7% were over 65; 72.5% male; 15.9% progressive stage A, 48.3% stage B and 35.7% stage C. 16.4% had a creatinine clearance under 60ml/min. 66.3% (114/172) had unmutated Ig genes; 7.0% (13/186) 17p deletion; and 14.4% (27/187) 11q deletion. 87.4% (181/207) of patients received greater than 3 cycles of treatment with 73.4% (152/207) receiving all 6 cycles. 102 (51.5%) of 198 received G-CSF during treatment. 132 (63.8%) patients experienced a dose modification. Of the assessable 187 patients, 137 (73.3%) achieved CR/CRi and 187/193 (96.9%) achieved at least a PR. MRD by flow cytometry (sensitivity 10^{-4}) was assessed in marrow 3 months post treatment, 58.1% (108/186) of assessable patients had undetectable MRD (108/186).

Summary and Conclusions: The response rates (CR/CRi and MRD eradication) to FCR given orally in 207 patients in ARCTIC and ADMIRE are much higher than previous multicentre studies of intravenous therapy. We report response rates of 73.3% for FCR and 69.7% for the whole cohort of 415 patients. This compares favourably to CR rates of 38% for FC in LRF CLL4 Trial and 44% for FCR in the GCLLSG CLL8 Trial. MRD in the marrow was undetectable in 58.1% of patients receiving FCR in control arm and 53.1% in the whole cohort. The overall response rate was 96.9% (CR/CRi+PR) in FCR control arm and 96.2% in the whole cohort. Explanations that may contribute to this improved efficacy maybe that: the (1) FC chemotherapy is spread over 5 rather than 3 days per cycle; (2) dose intensity and timing was prospectively supported by the use of G-CSF (51.5% patients) which along with appropriate dose modification enabled ¾ of patients (73.4%) to receive 6 cycles of FCR; and (3) patients received prophylaxis with co-trimoxazole and aciclovir. Our results suggest that FCR is more effective when given orally with greater convenience to patients and reduced cost justifying the use of this oral regimen.

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DEVELOPMENT AND VALIDATION OF COMPREHENSIVE CLL MUTATION DETECTION PANEL USING ION TORRENT NEXT GENERATION SEQUENCING PLATFORM

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Background: Mutations of multiple genes have emerged as important risk stratification and prognostication indicators in chronic lymphocytic leukemia (CLL). These mutations affect a heterogeneous population of genes involved in a wide variety of functions including sensing of DNA damage, DNA repair, signal transduction and spliceosome assembly and function. Several of these mutations show an association with poor response to certain types of therapy in CLL. Moreover, some of these mutations are absent at initial presentation and appear rapidly in a subset of patients who become therapy resistant or show disease progression including those with Richter's transformation. These findings create a need for a rapid, reproducible and sensitive method for mutation identification occurring on a sub clonal level obtained at initial presentation and during therapy allowing for "real time monitoring" of high risk CLL patients involved in clinical trials. Considering the high number of mutations and that some of these mutations show a wide distribution throughout large genes such as ATM, the Sanger sequencing method currently used in most clinical laboratories is not suitable for such rapid analysis.

Aims: To address this method challenge, we developed and validated a comprehensive CLL focused mutation analysis panel using Next Generation Sequencing/Ion Torrent platform.

Methods: A CLL focused mutation panel was developed using the AmpliSeq design site. The following genes were included: ATM, BIRC3, BRAF,BTK, CCND1, CCND2, CRM1, DDX3X, ERK1, ERK2, FBXW7, KLHL6 exon1, KRAS, MDM2 snp309, MYD88, NOTCH1 exon34, TP53, PLCG2,SF3B1, SMARCA2 and ZMYM3 with in silico coverage 98.9% of targeted sequence. Barcoded libraries were prepared in two pools using 15ng of DNA derived from isolated CLL cells from baseline samples of 64 patients with previously untreated CLL treated on a Sarah Cannon Research Institute clinical trial. Following standard processing, samples were analyzed on the Ion Torrent using 316 and 318 chips. Raw data was analyzed by Ion Torrent Server Suite v.3.6.2. Calls were made using variant Caller v3.6.63335 using Somatic Low Stringency setting. Coverage analysis was performed using Coverage Analysis v.3.6.633324. For a subset of specimens, multiple runs were performed to evaluate reproducibility and precision.

Results: With the exception of NOTCH1 exon34 and MDM2 snp309 (that will be reported separately) all 64 samples were successfully analyzed with an average depth of 685 (range 308 to 2123) and average uniformity of 90% (range 65.8 to 95.3). Comparison of in silico and analytical runs showed highly concordant on target coverage of 98.1% (range 96.3-99.4). Detected mutations and SNPs were observed with expected frequencies consistent with those reported in the literature and SNP databases. There was 100% complete agreement between multiple runs of samples analyzed in separate experiments. Complete analysis of 5 samples of DNA performed by a single technologist was reproducibly achieved in 5 working days.

Summary and Conclusions: The Ion Torrent platform based NGS comprehensive CLL mutation detection panel as noted above allows for the rapid, accurate, reproducible and sensitive detection of multiple mutations a in large number of genes. After further validation in a clinical CLIA certified molecular laboratory (ongoing) this approach will offer real time analysis of mutations of multiple genes in high risk CLL patients participating in clinical trials to help guide appropriate initial therapy and modification of therapy during treatment.

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PHASE 1 STUDY OF SINGLE AGENT CC-292, A HIGHLY SELECTIVE BRUTON'S TYROSINE KINASE (BTK) INHIBITOR, IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: CC-292 is an oral, highly selective small-molecule covalent inhibitor of Btk, which plays an important role in the biology of B-cell malignancies.
Aims: This phase 1 trial investigated the safety, dose limiting toxicities (DLT), and activity of single agent CC-292, administered once (QD) or twice daily (BID) to pts with relapsed or refractory (R/R) CLL and NHL. Here, we focus on the safety and clinical activity in CLL and small lymphocytic lymphoma (SLL) pts.
Methods: Eligible pts with R/R CLL/SLL after ≥1 prior therapy received CC-292 at doses of 125, 250, 400, 625, 750, and 1000mg QD or 375 and 500mg BID. As the maximum tolerable dose was not reached, CLL pts were enrolled in two expansion cohorts at 750mg QD and at 500mg BID. All pts received continuous dosing in 28-day cycles until progressive disease or intolerable toxicity. Clinical activity was investigator assessed per 2008 IwCLL criteria. This analysis reports safety and efficacy on all pts enrolled as of cutoff date 12-31-2013.
Results: A total of 84 CLL/SLL pts were evaluated across all doses for safety, including 29 and 28 CLL pts at 750mg QD and 500mg BID, respectively, in the expansion cohorts. Median age was 66.5 (34–89) and median number of prior therapies was 3 (1–12). 56 (67%) pts had ≥1 high risk factor, including 45 (54%) with unmutated IgHV, 18 (21%) with del11q22, and 20 (24%) with del17p. Median time on therapy was 254 days (16–797). Only one CLL patient at the 500mg BID dose level experienced a DLT (grade 4 thrombocytopenia) during Cycle 1. Otherwise, grade 3/4 adverse events (AEs) occurring in ≥2 pts in the 750mg QD, 1000mg QD, 375mg BID, and 500mg BID dose cohorts included pneumonia (17%), febrile neutropenia (7%), diarrhea (7%), neutropenia (5%), thrombocytopenia (5%), fever (5%), sepsis (4%), anemia (4%), respiratory failure (3%), and confusion (3%). Overall, 67 SAEs were observed in 37 pts; 21 SAEs were deemed possibly related to CC-292. Results are summarized for 70 efficacy-evaluable pts at dose levels 750mg QD, 1000mg QD, 375mg BID, and 500mg BID (29, 7, 6, 28 pts, respectively). The PR rate at the 4 dose levels was 34%, 57%, 67%, and 39%, respectively. In addition, PR with lymphocytosis was achieved by 7 (24%) pts at the 750mg QD dose level and 6 (21%) pts at the 500mg BID dose level. In pts with del11q, del17p, unmutated IgVH, and no high-risk genomic factors, the PR rates were 46% (6/13), 47% (7/15), 61% (20/33), and 27% (6/22), respectively. 87% of pts experienced a ≥25% increase in absolute lymphocyte count (ALC), which usually resolved with continued treatment. Importantly, nodal responses were induced in the majority of pts receiving BID dosing (375mg: 67%; 500mg: 61%) with lymph node size reduction over time from cycle 2 (mean reduction of 42% and 40%, respectively) to cycle 7 (mean reduction of 60% and 60%, respectively). At time of data cut, 41%, 43%, 83%, and 73% of pts were still on study at dose levels 750mg QD, 1000mg QD, 375mg BID, and 500mg BID, respectively.
Summary and Conclusions: CC-292 is well tolerated as an oral daily monotherapy. Single-agent therapy with CC-292 is sufficient to achieve high nodal and partial response rates in R/R CLL pts, including those with high-risk cytogenetic features. These results support continued development of CC-292 for the treatment of pts with CLL/SLL. Two Phase 1b studies of CC-292 in combination with lenalidomide or rituximab, respectively, are ongoing to determine safety and tolerability, evaluate preliminary efficacy, and ascertain whether these combination regimens might improve outcomes in CLL pts.

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PRE-TREATMENT WITH IDELALISIB MARKEDLY REDUCES RITUXIMAB INFUSION-RELATED REACTIONS AND INFUSION INTERRUPTIONS IN PATIENTS WITH CLL

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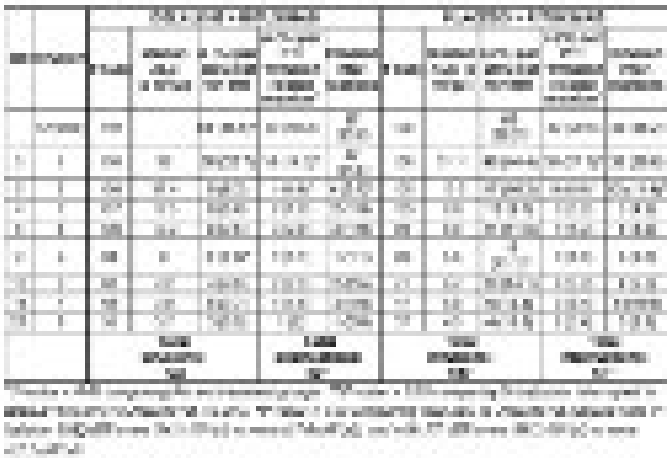
Background: Rituximab (R) and other anti-CD20 antibodies are important agents in the treatment of CLL. However, infusion-related reactions (IRRs) during the first infusion of R in patients with CLL have been reported to occur as frequently as 94% (O'Brien 2001), presenting a significant medical burden. IRRs may be more frequent in patients with high absolute lymphocyte counts (ALCs) (Winkler 1999). The combination of the oral selective inhibitor of PI3Kδ idelalisib with R has been demonstrated to be highly active in heavily pretreated patients with CLL in a Phase 3 trial (Furman, NEJM 2014).
Aims: As PI3Kδ is expressed in various cell types that may be involved in cytokine release and response, we assessed if IDELA has an effect on the frequency of R IRRs.
Methods: A Phase 3 double-blinded study evaluated IDELA+R *vs* placebo+R in pts with relapsed CLL who experienced progression within 24 months since completion of the last therapy and who were considered unfit to receive cytotoxic therapy. R was administered at 375 mg/m^2 (1st dose) and then at 500 mg/m^2, every 2 weeks for the next 4 doses and then every 4 weeks for a total of 8 doses. Infusion premedication as recommended by the protocol included an antipyretic and an antihistamine; local procedures often also included steroids. Per protocol, the 150 mg standard dose of idelalisib/placebo (150 mg BID) was recommended to be administered 30 minutes before the start of each R infusion. IRRs were analyzed in two ways: 1) per MedDRA preferred term (PT) "infusion-related reaction" (PT-IRR), and 2) per any adverse event (AE) starting on the day of infusion and on the list of Standardised MedDRA Queries (SMQ) as related to IRRs (SMQ-IRR). The data presented are from an exploratory analysis based on an interim analysis of the Phase 3 study.
Results: 220 subjects were randomized 1:1 and 218 received at least one dose of rituximab, 110 with IDELA and 108 with placebo. 99.7% of infusions were preceded by premedication. The Table 1 depicts the frequencies of the IRRs, along with the respective median absolute lymphocyte count (ALC). On both arms, IRRs were most common after the 1st and 2nd R infusions. Placebo+R subjects who experienced dose #1 infusion reactions had higher ALCs than those who didn't ($p<0.05$). Subjects on IDELA+R experienced significantly fewer ($p<0.05$) PT-IRRs during the 1st and 2nd infusions despite a 3.5 fold higher median ALC at the 2nd infusion. In addition, when comparing the overall incidence of SMQ-IRR AEs, subjects on IDELA+R experienced significantly fewer ($p<0.05$) events. Fewer infusion interruptions were required in the IDELA+R arm than in the placebo+R arm (5.8% *vs* 11.1%, $p<0.05$). Gr 3 IRR AEs were reported for 3 infusions (in 3 subjects) in those receiving IDELA and for 7 infusions (in 5 subjects) in those receiving placebo.

Table 1.



Summary and Conclusions: In this randomized double-blinded Phase 3 study, IDELA treatment given only 30 minutes before R reduced the frequency of IRRs and infusion interruptions. This protective effect was even more marked with the second R infusion when subjects had been receiving IDELA for 2 weeks, despite a 3.5-fold higher ALC in the IDELA group. This finding is consistent with the known mechanism of action of IDELA, and indicates that IDELA reduces the significant burden of IRRs in patients receiving R, and could potentially do so with newer generation anti-CD20 antibodies.

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SEVERE INFECTIONS ARE MORE COMMON IN PHYSICALLY FIT CLL PATIENTS WITH FCR-THERAPY COMPARED TO BR: RESULTS OF THE INTERMIN-ANALYSIS OF THE CLL10 TRIAL OF THE GERMAN CLL STUDY GROUP

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Background: Infections are common complications in CLL patients correlating with immune defects caused by the disease itself as well as treatment-related immunosuppression. Fludarabine, cyclophosphamide and rituximab (FCR) as first-line treatment is associated with a higher risk of infection compared to bendamustine plus rituximab (BR).
Aims: This analysis was performed to prospectively assess frequency, characteristics and risk factors of infections in advanced CLL during and after first-line with FCR *versus* BR in physically fit patients.
Methods: Within the multicenter phase-III CLL10 trial detailed information on the incidence of infections were prospectively evaluated. Except for PCJ-prophylaxis in longer lasting neutropenia the protocol did not recommend routine anti-infective prophylaxis during and after chemoimmuntherapy.
Results: 561 patients were included in the study, 282 (50.3%) were randomised to the FCR and 279 (49.7%) to the BR arm. Median observation time was 27.9 months. 395 (70.4%) of all patients developed an infection. A total of 1050 infections were reported and 26.2% were defined as severe (CTC grade 3-5). When comparing the different treatment arms significantly more patients treated with FCR developed an infection (53.2 *vs.* 46.8%, p=0.034) and infections were more severe (29.8 *vs.* 21.3%, p=0.002). The average number of infections in all affected patients was 2.6. Median time from registration to onset was 4.6 months in the FCR-group compared to 5.0 months in the BR-group. Late infections occurred significantly more in the FCR-treated patients after initial response (23.8 *vs.* 10.7%, p<0.000) and final restaging (19.8 *vs.* 10.8%, p=0.008). The causative pathogen was identified as bacterial in 15.7%, as viral in 14.8%, as fungal in 2%, and as other in 2.3%. The pathogen was unknown in 66.8%. Viral infections were significantly more common in patients treated with FCR (20.8% *vs.* 12.3%, p=0.007). 13.9% of all infections were classified as fever of unknown origin, followed by pneumonia in 8.9%, and bacteremia in 3%. Severe pneumonia was more frequently observed in patients treated with FCR (32 *vs.* 17, p=0.027). Antibiotic treatment was administered in 76.1% of all documented infections, followed by antiviral, and antifungal treatment in 15.3, and 4.6% respectively. G-CSF was administered in 85 (8.3%) cases and was significantly more frequently given with FCR treatment (10.8 *vs.* 4.8%, p=0.001). Inpatient treatment was necessary in 141 and intensive care treatment in 12 patients, there was no significant differences in both treatment arms. Six patients in the BR arm and seven patients in the FCR arm died due to treatment related infectious complications. Pneumonias and sepsis were the reason for death in five patients each; one patient each died because of neutropenic colitis, hepatitis B and progressive multifocal leukencephalopathy.
Summary and Conclusions: Infections are more frequently observed in CLL patients treated with first-line FCR than BR. The causative pathogen is rarely detected and infections occur rather late during the course of treatment. In conclusion we recommend anti-infective prophylaxis in neutropenic patients during first-line chemoimmuntherapy.

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LATE ONSET NEUTROPENIA IS A COMMON COMPLICATION OF FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB (FCR) THERAPY AND IS ASSOCIATED WITH SUBSTANTIAL MORBIDITY AND HOSPITALIZATION

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Background: Neutropenic complications, including late-onset neutropenia (LON), are known to complicate FCR chemotherapy. However, the incidence, clinical significance and risk factors for LON remain unclear.
Aims: Determine the incidence and risk factors for the development of haematological complications associated with FCR.
Methods: We performed a retrospective analysis of 104 consecutive patients (pts) who received FCR treatment across 2 major cancer centres between 01/2004 and 11/2012, in order to determine the incidence, clinical consequence and risk factor for development of neutropenic complications (a) during therapy, and (b) in the first year after treatment completion. Eight-six pts received follow-up at their primary treatment center, with a minimum of 3 complete blood count over the 12 month study period. LON was defined as grade III-IV neutropenia (G3-4N) developing at least four weeks after cessation of therapy. Severe prolonged cytopenias are defined as grade III-IV cytopenias persisting for >4 weeks post completion of treatment.
Results: DURING THERAPY: the median age of the 104 pts (71M, 33F) was 66 years (range: 40-83). 69 received FCR for CLL; 35 for low-grade non-Hodgkin lymphomas. 44 previously untreated and 60 had prior chemotherapy [median 2 (range 1 – 6) regimens]. The median number of FCR cycles received was 4 (range: 1-6), and 16 pts received maintenance rituximab. G3-4N occurred in (43%), and febrile neutropenia in 20% of pts (5.8% per cycle). ONE YEAR POST THERAPY: Of the 86 pts assessable, LON was documented in 25 (29%) after a median of 95 (range 53-326) days from treatment cessation. The median LON neutrophil count was $0.4x10^9$/L (range: 0.0-0.9). LON was associated with substantial morbidity: 28% of pts were hospitalized for neutropenic complications and 32% were administered antibiotics and G-CSF. There was no significant association between the risk of LON development and age, ECOG, disease subtype, previous therapy, number of FCR cycles, maintenance rituximab, baseline hemoglobin / neutrophil / platelet or baseline creatinine. However, there was a positive association between LON and the occurrence of G3-4N during treatment: of those pts who developed G3-4N during therapy (n=35), LON developed in 15 (43%; p=0.017 compared with 19% in pts without G3-4N, Figure 1); this risk of LON rises to 57% for pts who developed G3-4N and received GCSF support. Among pts who did not develop G3-4N during treatment, female sex emerged as the dominant risk factor for LON (33% risk, *vs* 13% for males, p=0.07). Prolonged severe cytopenias occurred in 20 pts and similarly was more common in women (p<0.001) and those with grade 3-4N during treatment (p-0.005).

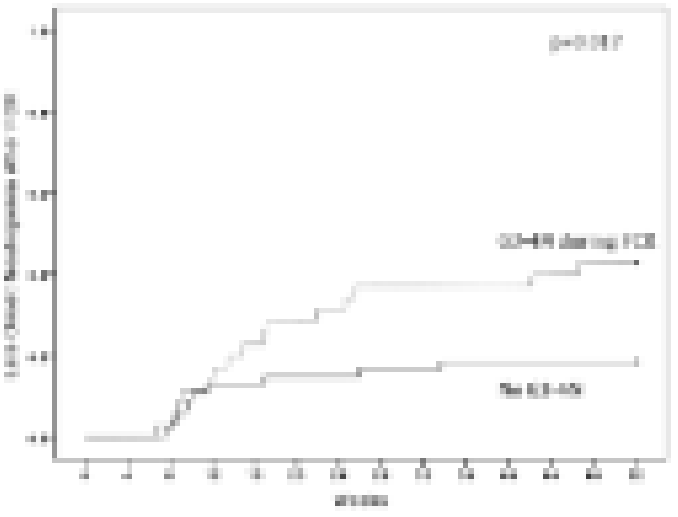

Figure 1.

Summary and Conclusions: Neutropenic complications, particularly LON, is an under-recognized and poorly reported complication of FCR. LON in pts receiving FCR is associated with high morbidity and frequent hospitalization. Pts who develop G3-4N during FCR chemotherapy are at high risk of subsequent LON, particularly if their chemotherapy was supported using GCSF. Our results demonstrate that the onset of G3-4N during FCR chemotherapy identifies pts at high risk for late neutropenic complications, and sound a cautionary note regarding the use of GCSF support during FCR therapy.

P239

EFFECT OF IDELALISIB/RITUXIMAB COMBINATION TREATMENT OF RELAPSED CLL ON THE BCR SIGNALING-RELATED CHEMOKINES CCL3 AND CCL4: DATA FROM A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background: Idelalisib (IDELA) is a potent and selective inhibitor of PI3Kδ, which is critical for activation, proliferation and survival of B cells and their homing and retention in lymphoid tissues. Inhibition of PI3Kδ modulates BCR signaling as well as signaling through cytokine and chemokine receptors. Data

generated both *in vitro* and in clinical studies have demonstrated that IDELA reduces the levels of CCL3 and CCL4 (MIP-1α/β) (Hoellenriegel *et al.*, Blood, 2011), two chemokines secreted by CLL cells in response to BCR activation. Importantly, high levels of CCL3 and CCL4 have been associated with shorter PFS in CLL and therefore have been suggested as BCR-related risk factors with cut-off levels for negative prognosis of >10 pg/mL and >60 pg/mL, respectively (Burger *et al.*, Trends in Immunology 2013).

Aims: This report describes the effect of IDELA treatment on CCL3 and CCL4 levels in patients enrolled in a randomized, placebo controlled phase 3 study.

Methods: GS-US-312-0116 is a phase 3, randomized double-blind placebo-controlled study evaluating the efficacy and safety of IDELA in combination with rituximab (R) for previously treated CLL. The plasma levels of CLL3 and CCL4 were assessed at baseline (BL) and at Week 4 (Wk4) of treatment with IDELA+R or placebo+R. CLL3 and CCL4 quantification was performed by EMD Millipore, at St. Charles, MO, USA, using a bead-based enzyme-linked immunosorbent assay.

Results: As shown in the Table 1 below, there was a prominent reduction of CCL3 and CCL4 plasma levels in patients on IDELA+R after 4 weeks of treatment. The reduction in CCL3 and CCL4 levels at Wk4 was superior with IDELA treatment (82.7% mean decrease from BL; median level of 11.9 pg/mL for CCL3 and 63.5% mean decrease from BL; median level of 35.7 pg/mL for CCL4) as compared to placebo (24.3% mean decrease from BL; median level of 53.2 pg/mL for CCL3 and 21.3% mean decrease from BL; median level of 74.0 pg/mL for CCL4). This difference was highly significant (p-value <0.0001). In addition, 37% and 47% of patients with BL levels above the respective negative prognosis cut-offs for CCL3 and CCL4, reached levels below the cut-off at Wk4 of IDELA+R treatment. In comparison, on placebo+R, only 4% and 12% of patients, respectively, achieved such a reduction.

Table 1.

Summary and Conclusions: The results from this prospective, placebo-controlled study confirm that in patients with CLL, treatment with IDELA rapidly and prominently reduces the levels of chemokines related to activated BCR signaling. Moreover, as high levels of CCL3 and CCL4 have been suggested as BCR-related risk factors in CLL, the rapid normalization of their levels may contribute to the strong clinical benefit observed with IDELA and other emerging novel therapies that inhibit BCR signaling.

P240

B2-MICROGLOBULIN IS A DYNAMIC MARKER OF PROGNOSIS DURING TREATMENT OF CLL WITH FCR OR IBRUTINIB-BASED REGIMENS.

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Background: In CLL, free plasma β_2-microglobulin (B2M) correlates with disease stage and tumor burden. Elevated B2M at diagnosis is independently associated with shorter time-to-first therapy and when treatment is required, is independently associated with poorer progression-free and overall survival. While the significance of initial B2M is established, changes in B2M during therapy and associations with patient outcomes have not been explored.

Aims: We analyzed patients (pts) treated on investigational protocols with FCR and ibrutinib-based regimens (Ib) at MDACC from 2008-2013, to determine whether patterns of change in B2M differed according to treatment protocol and would be predictive of outcome.

Methods: Patients treated with FCR or Ib (Ib monotherapy, n=35, Ib+rituximab, n=37 or Ib+bendamustine+rituximab, n=11), between 2008 and 2013 were eligible for inclusion, provided they had ≥3 months of follow-up, a baseline B2M level and at least one other B2M measurement. Follow-up B2M measurements were not mandated in these studies and were performed according to individual physician practice. The following variables were analyzed: B2M at 6 months (where available), lowest level achieved and time to B2M normalization or nadir.

Results: Baseline characteristics were as follows:

Characteristic	Ib-based (n=83), n(%)	FCR (n=198), n(%)	P value
Age (median, range)	65 (35-83)	59 (32-79)	0.02
B2M ≥4.0	47 (57)	82 (41)	0.02
Rai Stage III/IV	47 (57)	80 (40)	0.01
IGHV MS			
Unmutated	58 (75)	106 (54)	<0.001
Mutated	9 (12)	71 (36)	
ZAP70+	48 (71)	119 (65)	0.43
17p-	35 (42)	11 (6)	<0.001

There was no correlation between B2M at baseline, 6 months or nadir and any other baseline characteristic, except Rai stage. B2M fell rapidly during Ib-treatment despite a transient lymphocytosis during this time. Despite a higher median baseline B2M, and higher-risk genetic features, similar rates of normalization of B2M were seen for pts treated with Ib-based regimens and FCR (63.9 *vs.* 67.2). 88% of treatment-naïve Ib-treated pts normalized their B2M (p=0.04 *vs* FCR). When only patients with >1 year of follow-up were analyzed (to avoid bias from shorter follow-up in the Ib patients), median time to normalization or nadir was shorter for Ib than FCR: 335 *vs.* 433 days, p=0.001 for Ib *vs.* FCR). Landmark PFS analysis was performed from the date of B2M nadir; normalization of B2M was associated with superior PFS in both Ib and FCR-treated pts (see Figure 1). This remained significant when stratifying for pts with baseline B2M <4.0 or ≥4.0. Achieving minimal residual disease (MRD) negativity by flow cytometry in FCR-treated pts is associated with superior survival. When only FCR pts achieving MRD-negativity were analyzed (n=112), a trend remained for improved PFS in pts who also normalized of B2M (n=83) *vs.* those who did not (n=29), p=0.08.

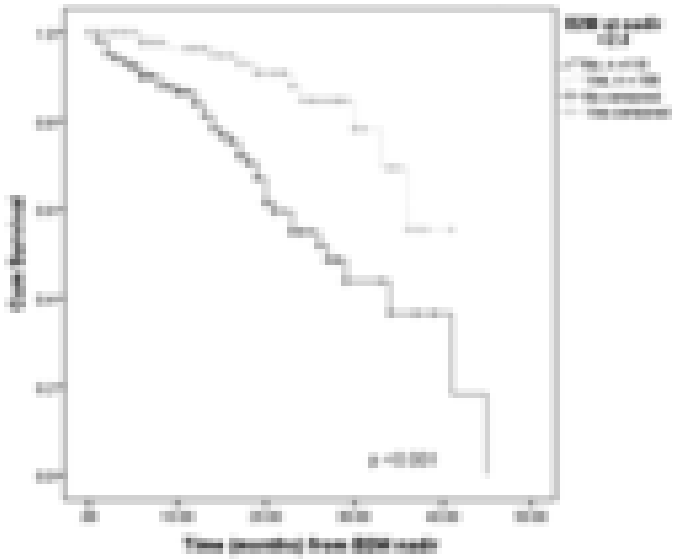

Figure 1. Landmark PFS from B2M nadir in all patients.

Summary and Conclusions: A more rapid and complete normalization of B2M level was observed with Ib treatment than with FCR, the reasons for which are unclear. B2M evaluated during treatment is a robust tumor marker and normalization of B2M was associated with improved PFS in both Ib- and FCR-treated pts. Serial B2M assessment may be particularly useful in Ib-treated patients to identify patients early who are at higher risk of relapse, especially as utility of MRD assessment is limited due to low rates of CR. Its ability to further subdivide patients achieving MRD-negativity after FCR requires further study. We intend to confirm these results in Ib-treated patients prospectively in a 200 patient study of Ib *vs.* Ib+rituximab.

P241

FIRST-LINE TREATMENT STRATEGIES FOR PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: PRELIMINARY RESULTS OF A NETWORK META-ANALYSIS

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Background: Chronic lymphocytic leukaemia (CLL) accounts for 25% of cases and is the most common lymphoid malignancy in Western countries. This disease affects predominantly elderly patients with a median age of 72 years at diagnosis. The highly variable course of CLL leads to a variety of therapy options for patients needing treatment. Chlorambucil (Clb) was considered standard first-line therapy for many years, but the implementation of newer treatment options have increased patient-related outcomes in comparison to Clb.
Aims: To assess the efficacy of different first-line therapy regimens and to provide a hierarchy of the best treatment options for previously untreated patients with CLL.
Methods: Sensitive search strategies for CENTRAL, EMBASE, and MEDLINE (1946 to 02.2014) were conducted to identify randomized controlled trials (RCTs). Furthermore, relevant conferences (ASCO, ASH, EHA) were searched up to 2013 and the German CLL Study Group provided unpublished data. RCTs that compared at least one treatment regimen to another regimen or to observation only in previously untreated CLL patients were included. Two authors independently assessed studies for their eligibility, extracted data, and assessed the quality of the trials according to the methodology standards of the Cochrane Collaboration. Overall survival (OS) and progression-free survival (PFS) were regarded as primary endpoints, secondary endpoints included treatment-related mortality and secondary malignancies. A Bayesian random-effects model was used for the network meta-analysis, combining head-to-head trials with indirect evidence. The results are reported relatively to Clb, a hazard ratio (HR) >1 indicating superiority of Clb.
Results: The sensitive search resulted in 6,871 relevant references, of which data from 33 RCTs were included. The final network was constructed from 21 different first-line treatment regimens and comprised a total of 11,638 patients. Overall, the quality of the evidence was judged as moderate. Data from 18 different regimens were available for analysis of OS in comparison to Clb. Seven regimens had a more than 50% probability to be better than Clb. Obinutuzumab (GA101) plus Clb (HR 0.41, 95% credible intervals (Crl) 0.22 to 0.78) showed the highest probability to be better than Clb. Treatment with Clb-rituximab (R) (HR 0.66, 95% Crl 0.33 to 1.32) showed a probability of 88%, bendamustine (B)-R (HR 0.64, 95% 0.30 to 1.44) of 87% and treatment with fludarabine, cyclophosphamide, rituximab (FCR) (HR 0.76, 95% Crl 0.47 to 1.27) reached a probability of 86% to be better than Clb. Regarding PFS, GA101 plus Clb (HR 0.18, 95% Crl 0.05 to 0.67) had a 99% probability to be better than Clb, followed by FCR (HR 0.42, 95% Crl 0.15 to 1.13) with a probability of 96%. Subgroup analysis for patients in Binet stages B/C only showed similar results for OS and PFS. Overall, the between trial heterogeneity was low τ^2=0.016 for OS. Results regarding treatment-related mortality and secondary malignancies will be awaited (Figure 1).

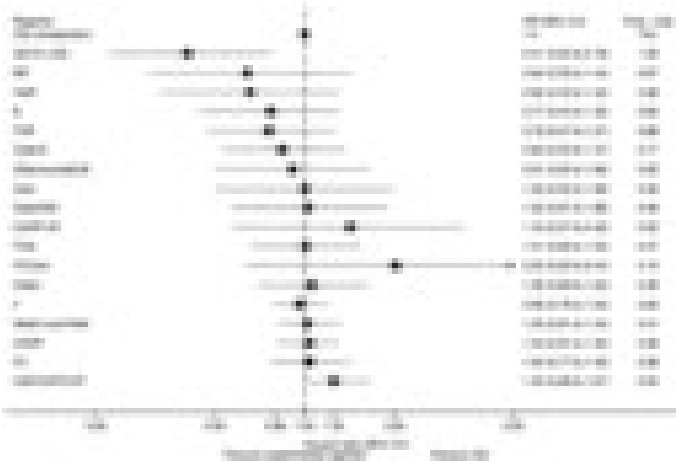

Figure 1.

Summary and Conclusions: This network meta-analysis shows a statistically significant improvement in OS following treatment with GA101 plus Clb for treatment-naïve patients with CLL. Also, FCR, BR and Clb-R shows a high probability to be better than Clb alone regarding OS. These results were similar for PFS. However, patients characteristics of the included trials were heterogeneous (fit *versus* less fit patients). Longer follow-up results of newer recent trials will give further information for patient-related outcomes.
This project was funded by the Federal Ministry of Education and Research, grant number: 01KG1015.

P242

CHRONIC LYMPHOCYTIC LEUKEMIA: A SMALL PROPORTION OF LONG-TERM SURVIVORS COULD ACTUALLY BE CURED

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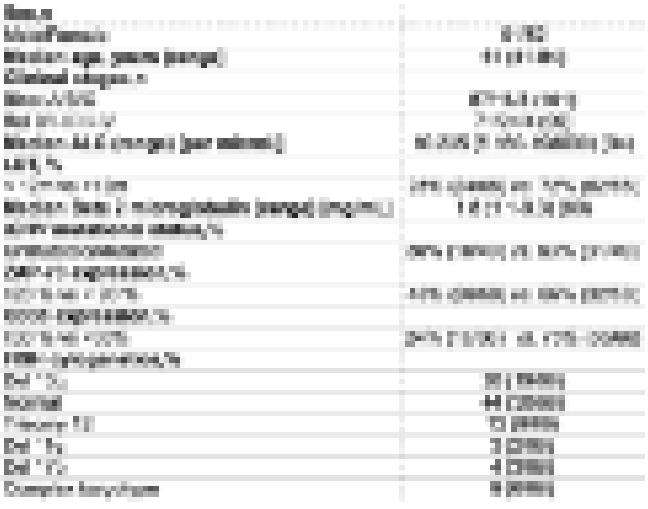
Background: Progress in the general management and treatment of patients with CLL has resulted in a longer overall survival. There is little information however on whether such an improvement is due to the favorable characteristics of patients at diagnosis and the indolent clinical course of the disease or to modern treatment approaches. Likewise, whether some patients with sustained response and long survival are actually cured is not known. The analysis of unselected series of patients with a long follow up can contribute to shed light on these issues.
Aims: To analyze the characteristics at diagnosis, over the course of the disease, and at last follow up of patients with CLL surviving more than 10 years from diagnosis.
Methods: One-hundred and three patients (24% of the whole series) from the Sant Pau Hospital from Barcelona with a survival longer than 10 years from the time of diagnosis were analyzed
Results: The main characteristics of the 103 patients are shown in the Table 1. Treatment-free survival at 10 and 20 years was 62% (95% CI, 52-70) and 39% (95% CI, 28-50), respectively. After a median follow-up of 14 years (range, 11-32), 56 patients required therapy. Most of these patients had unmutated IGHV genes and poor cytogenetics at the time of diagnosis. Front-line therapy consisted of alkylating agents (n=32) or fludarabine-based treatments (n=24). Thirty-eight patients (76%) achieved complete remission, 11 patients partial remission (22%), 1 patient (2%) had no response, and 6 were not evaluable. No significant differences in the response rate were observed among different prognostic subgroups. The median duration of response to first therapy was 33 months (range, 2-173). Duration of response was significantly longer in patients with good *vs.* poor cytogenetics and low *vs.* high β-2 microglobulin at treatment. Thirty-two of 56 patients had a second progression and received further treatment, and 21 patients were given ≥3 lines of therapy. Ten patients underwent stem cell transplantation (2 autografts and 8 allografts). Richter's transformation was documented in 3 out of 103 patients, while 8 patients developed a second malignancy and 5 patients developed autoimmune hemolytic anemia during the evolution of the disease. At last follow-up, 7 patients were free of disease (of which 3 had been treated with allogeneic stem cell transplantation, and the remaining with different modalities of therapy) and 80 patients remained alive but with signs of persistent disease.

Table 1. Clinical and biological characteristics at diagnosis (num).



Summary and Conclusions: Not unexpectedly, most patients with CLL enjoying very long survival had good clinical and biological features at diagnosis. Long-term survivors with poor prognostic factors (*i.e.*, unmutated IGHV genes, high expression of ZAP-70, and/or poor cytogenetics) were likely to progress

and require therapy. In patients requiring therapy, poor cytogenetics and high β-2 microglobulin were associated with a shorter duration of response. Further studies are needed to identify those patients in which achieving CR is a necessary condition for prolonging survival, as opposed to those in whom disease control (without aiming for a CR) is a reasonable treatment goal.

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RICHTER'S TRANSFORMATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): ANALYSIS OF BIOLOGICAL AND CLINICAL RISK FACTORS AND OUTCOMES

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Background: Among CLL patients (pts), 5-10% develop an aggressive large B-cell lymphoma, Richter's transformation (RT) that results in rapid clinical deterioration with increased lymphadenopathy, splenomegaly and worsening B symptoms. The risk factors for the development of RT are not well defined due to limited data.
Aims: The purpose of this study was to perform actuarial analysis of biological and clinical risk factors and survival outcomes among CLL pts with RT compared to a CLL control group (grp) with no RT. The parameters included were age grp, Rai stage, diagnosis decade, lymphocyte count, lactate dehydrogenase (LDH) level, hemoglobin (Hb), platelet count, prior treatment, immunophenotype and FISH cytogenetics.
Methods: A population based retrospective analysis through an electronic search of pts within the St Paul's Hospital CLL and BC Provincial CLL databases between 1980-2013 was carried out. RT patients were either biopsy confirmed or considered to have RT by treating physician based on clinical presentations such as rapid increase in lymph node size and LDH, unusual disease site such as CNS, Liver. Chi square test was used for comparison of parameters and association with RT using a CLL control grp without RT. Survival analysis was performed by the Kaplan-Meier method.

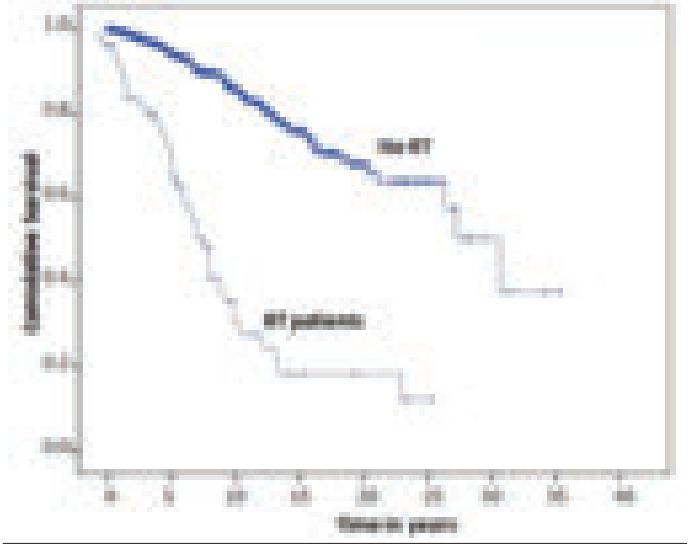

Figure 1. Overall survival of CLL patients with Ritchers Transformation (RT) *versus* control group of CLL patients with No RT.

Results: Among 1631 pts with CLL, 59 (3.6%) developed RT and of these 20 were biopsy confirmed. The median overall survival (OS) from the time of CLL diagnosis for the RT grp is 8.18 years (0-26 yrs) and for the CLL control grp is 30 years (0-35 years (yrs)), p=0.017. The median time from CLL diagnosis to RT was 4.9 years and the median OS post RT was 9 months (0-10.6 yrs). Within the RT grp, the median OS was worse for those with advanced Rai Stage - High: 2.0, Intermediate: 7.7 and low: 10.4 years (p=0.039) and those who had previous treatment for CLL had OS of 7.2 yrs *versus* 10.5 yrs with no previous treatments (p<0.001). However, there was no significant difference in OS for LDH>1.5 upper limit of normal (ULN), decade of CLL diagnosis and type of treatment. LDH>1.5ULN, advanced Rai stage, low Hb and low platelet counts at time of CLL diagnosis were significantly associated with the RT *versus* the CLL control grp (p<0.01). Among pts tested, flow cytometry and FISH at diagnosis showed CD38$^+$, (Odds ratio [OR] 4.76), trisomy 12 (OR 8.55), 11q- (OR 30.49) and 17p- (16.25) were significantly associated with RT (p<0.01 for all comparisons) but not for 13q- (OR 0.90). Within the RT grp, CD38$^+$ alone and flow pattern (typical/atypical) had no effect on OS. RT pts with high-risk FISH abnormalities (17p- or 11q-) had significantly worse OS compared to those without; while the presence of 13q- or +12 had no effect on OS (p<0.01). On multivariate analysis, only advanced Rai stage was significant with a Cox hazard ratio of 4.69 (p=0.01). Finally, using the BC province general population life tables, the 5 year relative survival ratio for the various age groups was 0.75 for the RT grp and 0.93 for the CLL control grp (Figure 1).
Summary and Conclusions: This study of 59 RT pts is the largest to date analyzing biological and clinical risk factors for outcomes. RT significantly shortened OS compared to CLL control grp. Pts with LDH>1.5ULN, advanced Rai stage, CD38$^+$ and >1 FISH abnormalities at diagnosis should be closely monitored for RT. Early treatment of CLL pts correlating to high risk of RT warrants further investigation. Expanding this study provincially and nationwide, along with a more comprehensive analysis of clinical and biological parameters and their combinations would help in better predicting RT among CLL pts leading to improved patient care.

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INTERIM RESULTS FROM A PHASE IIA STUDY OF THE ANTI-CXCL12 SPIEGELMER OLAPTESED PEGOL (NOX-A12) IN COMBINATION WITH BENDAMUSTINE/RITUXIMAB IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Olaptesed pegol (NOX-A12) is a novel, potent, L-stereoisomer RNA aptamer that binds and neutralizes CXCL12/SDF-1, a chemokine which attracts and activates immune- and non-immune cells via interaction with the receptors, CXCR4 and CXCR7. The signaling of CXCL12 is pivotal to the interactions of leukemic cells with bone marrow microenvironment. The therapeutic concept of olaptesed is to inhibit such tumor-supporting pathways and thereby mobilizing and sensitizing CLL cells to chemotherapy.
Aims: Here we aim to assess the activity and safety of olaptesed in combination with rituximab and bendamustine in patients with relapsed / refractory CLL.
Methods: This multicenter Phase IIa study investigates olaptesed alone and in combination with BR in relapsed / refractory CLL patients. The study population was split into a pilot and expansion group. Patients were treated using a dose titration design with intravenous (IV) olaptesed at doses increasing from 1 mg/kg to 2 mg/kg and 4 mg/kg at cycles 1, 2 and 3, respectively at 1 hour before rituximab treatment. During cycles 4 to 6, olaptesed was dosed at the highest individually titrated dose. Rituximab was administered IV at doses of 375 mg/m² on day 1 of 1st 28-day cycle and 500 mg/m on day 1 of subsequent cycles. Bendamustine (70 - 100 mg/m²) was given IV on days 2-3 (cycle 1) or days 1-2 (cycles 2-6) of each 28-day cycle following administration of rituximab. To study PK/PD, a pilot dose of 1 to 4 mg/kg olaptesed alone was administered to the initial 10 patients before start of the regular treatment regimen. Clinical response was assessed according to NCI-WG Guidelines (Hallek M *et al.* Blood 111; 2008: 5446-56). To date, 20 patients are evaluable (11 women, 9 men) with a median age of 67 years (range 52 to 79). At screening 4, 7 and 9 patients presented with Binet stage A, B and C, respectively. The median prior treatment line was one. 7 patients presented an unfavorable disease state being relapsed within 24 months after fludarabine or bendamustine treatment (4 patients) or presenting a deletion/mutation of the TP53 gene (3 patients). Most patients (15 of 20) were previously treated with fludarabine or bendamustine.
Results: Flow cytometric analysis of CD19$^+$/CD5^{high} CLL cells showed a rapid mobilization of these cells into the peripheral blood which lasted throughout the observational time of 72h. Reduction of lymphadenopathy by ≥50% occurred in 11 out of 14 evaluable patients by the end of treatment. Concomitantly, rapid reduction of lymphocytosis in peripheral blood with normalization by treatment cycle 2 – 3 was observed. Also, the CLL to leukocyte ratio improved on average from >60% in cycle 1 to <2% at cycle 4. Olaptesed at 1, 2 and 4 mg/kg BW at a single dose and in combination with BR was safe and well tolerated. The maximum tolerated dose was not reached and all patients could have been titrated up to 4 mg/kg. With 4 patients (20%) achieving a complete response and 14 patients (70%) achieving a partial response, the overall response rate was 90%.
Summary and Conclusions: Olaptesed as a single dose and in combination with BR was safe and well tolerated. Compared to historical data, olaptesed shows superiority over baseline therapy with regards to overall response rate and increasing rates of high quality responses. Moreover, the evaluation of the baseline characteristics indicates that "relapsed / refractory patients", a hard-to-treat patient population, were enrolled in the study, which underlines the clinical relevance of the observed effects and warrants further development of this Spiegelmer in CLL.

P245

A SYSTEMATIC REVIEW AND EVIDENCE SYNTHESIS OF RANDOMISED CONTROLLED TRIALS (RCT) FOR THE TREATMENT OF RELAPASED OR REFRACTORY CHRONIC LYMPHOCTYIC LEUKEMIA (CLL)

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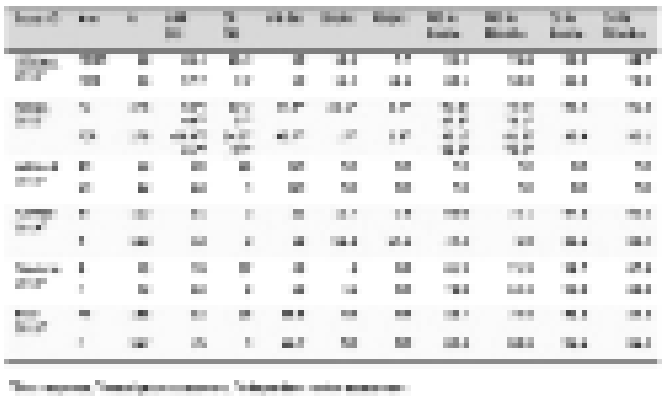
Background: Patients with relapsed CLL often have limited treatment options due to toxicity or resistance to first-line therapies. Frailty and comorbidities are also important consideration in the elderly population. Idelalisib is a novel oral inhibitor of PK13K δ for the treatment of relapsed or refractory CLL.
Aims: To evaluate the relative efficacy of commonly used treatments for relapsed/ refractory CLL.
Methods: Relevant RCTs were identified via formal searches of the EMBASE, MEDLINE, MEDLINE-in-process and CENTRAL databases. Treatments of interest were idelalisib (I), ibrutinib (Ib), alemtuzumab (A), fludarabine (F), rituximab (R), ofatumumab (O), methylprednisolone (MP), chlorambucil (Cl), cyclophosphamide (C), lenalidomide (L) alone or in combination in adult patients with relapsed/refractory CLL. Conference abstracts from EHA and ASH 2012/2013 were also searched. Screening was carried out independently by two reviewers. Meta-analysis was planned to generate relative efficacy estimates for all endpoints of interest. Response, Overall Survival (OS) and Progression Free Survival (PFS) were outcomes of interest.
Results: 1464 abstracts were review and from this 20 full papers were screened, with five full papers and a conference abstract included in the review. Three studies restricted participants to one previous line of treatment and patients in another had an average of 2.2 previous lines of therapy. Patients receiving I had received, on average, three previous treatments. Eligibility criteria were not reported in the remaining study. Imbalances in del 17p also existed in all identified studies, with ~25% for I, <10% all other treatment options. Evidence networks containing I could not be created for any endpoint of interest and so no formal evidence synthesis was possible. Despite the baseline imbalances, the overall response and stable disease rates for idelalisib were comparable to those observed for other therapies (see Table 1). PFS and OS rates at 6 and 12 months were also comparable with those observed for other therapies (Table 1). The hazard ratios derived for I+R *vs.* R (PFS: 0.15 [0.08, 0.28] OS 0.28 [0.09, 0.86]) were better than those for other pairwise comparisons (PFS range 0.65 [95% CI 0.51, 0.82] to 0.87 [0.6, 1.27]; OS range 0.65 [0.45, 0.94] to 0.83 [0.59, 1.17]).

Table 1. Results of systematic review of RCTs.



Summary and Conclusions: The availability of randomised evidence of treatments for relapsed/ refractory CLL is limited and currently does not support formal evidence synthesis. Overall, despite being used in a harder to treat population, idelalisib appears to have a comparable response profile and PFS and OS rates at 6 and 12 months to other available treatments.

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PROSPECTIVE VALIDATION OF A RISK SCORE BASED ON BIOLOGICAL MARKERS FOR PREDICTING PFS IN BINET STAGE A CLL PATIENTS: RESULTS OF THE MULTICENTER O-CLL1-GISL STUDY

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Background: The clinical course of chronic lymphocytic leukemia (CLL) patients is characterized by marked variability, with some cases requiring therapy shortly after diagnosis and others living for decades without ever requiring therapy. A risk score based on three biological features (CD38, ZAP-70 and IGHV mutational status) was previously developed to predict progression-free survival (PFS) in untreated Binet A CLL patients. Using this scoring system patients were stratified into 3 risk groups: low-risk (absence of unfavorable markers or score 0), intermediate-risk (score 1, presence of 1 unfavorable marker) and high-risk (score 2-3, presence of 2/3 unfavorable markers). According to this score system the patients had significantly different clinical courses.
Aims: Here we perform a score validation analysis in a prospective and independent cohort of patients.
Methods: Biological markers (CD38, ZAP-70 and IGHV mutational status) and gene expression profiles (GEP) of leukemic cells from CLL patients included in a prospective multicenter observational study (O-CLL1-GISL protocol, clinicaltrial.gov ID:NCT00917549) were used to assess the value and reproducibility of this score. Sub-analyses were also performed in the setting of Rai low-risk and clinical monoclonal B-cell lymphocytosis (cMBL) cases, included in this cohort.
Results: To date, 468 Binet A patients have been enrolled. According to the progression risk score, 237 patients (50.6%) were classified as low-risk, 99 (21.2%) as intermediate-risk, and 132 (28.2%) as high-risk (69 cases had score 2 and 63 score 3). The high-risk group included more numerous patients with unfavorable cytogenetic alterations (1.4% *vs* 4.9% *vs* 20.1%; P<.0001), with higher β2-microglobulin levels (34.1% *vs* 28.4% *vs* 52.9%; P=.002) and with NOTCH1 mutations cases (4.8% *vs* 5.6% *vs* 30.4.%; P<.0001) than the low-risk and intermediate-risk group. The 3-year PFS probability was 91.7%, 82.9% and 57.4% for low-, intermediate- and high-risk (P<.0001) cases, respectively. These values were similar to those found in the original cohort. At Cox multivariate analysis, Rai stage, absolute lymphocyte count, progression risk score and β-2 microglobulin maintained an independent prognostic impact on PFS. This score remained a predictor of progression when analysis was limited to 371 Rai 0 cases (P<.0001). While high-risk cMBL patients (3-year PFS probability 84.3%, HR=7.3, 95% CI 1.9–27.6, P=.003), but not intermediate-risk (3-years PFS probability 89.5%, HR=3.5, 95% CI 0.8-15.9, P=.1), showed a significantly shorter PFS than low-risk (3-year PFS probability 98.6%, HR=1). Gene expression profiling indicated that the risk groups identified here were characterized by specific transcriptional patterns that could distinguish between low- and high-risk groups. Among the genes found to be modulated in multiclass SAM analyses some are known to have prognostic relevance and showed the higher value in score 3 group, *i.e.* ZAP70, CD38, LPL, CRY1 and LDOC1 genes.
Summary and Conclusions: These results confirm the ability of this progression risk score to predict PFS among Binet A patients. The utility of the score was also extended by demonstrating that it retains prognostic value when applied exclusively to Rai 0 patients. Specific transcriptional patterns were significantly associated with risk groups.

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SALVAGE TREATMENT WITH BENDAMUSTINE, OFATUMUMAB, HIGH-DOSE METHYLPREDNISOLONE (BOMP) IN HIGH-RISK R/R CLL: INTERIM RESULTS OF THE FRENCH CLL INTERGROUP (GCFLLC/MW - GOELAMS) ICLL01 PHASE 2 TRIAL.

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Background: For relapsing or refractory (R/R) CLL patients, combination of bendamustine and rituximab (BR) has previously been evaluated as safe and efficient with an overall response (ORR) and complete response (CR) rates of 59% and 9% respectively in the CLL2M trial (Fischer 2011). Ofatumumab is a recently developed anti-CD20 immunotherapy providing an ORR of 58% in fludarabine/alemtuzumab-R/R CLL patients if used as monotherapy (Wierda JCO 2010). High doses methylprednisolone (HDMP) alone or in combination with rituximab have also been used in poor prognosis patients with bulky nodal involvement or p53 impairment. (Castro 2008, Xu 2010).
Aims: Based on these results, we tested the combination of bendamustine, ofatumumab and HDMP (the BOMP regimen) for the treatment of R/R patients, especially following fludarabine cyclophophamide rituximab (FCR) as previous treatment(s), an increasing challenge of our current practice.
Methods: We report a planned interim analysis of the phase II ICLL01 trial (NCT01612988) evaluating the efficacy and toxicity of the BOMP in fit R/R CLL patients, pretreated with 1 to 3 previous lines. Treatment consisted on a prephase of ofatumumab (300 mg) followed by 6 monthly courses of BOMP including bendamustine (70 mg/m2 d1-2), ofatumumab 1000 mg TD (d1 and d15 on 1st and 2nd courses) and high dose methylprednisolone (1 g/m2 d1-3). Primary endpoint was CR rate at 6 cycles of BOMP according to IWCLL 2008 guidelines.
Results: Data from the first 55 pts (enrolled between july 2012 and may 2013) are available. Median age was 63.8 yrs. Patients had been previously pretreated with a median of 1 (1-3) lines, including FCR-based regimens in 51/55 (93%) and 22/55 (42%) had experienced high-risk relapses within 24 months post-FCR, with 7/55 (13%) being fludarabine-refractory. *IGVH* gene status was unmutated in 90%. Karyotypes were complex (≥3 abnormalities) in 18/46 (39%) successful cases. Using FISH, we found 15/55 (27%) del17p, 6/55 (11%) tri12, 18/55 (33%) del11q, 35/55 (64%) del13q. *TP53* gene mutations were observed in 17/55 (31%). Hence p53 impairment by deletion and/or mutation was found in 19/55 (34%). Patients with either a high-risk relapse (≤2 years post-FCR) and/or p53 impairment accounted for 40/55 (73%). A total of 292 and mean number of 5.3 cycles were administered. Safety analysis available for the first 268 cycles recorded 119 grade 3-4 adverse events (AE) including neutropenia (15.3%), thrombocytopenia (10.1%), infection (7.5%) and anemia (2.2%). Non-hematologic/infectious grade 3-4 toxicity included hyperglycemia (3,4%), digestive AE (nausea, vomiting and/or diarrhea) (1.5%) and cardiovascular AE (1.11%). Overall, 36 out of 55 pts (65.5%) had at least one grade 3-4 AE. Twenty eight severe adverse events were reported in 18 patients. With a median follow-up of 340 (39-558) days following inclusion, we observed 7 deaths, related to disease progression (4), EBV-induced lymphoproliferation (1), progressive multifocal leuco-encephalitis (1) and sepsis (persistant pancytopenia) (1) with 5,5% TRM. We recorded 14 relapses including 4 Richter Syndromes. Following evaluation after 6 BOMP cycles, 5 pts proceeded to allogeneic transplantation. Response was evaluable in 52/55 patients. The ORR was 74.5% with 20% CR (11/55), 54.5% PR (30 pts including 5 nPR, 1 CRi and 4 CRu), 9,1% stable disease (5/55) and 10,9% progressive disease (6/55). Blood and bone marrow 9-color flow MRD analysis as well as statistical analysis on the impact of molecular biomarkers will be available.
Summary and Conclusions: Taking into account the very adverse profile of the treated population, this analysis shows promising results of the BOMP regimen, as compared with the results of both CLL2M (BR) and Gimema (bendamustine plus ofatumumab) trials (Cortelezzi 2013) and constitute an important set of information, for forthcoming comparison with next emerging therapy of CLL.

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NOVEL GENE MUTATIONS IN CHINESE CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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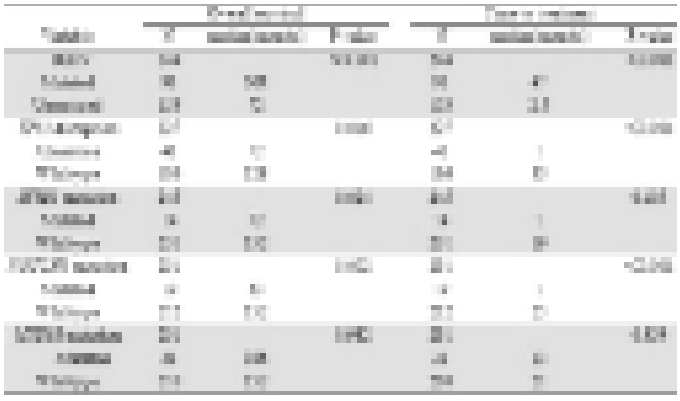
Background: Chronic lymphocytic leukemia (CLL) is a clinical heterogeneous disease with different molecular and cytogenetic abnormities. Several recurrent gene alterations detected by next-generation sequencing (NGS) have shown promising prognostic value in CLL including *SF3B1*mut and *NOTCH1*mut. However, the precise prognostic value of novel genomic mutations is still under dispute and there is no integrated study of these mutations in Chinese CLL patients.
Aims: The aim of this study is to assess the incidence and prognostic value of *SF3B1*, *NOTCH1* and *MYD88* along with *TP53* mutation in Chinese CLL cohort.
Methods: A total of 247 patients were included in this study. The median age was 61 years old with a male/female ratio of 2.05. The median follow-up was 35 months. Sanger sequencing was performed for exon4-9 of *TP53*, exon14-16 of *SF3B1*, exon 3-5 of *MYD88* and PEST domain of *NOTCH1*.
Results: Significant high incidence of *MYD88*mut (8.3%, 19/229) was observed, while *SF3B1*mut was relatively infrequent (5.3%, 14/245). 8.2% (19/231) and 16.3% (40/245) patients carried *NOTCH1*mut and *TP53*mut respectively. Detailed mutation rates regarding disease course are listed in Table 1. Of 226 patients who were analyzed for all four genes, 66.4% (150/226) patients had no gene alterations. 29.6% (67/226) showed single, 3.1% (7/226) two and 0.8% (2/226) three different mutations. Of note, *MYD88*mut were mutually exclusive of *NOTCH1*mut and *SF3B1*mut in our cohort. No correlation of any mutation with age or gender could be found. *SF3B1*mut, *NOTCH1*mut and *TP53*mut showed significant association with unmutated IGHV status, whereas *MYD88*mut were more frequent in CLL patients harboring mutated IGHV gene. Moreover, *NOTCH1*mut were associated with IGHV4-39 (P=0.012) and IGHV1-69 (P=0.006), while *SF3B1*mut correlated with IGHV4-59 (P<0.001). For the cytogenetic part, *NOTCH1*mut correlated with trisomy 12 (P=0.033), so did *TP53*mut with del(17p13) (P<0.001). *SF3B1*mut and *NOTCH1*mut were associated with shorter OS (overall survival), with median OS of 72 (P=0.024) and 63 (P=0.002) months. For the TP53 disruption patients, we further divided them into 2 groups according to their IGHV mutational status. Those carried unmutated *IGHV* gene showed significant worse prognosis compared to those with mutated IGHV gene (median OS: 71 *vs* 152 months, P=0.014). *MYD88*mut showed no impact on OS (median OS: NR *vs* 152 months, P=0.503). To our surprise, *SF3B1*mut did not affect time to treatment (TTT) in our cohort (P=0.105). Detailed data are listed in Table 2. Multivariate analysis of TTT (Time to treatment) only identified unmutated *IGHV* status (P=0.005) and TP53 disruption (P=0.019) as independent prognostic factors, while *NOTCH1*mut (P=0.088) had no statistical significance.

Table 1. Different frequency of novel gene mutation regarding to disease stage.

Table 2. Overall survival and time to treatment of Chinese CLL cohort.



Summary and Conclusions: Our data suggest remarkable differences of both incidence and prognostic value of novel gene mutations between Chinese and western CLL patients. Of particular interest, *SF3B1*mut were relatively rare even in the relapsed/refractory group and held limited prognostic value of TTT in Chinese CLL cohorts. In contrast, *MYD88*mut seemed to be more common but showed no prognostic impact. The reason of the discrepancy is yet unknown. The application of NGS might help to elucidate the underlying mechanism.

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A PHASE II MULTI-CENTER TRIAL OF PENTOSTATIN PLUS CYCLOPHOSPHAMIDE WITH OFATUMUMAB (PCO) IN OLDER PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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Background: The addition of anti-CD20 monoclonal antibody [mAb] rituximab to the FC platform (FCR) improved response rates, progression free survival and also overall survival. However, FCR showed considerable hematologic toxicity, particularly among patients over age 70. Pentostatin in CLL demonstrated similar activity with minor myelotoxicity compared to other purine analogues. Moreover, when administered with other myelotoxic agents such as cyclophosphamide resulted in a better toxicity profile. Ofatumumab is a fully human anti-CD20 mAb more effective than rituximab when used as single agent in patients with previously treated CLL being also effective in rituximab-refractory patients.
Aims: Given the reported efficacy of chemo immunotherapy [CIT] in CLL and the activity and toxicity profile of pentostatin combinations, we designed a trial of pentostatin, cyclophosphamide, and Ofatumumab for previously untreated older patients with CLL.
Methods: Patients with CLL requiring therapy (2008 NCI-WG guidelines) aged ≥65 years and ECOG PS of 0-2 were enrolled to receive Pentostatin 2 mg/sqm and Cyclophosphamide 600 mg/sqm both as intravenous infusions at day 1 of each 21 day cycle and Ofatumumab administered as intravenous infusions (Cycle 1: 300 mg day 1 and 1000 mg day 2, subsequent cycles: 1000 mg at day 1). Patients received up to 6 courses of treatment. The primary endpoint was overall response rate (ORR) including detection of minimal residual disease (MRD) and secondary endpoints included, progression-free survival (PFS) overall survival (OS) and safety.
Results: Forty-seven patients from 12 centres from the italians regions of Lombardy and Piedmont were included. Median age was 72.2 years with 60% aged over 70, 31 patients were males (66%). Twelve patients (25%) presented a Binet stage C; 6 patients (13%) had a bulky disease; 10 (21%) showed an unfavorable FISH aberration such as 17p or 11q deletion (respectively in 3 and 7 patients). Unmutated IGHV status was detected in 49%. ORR was 89% with 51% CR (18)/CR with incomplete marrow recovery [CRi] (6). Nor IGHV unmutated status, neither 17p/11q deletion had a unfavorable impact on quality of response. The six intended courses of treatment were administered in 44 (94%) patients, and 93% of these received full-dose treatment. Reasons for discontinuation before treatment completion were: 1 acute renal failure (2 courses completed); 1 septic shock (2 courses); 1 persistent skin rash (5 courses). Main reason for dose reduction was myelosuppression. At least 1 episode of grade ≥3 AEs was experienced by 59,6% of patients, with the most common being neutropenia [grade 3, 10 pts; grade 4, 17 pts]; anemia in 1 case, while thrombocytopenia was detected only as grade 1. Grade 3-4 infusion-related AEs were reported in 4% of patients and included skin rash, dispnoea, lipotimia, chills, glottis edema, hypotension, nausea. Five patients (10,6%) experienced infection episodes with two major events: 1 pneumonia and 1 septic shock leading to death. Median follow up from the beginning of treatment is 17 months.
Summary and Conclusions: Ofatumumab added to Pentostatin and Cyclophosphamide demonstrated clinically important results and is well tolerated in patients with previously untreated CLL. In this preliminary report the efficacy of this ofatumumab-based CIT compares favorably to the historical rituximab-based CIT using the same chemotherapeutic agents with a more manageable side effect profile in this older population. Further data, including MRD detection will be presented at the Meeting.
The project has been realized by Rete Ematologica Lombarda (REL) with the support of Lombardy Region.

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TGR-1202, A NOVEL ONCE DAILY PI3K DELTA INHIBITOR, DEMONSTRATES PROMISING CLINICAL ACTIVITY WITH A FAVORABLE SAFETY PROFILE IN PATIENTS WITH RELAPSED OR REFRACTORY HEMATOLOGIC MALIGNANCIES

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Background: TGR-1202 is a novel, once-daily PI3Kδ inhibitor with unique pharmacologic properties including an extended half-life and structural design which differs significantly from other leading PI3Kδ inhibitors in development. Preliminary data from an ongoing Ph I study of TGR-1202 demonstrated clinical activity in patients with advanced hematologic malignancies with a favorable safety profile.
Aims: Herein we present updated results from this Phase I, first in human study of TGR-1202.
Methods: TGR-1202 is administered orally daily following a 3+3 dose escalation design. Previously treated patients with an ECOG PS <2 and confirmed diagnosis of B-cell non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), Hodgkin's Lymphoma or other lymphoproliferative disorders are eligible. Endpoints include safety, PK/PD, and efficacy.
Results: 27 patients have been enrolled to date across 7 dose levels: 50, 100, 200, 400, 800, 1200, and 1800 mg QD. Informed consent obtained for all patients. 74% male, ECOG 0/1: 33%/67%, median age of 59 years (range: 28-82), median 3 (range: 1-14) prior treatment regimens, and 40% were refractory to prior treatment. Evaluable patients include 7 indolent NHL (iNHL), 11 CLL/SLL, 4 Hodgkin's lymphoma, 2 mantle cell lymphoma (MCL), 1 each of lymphoplasmacytic lymphoma, DLBCL, and atypical hairy cell leukemia. TGR-1202 was well tolerated. Grade ≥3 AE's in >5% of patients were limited to: dyspnea (7%), neutropenia (15%), rash (7%), and thrombocytopenia (7%). Two DLTs were observed: 1 Grade 3 rash at 800 mg (pt rechallenged with no recurrence), and 1 Grade 3 hypokalemia at 1800 mg (patient discontinued due to non-compliance). Notably, no hepatotoxicity has been observed to date. Of the 27 enrolled, 23 were evaluable for efficacy. A significant dose-response relationship was observed. Of the 13 patients treated at <800 mg QD who completed 2 cycles of treatment, 7 achieved stable disease. Of 10 patients treated at ≥800 mg QD: 4/6 CLL patients (67%) achieved a nodal partial response, 1/3 Hodgkin's patients (33%) achieved a partial response. Nodal reductions occurred rapidly in patients with CLL and were accompanied by marked lymphocytosis.
Summary and Conclusions: TGR-1202 is well tolerated in patients with relapsed/refractory hematologic malignancies with no reported hepatic toxicity and promising clinical activity at doses ≥800 mg QD. Long term tolerability has been established with patients having been on TGR-1202 for 12+ months with no safety concerns identified. Enrollment continues in expansion cohorts and at higher dose cohorts. Updated safety, efficacy, PK, and PD data will be presented.

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THE ROLE OF ALLOGENEIC STEM-CELL TRANSPLANTATION FOR RICHTER'S SYNDROME - META-ANALYSIS

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Background: Richter's syndrome (RS) is transformation of chronic lymphocytic leukemia (CLL) into aggressive lymphoma and occurs in 2% to 8% of patients with CLL. Despite use of multiagent immunochemotherapy regimens, median survival has generally not exceeded 1 year. Because of that allo stem cell transplantation (SCT) is of considerable interest having in mind dose intensity delivered by high-dose cytotoxic therapy and graft-*versus*-leukemia activity. Also, important question is about time for alloSCT: as postremission therapy in patients with chemotherapy-sensitive disease and in patients refractory to chemotherapy as salvage therapy?
Aims: There is lack of studies with reasonable sample size about role of allo SCT in patients with RS. Therefore, we decided to obtain meta analysis comparing value of allo SCT in patients with chemotherapy-sensitive disease and in patients refractory to (immune)chemotherapy.
Methods: Literature search: A search strategy was developed to identify publications reporting on allo SCT in patients with RS available through MEDLINE, EMBASE and Cochrane Library databases. Reference lists of articles identified during electronic searches were searched to identify other articles of potential relevance. In addition, hand searching of conference proceedings was conducted. Experts of the field were also contacted. Statistical analysis: The meta-analysis of studies has been developed in accordance with the rules accepted by the Cochrane Collaboration. The odds ratio (OR) of the effect of alloSCT in patients with chemotherapy-sensitive disease relative to alloSCT salvage approach was estimated by pooling individual trial results using the fixed effects method of Mantel-Haenszel. The automatic "zero cell" correction was utilized so that the studies with no events in a given arm would still be included for analysis. Result of evaluated end point (overall survival) have been demonstrated in the form of OR with 95% confidence interval (CI). Patients with complete (CR) or partial remission (PR) considered as chemotherapy-sensitive disease, while patients with stable disease (SD) or disease progression (PD) considered as refractory to chemotherapy.
Results: Three studies with data on alloSCT in CR/PR or salvage alloSCT were identified and all had accomplished 3 years of follow up. Of 49 patients involved, 24 patients received alloSCT in CR/PR and 25 received salvage alloSCT. Patients with chemotherapy-sensitive disease who underwent allogeneic SCT as postremission therapy had longer survival than patients who underwent allogeneicSCT as salvage therapy (p=0,007) (3-years OS 58.3% *vs* 20%, respectively). Meta analysis identified alloSCT derived as postremission therapy as a factor with significant impact on survival of patients with RS (OR=0,16;95%CI 0,04-0,60).
Summary and Conclusions: First meta analysis regarding to role of alloSCT in RS shows that RS patients with alloSCT as postremission therapy had significantly prolonged survival than patients with allo SCT as salvage approach. Therefore, alloSCT absolutely need to be part of therapeutic approach in chemotherapy-sensitive RS patients.

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HEALTH-RELATED QUALITY OF LIFE IMPACT OF IDELALISIB (IDELA) IN PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): PHASE 3 RESULTS

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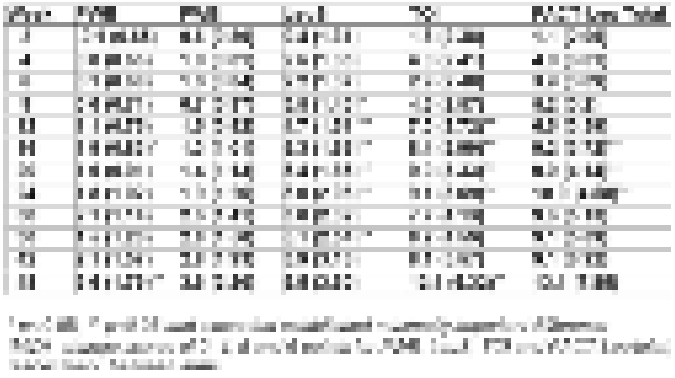
Background: Patient-reported outcomes (PROs), including health-related quality of life (HRQL), from randomized clinical trials may be used to inform clinical decision making and reimbursement decisions. Idelalisib (IDELA), an oral inhibitor of PI3Kδ, is highly active in frail, heavily pretreated patients with CLL as single agent or combined with rituximab (R).
Aims: The aim of this study was to use PROs to evaluate HRQL among patients with relapsed CLL being treated with idelalisib in Study 116, a double-blind, placebo-controlled phase 3 trial (Furman *et al.*, NEJM, 2014).
Methods: Patients were randomized to IDELA+rituximab (R) (n=110) *vs.* placebo+R (n=110). The 44-item Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) scale was used to measure physical (PWB), functional (FWB), social (SWB) and emotional (EWB) well-being and leukemia-specific concerns (LeuS). The FACT-Leu total score is the sum of subscales; Trial outcome index (TOI) is the sum of PWB, FWB and LeuS. Higher scores reflect better HRQL. Repeated measures mixed-effects models assessed change from baseline within and between arms.
Results: IDELA+R was superior for OS: HR=0.28 (0.09, 0.86), p=0.018. In the mixed-effects model analysis, PWB (p=0.015), FWB (p=0.014), LeuS (p=0.001), TOI (p=0.002), and FACT-Leu total (p=0.006) scores were significantly higher for IDELA+R. EWB/SWB scores did not change significantly over time. Repeated measure mixed-effects model results are shown in the Table 1.

Table 1.



Summary and Conclusions: In this frail CLL population, IDELA+R had superior efficacy, clinically significant improvements in HRQL, and superior symptom control occurring by 8 weeks compared to R+placebo.

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BLOOD STREAM INFECTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA: STABLE DISTRIBUTION BUT WITH INCREASED MORTALITY RATE?

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Background: The clinical management of chronic lymphocytic leukemia (CLL) has changed considerably during the last three decades. This has led to an improvement in overall survival in CLL. Infections, mainly due to hypogammaglobulinemia and therapy-related T- and B-cell immunodeficiencies, represent a major cause of morbidity and mortality in CLL.
Aims: The aim of the study was to elucidate potential temporal trends in blood stream infections among CLL patients; association to mortality and introduction of new anti-tumoural treatments.
Methods: The study cohort included 120,038 blood cultures analyzed 1988-2006 at the Clinical Microbiology laboratory Karolinska University Hospital Solna. These individuals were linked to the nationwide Swedish Cancer Registry to identify all persons with CLL (ICD-10 code C91.1) diagnosed before blood cultures analysis. Blood stream infections were divided into three calendar periods (1988-1993, 1994-1999 and 2000-2006) according to year of CLL diagnosis. A positive clinical relevant blood stream infection excluded possible skin contaminants. Survival was analyzed using all-cause mortality with follow-up to December 31, 2009. Cox proportional hazards modeling was used to estimate relative risks, yielding hazard ratios (HR) with 95% confidence intervals (CI).
Results: A total of 215 episodes of blood stream infections in 115 CLL patients were identified. Mean age at first blood culture following diagnosis was 72.9 years, with a male predominance (66%). Dominating pathogens were coagulase-negative staphylococci (for 1988-1993, 1994-1999 and 2000-2006 corresponding numbers were 22 (31%), 23 (22%) and 5 (12%), respectively), *Escherichia coli* (11 (16%), 15 (14%) and 9 (22%)), *Streptococcus pneumoniae* (7 (10%), 13 (12%) and 6 (15%)), *Pseudomonas aeruginosa* (2 (3%), 8 (8%) and 3 (7%)), *Staphylococcus aureus* (1 (1%), 6 (6%) and 6 (15%)) and viridians streptococci (5 (7%), 6 (6%) and 2 (5%)). The number of blood stream infections was stable as well as the relative proportions of Gram-positive and Gram-negative bacteria, over the three calendar periods. There was a significantly increased risk of dying in the last calendar period in CLL patients with a positive clinically relevant blood culture (HR=2.52; 95% CI 1.44-4.41) compared to others (possibly clinically relevant and negative blood cultures), as well as compared to negative blood cultures alone (HR=2.44; 95% CI 1.38-4.30). When analyzing the exposure as time varying, the mortality rates were significantly increased after a positive clinically relevant blood culture compared to before in patients diagnosed 1994-1999 (HR=2.59; 95% CI 1.74-3.85) and 2000-2006 (HR=3.21; 95% CI 1.83-5.62).
Summary and Conclusions: The distribution of blood stream infections in the last decades remained stable despite advances in management of CLL patients. Importantly, in recent years, an increased risk of dying was observed in patients with a positive clinical relevant blood culture. A possible explanation for this finding is that CLL patients, despite improved survival, are more immunocompromised with the expansion of the anti-tumoural armamentarium in the later calendar periods. Treatment-related infections and the role of prophylaxis need to be studied further in CLL.

Chronic myeloid leukemia - Biology

P254

TARGETING OF STAT3 AND STAT5 BY A NOVEL DRUG- COMBINATION EMPLOYING PONATINIB AND BARDOXOLONE METHYL (CDDO-ME) LEADS TO SYNERGISTIC EFFECTS IN BCR/ABL-T315I-MUTATED CELLS IN PH+ CML

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Background: In chronic myeloid leukemia (CML), resistance against imatinib is a major challenge in clinical practice. In many of these patients, *BCR/ABL* mutations are detected. These patients are candidates for second- or third-generation BCR/ABL tyrosine kinase inhibitors (TKI). However, even when treated with such novel TKI, patients often relapse which is best explained by activation of additional oncogenic pathways in leukemic cells. Therefore, current research is seeking drug combinations involving second- or third-generation TKI and other targeted drugs, with the aim to overcome resistance and to prolong relapse-free surival. Bardoxolone methyl (CDDO-Me) is a novel agent that suppresses survival of leukemic cells by targeting several pro-oncogenic molecules, including Akt, mTOR, and STAT3.
Aims: In the current study, we examined potential cooperative effects of bardoxolone methyl and BCR/ABL TKI. Drug interactions were analyzed at the molecular level (deactivation of critical target-proteins) and in functional assays determining growth and survival (apoptosis) of leukemic cells.
Methods: Primary CML cells were obtained from the blood of 12 CML patients. Four patients had developed resistance against at least two TKI. Furthermore, we examined drug effects in four human CML cell lines, namely KU812, K562, imatinib-resistant K562 cells and imatinib-resistant KCL22, as well as in Ba/F3 cells expressing various BCR/ABL mutants (E255K, H396P, F359V, Y325F, G250E, T315I). Protein expression and phosphorylation was analysed by Western blotting. Cell proliferation was assessed by ^{3}H-thymidine-uptake and apoptosis was determined by flow cytometry.
Results: Bardoxolone methyl was found to inhibit the proliferation and viability of all BCR/ABL+ cell lines (human and Ba/F3) tested and also in primary leukemic cells isolated from pre-treated patients, including those harbouring BCR/ABL T315I. IC_{50}-values ranged between 0.1 and 0.5 µM without a significant difference when comparing imatinib-naïve with imatinib-resistant cells. We next combined bardoxolone methyl with two novel BCR/ABL TKI, dasatinib and ponatinib. Clear synergistic effects were seen with both TKI in all imatinib-sensitive and imatinib-resistant cell lines and in all primary cell samples tested. In Ba/F3 cells harbouring BCR/ABL T315I, the most impressive synergy was seen with bardoxolone methyl and ponatinib. Synergistic effects were accompanied by simultaneous inhibition of multiple signalling molecules, including STAT3 and STAT5. By contrast, exposure to Bardoxolone methyl resulted in deactivation of STAT3 but not deactivation of STAT5 and BCR/ABL; and exposure to the TKI caused deactivation of STAT5 but not STAT3. Bardoxolone methyl is also known to increase expression of heme oxygenase-1 (HO-1), a heat-shock-protein that is associated with drug resistance and abnormal cell survival. Therefore we combined bardoxolone methyl with the HO-1 inhibitor SMA-ZnPP, which also resulted in synergistic growth-inhibitory effects in all primary CML cells and all cell lines tested. Finally, SMA-ZnPP was found to sensitize CML cells against the drug-combination "bardoxolone methyl+TKI". In fact, the combination "bardoxolone methyl+TKI+SMA-ZnPP" produced highly potent anti-proliferative and pro-apoptotic effects in BCR/ABL+ leukemic cells.
Summary and Conclusions: Bardoxolone methyl and SMA-ZnPP enhance TKI effects in CML cells by blocking STAT3 and HO-1 activity. Whether such drug combinations are effective *in vivo* in TKI-resistant CML remains to be elucidated.

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CD62L EXPRESSION ON T CELLS IS DECREASED IN PATIENTS WITH EARLY CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP) AND PREDICTS RESPONSE TO THERAPY WITH FRONTLINE NILOTINIB

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Background: Imatinib and dasatinib modulate immune responses *in vitro* and *in vivo*. Immunological surveillance in the MRD-situation might be of particular relevance for long-term control or even elimination of CML-repopulating stem cells. Little is known about potential immune-modulatory effects of nilotinib *in vivo*. The ENEST1st study (NCT01061177) is focused on examining the role of first-line nilotinib therapy in CML-CP. A comprehensive immunological monitoring program within this ENEST1st substudy allows characterization of nilotinib-induced immunological changes and their potential correlation with clinical response parameters.
Aims: The identification of immunological changes induced by nilotinib and definition of immunological surrogates for response prediction in newly diagnosed CML-CP patients.
Methods: Peripheral blood was taken prior to treatment initiation and after 6 and12 months (mo) from 50 patients treated on the ENEST1st study. Samples were analyzed by nine colour flow cytometry employing six panels of antibodies to determine various leukocyte populations including T cell subsets. Soluble factors in plasma were measured by ELISA. Changes in immune cell parameters were correlated to clinical endpoints.
Results: 55% of the patients included into this substudy achieved MMR at 6mo, 75% at 12mo and 79% at 12mo of therapy. $MR^{4.5}$ was achieved by 5%, 18%, 24% of patients at 6, 12 and 24 mo of therapy, respectively. The proportion of CD8+ T cells significantly decreased while CD4+ T cells increased during therapy. Expression of the known lymph node homing marker CD62L was very low at baseline (median 5,7%) and the proportion of CD62L-expressing cells among CD4+ and CD8+ T cells negatively correlated with SOKAL score. During treatment, CD62L expression on both T cell subsets significantly increased (median 72,3%) and was restored to normal levels. Moreover, higher expression of CD62L+ cells was negatively associated with lower BCR-ABL mRNA burden at different time points and also predicted the time to reach MMR. More than 60% of patients with CD62L expressing cells above 25% (upper third) but less than 10% of the other patients reached MMR within the first three months ($p \leq 0.001$). As CD62L is a marker for naïve T cells, low expression of CD62L seems to indicate increased proportions of memory cells. However, a detailed characterization of other T cell differentiation marker, such as CD45RA, CD45R0, CD28, CD27 and CD95, did not reveal significant T cell subset alterations. Thus we speculated that low levels CD62L expression levels might be the result of increased shedding. Indeed, levels of metalloproteinases (MMP), mediating proteolytic cleavage of CD62L, were increased in plasma before compared to after treatment initiation. Finally, plasma taken at baseline but not after treatment initiation down modulated CD62L on normal T-cells.
Summary and Conclusions: Here we show decreased expression of CD62L in patients with CML-CP, probably due to cleavage by aberrantly expressed/activated MMP. CD62L is an important adhesion molecule regulating migration and homeostasis of immune cells. This may partly explain why patients with relatively normal CD62L expression are able to rapidly reduce BCR-ABL mRNA burden upon initiation of therapy. Alternatively, other targets of MMP might be causally involved in this process and CD62L expression may just represent a surrogate marker for MMP activity.

P256

LASP1 IS A NOVEL BCR-ABL SUBSTRATE AND A PHOSPHORYLATION-DEPENDENT BINDING PARTNER OF CRKL IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is characterized by a genomic translocation generating the Philadelphia chromosome with a permanent active BCR-ABL oncogene and yielding a complex pattern of atypically tyrosine-phosphorylated proteins that ultimately drive the malignant phenotype of CML. Recently the LIM and SH3 domain protein 1 (LASP1), a protein involved in cell proliferation and migration, was identified as a component of a six gene signature strongly predictive for disease progression and relapse in CML patients. However, the underlying molecular mechanisms why LASP1 expression correlates with dismal outcome remained unresolved.
Aims: It is thought that leukemia stem cells (LSC) (primitive CML progenitor cells) may not be addicted to BCR-ABL and shift to other pathways when treated with tyrosine kinase inhibitors (TKI) and are the reason for insufficient responses, relapse or progression into advanced phase or blast crisis. The identification and inhibition of pathways implicated in LSC survival, in combination with conventional therapy is the new therapeutic ambition in CML treatment to overcome TKI resistance and eradicate minimal residual disease in CML. In this context, the role of LASP1 in aberrant BCR-ABL signaling and CML progression is elucidated.
Methods: Comprehensive transcriptomic microarray data, immunofluorescence imaging and biochemical analysis like phosphopeptide binding assay, Western blot, immunoprecipitation and protein overlays were used to elucidate the role of LASP1 in CML progression in BCR-ABL positive cell lines and in blood leucocytes from CML patients before and after TKI therapy.

Results: LASP1 as a novel and highly overexpressed direct substrate of BCR-ABL in CML. The protein is specifically phosphorylated by BCR-ABL at tyrosine-171 in CML patients, which is abolished by tyrosine kinase inhibitor therapy. pLASP1-Y171 binds to non-phosphorylated CRKL, another specific BCR-ABL substrate and bona fide biomarker for BCR-ABL activity, at amino acids 36-39 of the SH2 domain of CRKL. Immunofluorescence validated localization of LASP1 and colocalization with CRKL. Accordingly, the BCR-ABL-mediated pathophysiological hyper-phosphorylation of LASP1 in CML disrupts normal regulation of CRKL and LASP1, which likely has implications on downstream BCR-ABL signalling.
Summary and Conclusions: In summary, the results suggest that LASP1 phosphorylation might serve as an additional biomarker for assessment of BCR-ABL activity and point to an important role of LASP1 in aberrant BCR-ABL signaling. Moreover, these data provide a first mechanistic insight in why LASP1 overexpression might contribute to CML progression.

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DNA METHYLTRANSFERASE 1-DRIVEN HYPER-METHYLATION OF B CATENIN ANTAGONIST CHIBBY1 IS NOT AN EPIGENETIC MARK OF CHRONIC MYELOID LEUKEMIA RESISTANCE TO IMATINIB

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Background: Chibby1 is an antagonist of β catenin, a central component of chronic myeloid leukemia pathogenesis and progression to blast crisis. Our recently published study proved that Chibby1 down-modulation is a component of β catenin activation associated with the *BCR-ABL1* fusion gene, partly contingent upon transcriptional events and driven by DNA hyper-methylation at promoter-associated CpG islands of the Chibby1-encoding gene *C22orf2*. Moreover, it established that Chibby1 induction in response to imatinib in chronic myeloid leukemia cells proceeds, at least partly, from *C22orf2* promoter demethylation. Indeed, methylator phenotype encompassing genes involved in leukemic cell proliferation and survival is a common event in chronic myeloid leukemia, eventually associated with the disease progression and drug resistance outcome.
Aims: We investigated whether DNA methyltransferase 1 is responsible for epigenetic control on tumor suppressor genes (such as *CBY1* and *BCL2-like11*) in K562 cell line and mononuclear cell fractions from bone marrow samples of chronic myeloid leukemia patients. Moreover we assessed the correlation between DNMT1 activity and imatinib resistance in imatinib-responsive and -resistant chronic myeloid leukemia patients.
Methods: PCR amplification of DNA from chromatin immunoprecipitation products was used to assess the amounts of DNA methyltransferase 1 and 5 methylcytosine at a 205 bp promoter region encompassing nucleotides -85 to +120 of C22*orf*2 promoter and at a 342 bp sequence of the pro-apoptotic *BCL2-like11* (BIM) in *BCR-ABL1+* cell line K562 and mononuclear cell fractions from bone marrow samples of imatinib-responsive and -resistant chronic myeloid leukemia patients.
Results: We found that DNA methyltransferase 1 enhanced recruitment is involved in hyper-methylation of C22*orf*2 promoter, associated with Chibby1 transcript reduction in three out of five imatinib-responsive patients and all three imatinib-resistant patients, supporting that such epigenetic modification is not the cause of overt resistance to imatinib. Further investigation showing that the DNA methyltransferase 1-driven hyper-methylation of the pro-apoptotic *BCL2-like11*, a crucial gene for the prognosis and response of chronic myeloid leukemia to imatinib, is not strictly associated with drug resistance.
Summary and Conclusions: Those findings suggest that DNA methyltransferase 1-driven epigenetic control on *Chibby1* and other tumor suppressor genes such as *BCL2-like11* is not the cause of overt chronic myeloid leukemia resistance to imatinib. Still, the DNMT1-driven hyper-methylation at those critical genes for cell survival and proliferation may contribute to the disease clonal evolution, hence supporting the clinical use of de-methylating agents, in particular, in the disease advanced stages.

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MYELOID DERIVED SUPPRESSOR CELLS (MDSCS) AS POTENTIAL IMMUNE ESCAPE MECHANISM IN CML PATIENTS

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Background: In some solid tumors it has been demonstrated that a subpopulation of myeloid cells, defined as "myeloid-derived suppressor cells" (MDSCs), plays an important role in inducing T cell tolerance by production of arginase 1 (arg1) that depletes microenvironment of arginine, an essential aminoacid for T cell function. Since chronic myeloid leukemia (CML) patients have high levels of immature myeloid cells it is of interest to investigate if these cells have MDSCs phenotype and activity.
Aims: The aim of this study was to analyze MDSCs and investigate their activity in CML patients.
Methods: MDSCs were analyzed in peripheral blood (PB) of healthy donors (HD; n=20), 30 CML patients at diagnosis, 21 of which collected from diagnosis and followed during imatinib. Granulocytic MDSCs (G-MDSCs) were identified as CD11b+CD33+CD14-HLADR- cells, while the monocytic MDSCs (Mo-MDSCs) as CD14+HLADR by cytofluorimetric analysis. Arg1 expression was assessed using real time PCR and Western Blot and the circulating protein was measured by ELISA.
Results: CML patients showed high levels of Mo- and Gr-MDSCs at diagnosis in comparison to HD (41±8 and 82,5±12,2% respectively in CML *vs* 9±2,1 and 55±5,3% in HD; p<0.001), while after imatinib therapy both subpopulations decreased returning to normal values. Also T-reg (CD4+ $CD25^{high}$ Foxp3+ cells) was significantly increased at diagnosis in respect to HD (from 6,1±0,8% to 9±2%; p<0.001) with a significant correlation with the percentage of Gr-MDSCs (r=0,6254; p<0.001). In order to evaluate if at least in part MDSCs belong to the tumor clone, we analyzed BCR/ABL expression in both CD11b+CD33+CD14-HLADR- and CD14+HLADR- subpopulation cells and all 3 analyzed patients showed the oncoprotein expression in both the two subsets. Either in PB cells and purified Gr-MDSCs, we showed a significant increase in Arg1 expression in CML patients at diagnosis compared to HD (p<0.001) and decreased after therapy. The same data were confirmed by Western Blot analysis. The differences in Arg1 concentration in serum of HD and CML patients at diagnosis was statistically significant (64±37,5 *vs* 260,6±64,3 ng/ml; p<0.0001) and a significant correlation was observed with the percentage of Gr-MDSCs (r=0,61; p<0.05). To verify whether the population of cells with the phenotype of Gr-MDSCs and high levels of Arg1 expression in CML patients could be functionally defined as MDSCs, we determined their ability to inhibit the proliferation of CFSE+ T cells. A significant suppressive function was observed (p<0.001). Since CML patients at diagnosis have high levels of arg1 in serum, subsequent experiments were designed to further understand whether also circulating protein has immunosuppressive activity with CML MDSCs. After 72 h from mitogen stimulation and incubation with HD or CML serum, the percentage of proliferation of CFSE-labeled T cells was significantly inhibited with CML serum compared to positive control (HD T cells plus PHA) (p<0.05) and this inhibitory effect was lost by the addition of Nor.NOHA (p<0.05).
Summary and Conclusions: Our data indicate that CML cells create an immunotolerant environment associated to MDSCs expansion with immunosuppressive capacity mediated by arginase1 (arg1) that could hinder the use of immunotherapy in CML patients. MDSCs should be monitored in imatinib discontinuation trials to understand their importance in relapsing patients.

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BIN1 AND RIN1 MODULATION IN CHRONIC MYELOID LEUKEMIA

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Background: The role of *Bcr-Abl* in the pathogenesis of Chronic Myeloid Leukemia (CML) is well established, however the mechanisms involved in progression remain poorly understood. By making use of *Drosophila Melanogaster* transgenic model for human *Bcr-Abl* gene, we have identified the involvement in CML progression of different genes regulating the recycling and the degradation of tyrosine kinase receptors (RTKs) through the assembly of clathrin coated vesicles. Among them, we focused our attention on Bridging Integrator 1 (Bin1) and its activator Rin1.
Aims: We aimed to identify and characterize a new possible mechanism substaing BCR-ABL pathway acting through a defect in recycling and degradation of RTKs through the assembly of clathrin coated vesicles.
Methods: *Bin1* and *Rin1* expression was measured by quantitative Real Time PCR (qRT-PCR) in 82 samples from CML patients and in 10 healthy control samples. Among CML, 34 samples were collected at diagnosis and 45 during the tyrosine kinase inhibitors (TKIs) treatment, 11 of which in Complete Molecular Response (CMR), 17 in Major Molecular Response (MMR), 17 in <MMR and 3 Imatinib resistant patients. Western Blot (WB) analysis was performed to analyze Bin1 and Rin1 expression in control subjects and CML patients. Bin1 and Rin1 expression was also analyzed by qRT-PCR and WB in K562 and HEK-293 cell lines after treatment with Imatinib at different times (24 and 48 hours).
Results: qRT-PCR data indicated that *Bin1* expression was significantly down-regulated in CML patients at diagnosis compared to healthy subjects (p=0.0001). Overall, during TKIs therapy, the transcript levels of *Bin1* showed an up-regulation trend from <MMR (p=0.0001) to MMR (p=0.0001), to CMR (p=0.0001) compared with diagnosis. Interesting, *Bin1* was also down-regulated in three TKIs resistant patients as observed in diagnosis ones (Figure 1A). These results demonstrated an indirect correlation between *Bin1* and *Bcr-Abl*

transcript expression levels (Figure 1A and 1C). Bin1 is activated by Rin1, a RAS effector protein, to promote the formation of early endosome structures. Besides, Rin1 interacts with Bcr-Abl stabilizing the fusion protein in CML cell lines. To assess if Rin1 was correlated with Bin1 in CML patients, we analyzed *Rin1* transcript levels in the same cohort of subjects. Like *Bin1*, *Rin1* expression was down-regulated in CML patients at diagnosis respect to healthy subjects (p=0.01), and was up-regulated during TKIs therapy from <MMR (p=0.0001) to MMR (p=0.05), to CMR (p=0.001) compared with diagnosis. Moreover, *Rin1* remained down-regulated in resistant patients (Figure 1B). To confirm the qRT-PCR data on CML patients, we analyzed both protein expression by WB analysis and we observed the same trend of transcript levels. To better elucidate the inverse correlation between Bcr-Abl, Bin1 and Rin1, we performed i*n vitro* experiments by Imatinib treatment, in K562 and HEK-293 cells. WB data showed an up-regulation of Bin1 and Rin1 proteins in K562 cell line, after 24 and 48 hours of treatment; we did not observe any variation of their expression in non tumorigenic cell line, HEK-293.

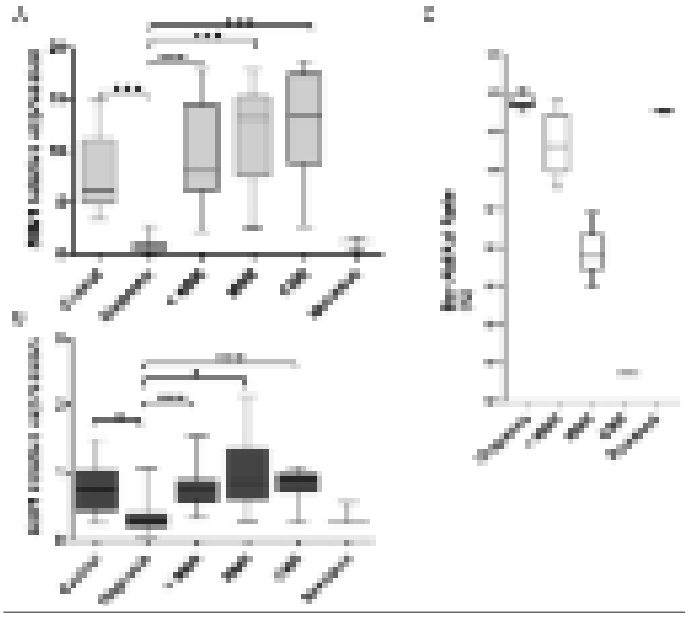

Figure 1. *BIN1* A) and *RIN1* B) expression levels were evaluated by qRT-PCR in control subjects, CML samples at diagnosis, and during TKIs treatment (<MMR, MMR, CMR and resistant patients). C) Numbers of *Bcr-Abl* transcripts were evaluated by qRT-PCR as Bcr-Abl/Abl Ratio (%) in CML saples at diagnosis and during TKIs treatment (<MMR, MMR, CMR and resistant patients).

Summary and Conclusions: Our results show a significant association between *Bin1* and *Rin1* expression and clinical phases of CML, suggesting an indirect correlation with Bcr-Abl levels. This study proposes a new deregulated mechanism in CML indicating Bin1 and Rin1 as possible players in the maintenance of the abnormal signaling in this haematological disease.

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PROMOTER METHYLATION OF THE TUMOR SUPPRESSOR GENES ON THE SHORT ARM OF CHROMOSOME 1 IN CHRONIC MYELOGENOUS LEUKEMIA

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Background: Chronic myelogenous leukemia (CML) is a hematological disorder characterized by Philadelphia chromosome and /or *BCR-ABL* fusion gene. We previously reported frequent loss of heterozygosity on the short arm of chromosome 1 (1p) in the progression of CML. Methylation (5-mC) in a promoter CpG of several tumor suppressor genes has been associated with loss or decreased expression in many tumors. Among candidate genes on 1p36, methylation of the *RIZ1, RUNX3,* and *p73* genes has been found, and the *RIZ1* gene was also inactivated by frameshift and missense mutations in solid tumors. We recently reported that RIZ1 methylation was more frequent in myelodysplastic syndrome and secondary leukemia. However, 5-mC was converted to 5-hmC by tet enzyme, and both 5-mC and 5-hmC were detected by conventional methylation specific polymerase chain reaction (MS-PCR).
Aims: To elucidate the relevance of tumor suppressor genes on 1p, we analyzed mutation and promoter methylation on the *RIZ1, RUNX3*, and *p73* genes in CML. To distinguish 5-mC from 5-hmC in promoter region, we performed oxidative MS-PCR.
Methods: Mononuclear cells were isolated from bone marrow or peripheral blood samples after obtaining written informed consent from 61 patients with CML. The 61 CML consisted of 43 chronic phase (CP), 2 accelerated phase (AP), and 16 blast crisis (BC). This study was performed according to ethical criteria of our institute. Genomic DNA was treated with bisulfite, and treated DNA was subject to amplification with HotStarTaq Master Mix Kit (Qiagen, Valencia, CA, USA) for MS-PCR. Bisulfite sequence was performed in both directions. Oxidative MS-PCR analysis was performed using $KRuO_4$ before bisulfite treatment. Samples that showed methylation more than twice were assessed as positive. Mutation was analyzed using PCR-single strand conformation polymorphism (SSCP) analysis. Quantitative real time reverse transcriptase-PCR was performed in 14 patients of which RNA was suitable for analysis. K-562 myeloid leukemia cells were grown in RPMI 1640 in the presence of various concentrations of 5-Aza-dC with or without tricostatin A (TSA). The correlation between the frequency of methylation and type of disease or clinical characteristics was analyzed using the chi-square test or Fisher's exact probability test. Analysis was performed using SPSS Software.
Results: MS-PCR showed methylation of the *RIZ1, RUNX3* and *p73* genes in 24 of the 60 (40%), 21 of the 61 (36%), and 28 of 60 (46%) patients, respectively. Methylation at all the three loci was detected in 19 of 60 patients (32%). Bisulfite sequence analysis revealed that methylation was present at many CpG sites in the *RIZ1, RUNX3,* and *p73* promoter regions in CML. *RIZ1* methylation was detected in 14 of the 43 CML in CP (33%), none of the 2 CML in AP, and ten of 15 CML in BC (67%). Methylation of the *RUNX3* gene was detected in 12 of the 43 samples of CP (30%), 0 of the 2 AP, and nine of the 16 BC (60%). Methylation of the *p73* gene was observed in 16 of the 43 samples of CP (37%), 0 of the 2 AP, and 12 of 15 BC (80%). *RIZ1, RUNX3*, and *p73* methylation was more frequent in BC than in first, CP. Oxidative MS-PCR analysis together with MS-PCR can distinguish 5-mC from 5-hmC: 5-mC in the *RIZ1, RUNX3,* and *p73* genes was detected in 10 of 22 (45%), 16 of 21 (76%), and 16 of 26 (62%) samples with methylation detected by conventional MS-PCR, respectively. PCR- SSCP analysis on the *RIZ1, RUNX3*, and *p73* genes showed mobility shifts in 2 CML patients in exon 3 of the *p73* gene, however sequence analysis reveals one-base deletion in intron 2. Quantitative real time reverse transcriptase-PCR analysis showed decreased expression of the *RIZ1, RUNX3,* and *p73* genes in several patients with or without 5-mC. Treatment of K-562 cells (that have 5-mC in *RIZ1* and *RUNX3*) with 5-Aza-dC and TSA induced growth suppression, demethylation, and reexpression of the genes.
Summary and Conclusions: Multiple tumor suppressor genes on 1p were inactivated in CML in part by methylation. In comparison with CP, higher incidence of the *RIZ1, RUNX3, and p73* methylation was found in BC. Oxidative MS-PCR analysis was useful to detect real 5-mC. Combination treatment of 5-Aza-dC and TSA resulted in reactivation of the repressed genes more efficiently.

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BONE MARROW (BM) MICROENVIROMENT FACTORS AS EARLY MARKERS OF RESPONSE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA (CML-CP) TREATED WITH NILOTINIB.

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Background: Treatment of patients with CML-CP with tyrosine kinase inhibitors (TKIs) can substantially improve the life expectancy of these patients. However, it is becoming evident that persistant leukemic stem cells, which in their quiescent state are insensitive to TKIs, can lead to a resurgence of CML. The Evaluating Nilotinib Efficacy and Safety in Trial as First-Line Treatment (ENEST1st) is a phase 3b is an open-label study of nilotinib 300 mg twice daily (BID) in adults with newly diagnosed BCR-ABL positive CML-CP.
Aims: The aim of the ENEST1st sub-study N10 is to investigate BM microenvironment markers that regulate leukemic stem cells in the BM niches of nilotinib-treated patients.
Methods: We enrolled 37 patients from 21 Italian ENEST1st centers, from whom written informed consent had been obtained, for participation in substudy N10. Patients were monitored by Real Time RT-PCR (RT-qPCR) for the expression of the fusion BCR-ABL mRNA. Response was based on ELN recommendations (Baccarani M, *et al.* Blood 2013 122:872-884). In an interim analysis, molecular and cytogenetics response by 24 months was assessed.Mononuclear cells were collected from BM and PB samples at the screening visit (V0) and after 3 months of treatment (V4). Total RNA from BM and PB mononuclear cells was purified. RT-qPCR for the expression of 10 genes (ARF, KIT, CXCR4, FLT3, LIF, NANOg, PML, PRAME, SET and TIE), involved in the regulation of the stemness and survival signaling of hematopoietic stem cells was conducted. RT-qPCR results were normalized by the expression of GUS mRNA (Normalized mRNA copy Number: NCN).

Results: Interim molecular analysis of MMR until the 24th month was available for 27 of the 37 patients, showing an optimal response in 20 patients, a warning response in 4 patients and a failure response in 3 patients. We observed a significant correlation in the expression of two genes involved in the regulation of stem cell pluripotency (NANOg) or cytokine signaling (SET) and patient's outcome. Indeed, NANOg and SET mRNA were significantly down-regulated in PB samples at diagnosis of patients with optimal response compared to patients with warning/failure response, (NANOg mRNA: 0.3±0.25 NCN by GUS mRNA *vs* 0.6±0.7 NCN by GUS mRNA, respectively; p=0.05; SET mRNA: 0.2±0.3 NCN by GUS mRNA *vs* 2.3±4.2 NCN, respectively; p=0.03).
Summary and Conclusions: These data suggest that expression analysis of genes involved in cell pluripotency (NANOg) and/or cell signaling (SET) at baseline, may assist in the early prediction of molecular response in patients treated with nilotinib. Further studies are planned to evaluate the role of stroma-secreted cytokines, such as SDF1 and VEGF, in the regulation of TKI-responsiveness in CML patients, since factors that modulate hematopoiesis may also promote leukemogenesis, enhance blast survival and make them resistant to treatment within the BM microenvironment.

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EFFECTS OF THE PI3-KINASE/MTOR INHIBITOR BEZ235 ON ONCOGENIC SIGNALING, PROLIFERATION AND SURVIVAL OF LEUKEMIC CELLS IN CML

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Background: In Ph+ chronic myeloid leukemia (CML) the BCR/ABL oncoprotein represents a major pro-oncogenic driver responsible for proliferation and accumulation of leukemic cells. The BCR/ABL tyrosine kinase inhibitor (TKI) imatinib produces long-lasting complete molecular remissions (CMR) in a subset of patients with CML. For patients with imatinib-resistant CML, novel second and third generation BCR/ABL TKI, such as nilotinib or ponatinib are available. However, not all patients achieve long-lasting CMR during TKI therapy. Therefore, current research is seeking novel targets in CML cells. The PI3-kinase and the mammalian target of rapamycin (mTOR) are two key downstream-signaling molecules considered responsible for BCR/ABL-induced proliferation of leukemic cells.
Aims: In the present study, we examined the effects of the dual PI3-kinase/mTOR blocker BEZ235 on growth and survival of CML cells.
Methods: A total of 7 patients with CML (chronic phase, n=6; accelerated phase, n=1) were examined. Proliferation of mononuclear CML cells was determined by ^{3}H-thymidine uptake and apoptosis by PI/AnnexinV-staining and staining for active caspase 3.
Results: BEZ235 was found to inhibit the proliferation of primary CML cells in a dose-dependent manner. In addition, BEZ235 suppressed the proliferation of various CML cell lines, including K562 (IC50: 50-100 nM), imatinib-resistant K562 (IC50: 50-100 nM), KU812 (IC50: 20-50 nM) and KCL22 cells (IC50: 10-20 nM). Moreover, BEZ235 induced growth-inhibition in Ba/F3 cells expressing various imatinib-resistant mutants of BCR/ABL, including T315I. Despite these impressive effects, BEZ235 did not induce apoptosis in CML cell lines. In subsequent experiments we found that BEZ235 downregulates the phosphorylation of the 40S ribosomal protein S6, and less effectively also Akt in BCR/ABL+ cells, but even upregulates the expression of activated STAT5 and the STAT5-target gene CD25. Based on these observations, we combined BEZ235 with nilotinib and ponatinib, both of which are known to induce apoptosis, growth inhibition and STAT5-downregulation in CML cells. We found that BEZ235 and nilotinib as well as BEZ235 and ponatinib exert strong cooperative growth-inhibitory effects in CML cells.
Summary and Conclusions: In conclusion, BEZ235 is a potent inhibitor of BCR/ABL-dependent proliferation of CML cells, but exert little if any effects on cell survival. Moreover, our data show that BEZ235 and various BCR/ABL TKIs synergize with each other in counteracting proliferation in CML cells. Whether BEZ235 alone or in combination with nilotinib or ponatnib, can also suppress the proliferation of leukemic cells *in vivo* in patients with CML remains to be determined in clinical trials.

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IMPACT OF CLONAL EVOLUTION ON THE OVERALL SURVIVAL OF PATIENTS WITH CML TREATED WITH IMATINIB: EXPERIENCE OF ONE CENTER

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Background: Occurrence of secondary chromosomal aberrations in patients with chronic myeloid leukemia (CML) is one of the factors influencing patient survival. These changes may appear on the background of treatment with tyrosine kinase inhibitors (TKI) both in Ph-positive (Ph+) and Ph-negative cells (Ph-). The influence of these abnormalities developing in the first category of cells was describes by many authors, whereas data concerning Ph-negative cells is limited.
Aims: Purpose of this study was to establish influence of chromosomal abnormalities in Ph+ and Ph- cells on the overall survival of patients with CML treated with imatinib.
Results: Cytogenetic examination was conducted in 101 patients with CML treated with TKI imatinib for median of 82 months (ranging from 9 to 166 months). Overall, median observation time for these patients from the diagnosis of CML was 94 months (from 25 to 296 months). The emergence of secondary cytogenetic abnormalities (clonal evolution) in Ph+ cells was revealed in 23 patients compared to 6 patients developing these changes in Ph–, cells. Spectrum of additional chromosomal aberrations in Ph+ cells was as follows: +der(22)t(9;22), +8, i(17)(q10), ider(22;22)t(9;22), idic der(22;22)t(9;22), -7, +19, +21, -Y and other structural or numerical aberrations. In Ph– cells +8 and del(7q) were revealed. Two peaks when the secondary chromosomal abnormalities were detected most frequently occurred between 12th and 36th month of imatinib therapy and after 60th month of this treatment. Among 23 patients with additional aberrations in Ph+, cells 9 subjects lost partial (PCyR) or complete (CCyR) cytogenetic response during treatment, and other 16 subjects did not reach any cytogenetic response at all. All patients with abnormalities in Ph- cells were diagnosed with these changes exceptionally during PCyR or CCyR. Thereafter all patients with cytogenetic aberrations in Ph– cells lost their cytogenetic response at different time points. Patient survival in two groups significantly differed; in particular, median survival was not reached in patients without clonal evolution, while in patients with clonal evolution it was detected at 100 months from the time of diagnosis (Figure 1).

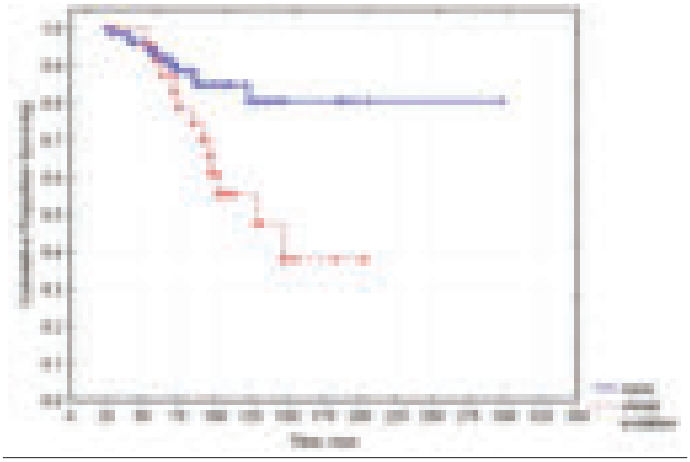

Figure 1. Overall survival of CML patiens with and without clonal evolution.

Summary and Conclusions: Occurrence of secondary changes both in Ph-positive and Ph-negative cells is a sign of unfavorable prognosis concerning treatment response and patient survival. The highest risk of clonal evolution was observed between 12th and 36th months of treatment with imatinib.

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A FUNCTIONAL POLYMORPHISM IN THE 3' UNTRANSLATED REGION OF THE ARHGAP26 GENE CONFERS AN INCREASED RISK OF CHRONIC MYELOID LEUKEMIA AND CAUSES ILLEGITIMATE REGULATION BY MICRORNA-18A-3P

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Background: MicroRNAs are small regulatory RNAs that control a wide variety of biological processes, such as proliferation, apoptosis and differentiation. MicroRNAs also play a crucial role in normal hematopoiesis by controlling the differentiation of hematopoietic stem cells into different types of mature blood cells, while deregulation of microRNA networks has been linked to hematological malignancies. Polymorphisms in microRNA binding sites (miRSNPs) in target genes may alter the strength of microRNA interaction with target transcripts thereby deregulating protein levels.
Aims: In this study we aimed at identifying miRSNPs associated with the risk of chronic myeloid leukemia and assessing the impact of these miRSNPs on the protein expression.
Methods: We analyzed with specialized algorithms (miRANDA, PITA, Patro-

cles and PolymiRTS) the 3' untranslated regions of 132 leukemia-associated genes and identified 111 putative miRSNPs, of which 10 were chosen for further investigation, based on the concordance of at least two applied algorithms. We genotyped patients with chronic myeloid leukemia (CML, n=140) and healthy controls (n=471). MiRSNPs found to be associated with leukemia risk were analyzed for their impact on the protein levels by luciferase reporter assay.
Results: Here we show that variant alleles of *ARHGAP26*_rs187729 T>C and *IRF8*_ rs10514611 C>T were associated with an increased risk of CML in the recessive model, with adjusted odds ratio (OR) and 95% confidence interval (95% CI)=1.63 (1.08-2.47) and 2.4 (1.12-5.15), respectively. Luciferase reporter assay revealed that the C allele of *ARHGAP26*_rs187729 creates an illegitimate binding site for miR-18a-3p, which leads to a 34% decrease in the protein levels (Figure 1).

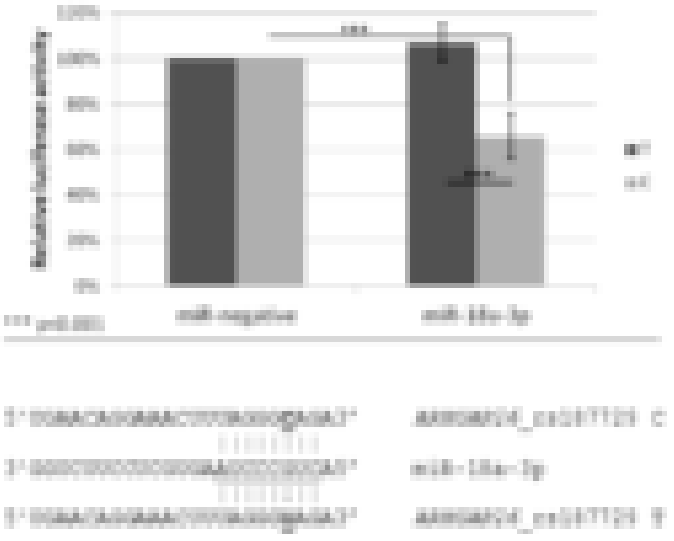

Figure 1. *ARHGAP26*_rs187729.

Summary and Conclusions: Our results demonstrate that the functional polymorphism in the 3'UTR of the tumor suppressor *ARHGAP26* leads to decreased protein levels mediated by miR-18a-3p, which may contribute to an increased risk of CML.
This project was supported by Ministry of Science and Higher Education grant no N N401 570740.

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PHARMACOGENETIC DETERMINANTS OF PLASMATIC AND INTRACELLULAR TYROSINE KINASE INHIBITORS
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Background: Tyrosine Kinase Inhibitors (TKIs) imatinib (IM), nilotinib (N) and dasatinib (D) are commercially available for the treatment of Ph+ Chronic Myeloid Leukemia. IM has a half-life of 12-20 hours (h), N of 15-17 h whereas D has a half-life of 3-5 h only. IM influx is dependent on hOCT1 protein and on other minor transporters. D and N have cellular uptake by diffusion. IM, D and N efflux is dependent on ABCB1 and ABCG2 transporters. Pharmacokinetic (PK)-related factors affecting exposure to TKIs may be involved in resistance to these drugs. TKI plasma level is correlated with efficacy. However there is a wide interpatient variability in drug exposure and this may be partly due to polymorphisms in transporter genes. Furthermore intracellular TKI accumulation and retention was identified as the underlying mechanism of induction of apoptosis but how this concentration is correlated to plasma concentration was never investigated.
Aims: We evaluated plasma and intracellular concentrations of the 3 drugs and we investigated the correlation between plasmatic and intracellular concentrations of IM, D and N, and genotypes of ABCB1 (3435C>T, 1236 C>T, ABCB1 2677 G>T).
Methods: Samples were collected after steady state was reached. HPLC-UV was used for plasma determination of IM and N; while for D analyses an HPLC-MS was used. For intracellular PK, drugs were extracted from isolated peripheral blood mononuclear cells (PBMC), after sonication. Chromatographic intracellular PK evaluation was performed on an HPLC-MS instrument. The allelic discrimination analysis was performed in PCR real-time (BIORAD, Milano, Italia) using the TaqMan assays (Applied Biosystems, Foster City, CA).
Results: We analyzed 56 patients from our Institution: 27 were treated with IM [8 females (F), 19 males (M)], 15 with D (7 F, 9 M) and 14 with N (7 F, 7 M). The median plasma level was 1116 ng/ml (744-1388) for IM, 2.48 ng/ml (0.78-5.74) for D, 1202 ng/ml (680-1638) for N. The median intracellular concentration was 5676 ng/ml (4474-8233) for IM, 452 ng/ml (133-767) for D, 4821 ng/ml (743-8697) for N. The PBMC/plasma ratio was 5.36 for IM, 167 for D and 4.7 for N. We found a statistically significant correlation between the PBMC/plasma ratio of IM and ABCB11236C>T polymorphism: patients carrying CC wild type (WT) had higher ratios *versus* heterozygotes/mutated patients (p=0.027). Significant correlations between plasma concentrations (p=0.011) and ABCB1 3435C>T SNP was observed, with higher concentrations for the TT group *versus* CC+CT group. Accordingly, the CC+CT group had higher PBMC/plasma ratio as compared with the TT group (p=0.018). No correlation was found either between IM and D intracellular concentrations and transporter polymorphisms, or between drug concentrations and genotypes for N. We are now expanding the number of samples in order to verify these data. Finally we found a positive Spearman correlation between plasmatic and intracellular concentration for the 3 drugs (R=0.6, p=0.001 for IM; R=0.559, p=0.024 for D; R=0.508, p=0.064 for N).
Summary and Conclusions: Drugs, particularly D, accumulate in cells, with a correlation between plasmatic and intracellular concentration. Bcr-Abl kinase activity is inhibited in a concentration-dependent fashion, indicating that the cytotoxicity of high-dose pulse exposure to TKIs correlates with the magnitude of target inhibition, and that high-dose pulse therapy and low-dose continuous therapy confer equivalent cytotoxicity to CML cells. Our experiments on a wider population may contribute to understand these mechanisms.

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MUTATED SETBP1 INCREASES THE NUCLEAR LOCALIZATION OF PP2A AND ITS PHOSPHORYLATED FORM
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Background: Mutations in the SET binding protein 1 (*SETBP1*) gene have been identified in patients with Schinzel-Giedion syndrome, in atypical chronic myeloid leukaemia (aCML) and in other proliferative disorders. These mutations strongly suggest an involvement of SETBP1 in the onset of these diseases. In literature there are evidences suggesting that SETBP1 over-expression leads to an increased proliferation of leukemic cells through its interaction with SET and secondarily to an inhibition of PP2A.
Aims: To analyze whether the presence of SETBP1 mutations alters the localization of PP2A within the cells.
Methods: HEK 293T cells were transiently or stably transfected to express SETBP1 wild type (WT) or G870S. Confocal imaging approach was applied to characterize the molecular mechanisms underlying the modification of PP2A expression and localization exerted by the SETBP1 G870S mutation recurrently identified in patients affected by aCML.

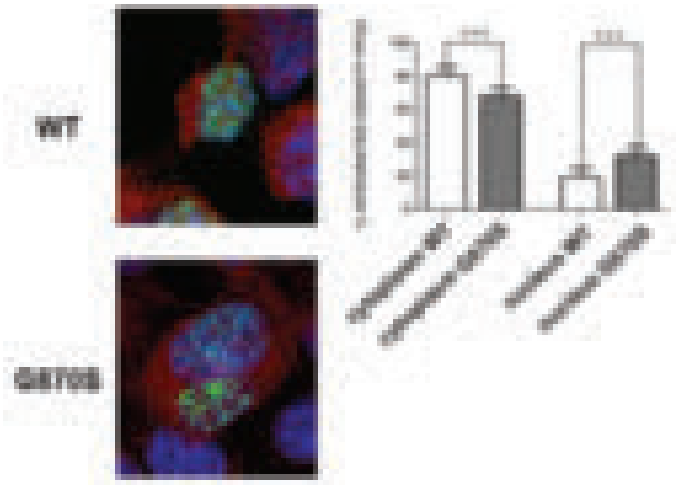

Figure 1. Blue: DAPI; Green: SETBP1; Red: Total PP2A.

Results: In HEK 293T cells transiently transfected with SETBP1 G870S we observed an increased localization of PP2A in the nucleus compared to its distribution in cells expressing SETBP1 WT as shown in Figure 1 (nuclear distribution of PP2A in SETBP1 WT cells *vs* SETBP1 G870S: 20%±5.6% *vs* 32%±5.1%; p<0.001 on three independent experiments). Moreover the antiproliferative effects

exerted by PP2A are reduced by its phosphorylation in Tyr307 (p-Tyr307) leading to a weak interaction with specific cytoplasmatic pathways. The localization analysis of p-Tyr307 PP2A confirmed that the over-expression of SETBP1 G870S predominantly causes retention of this fraction in the nucleus. (% p-Tyr 307 PP2A nuclear distribution in cells expressing SETBP1 WT 48%±4.6% and in cells expressing SETBP1 G870S 61%±4.6%; p<0.01 on three independent experiments). The same impaired localization was observed analyzing the distribution of total as well as phosphorylated PP2A in HEK 293T cells stably expressing SETBP1 WT or G870S (% PP2A nuclear distribution in cells expressing SETBP1 WT *vs* SETBP1 G870S 26%±5.3% *vs* 40%±3.5%; p-Tyr 307 PP2A nuclear distribution in cells expressing SETBP1 WT *vs* SETBP1 G870S 52%±4.7% *vs* 64%±3.9%; p<0.001 on three independent experiments).
Summary and Conclusions: These findings suggest a mechanism of action by witch SETBP1 G870S mutation causes nuclear accumulation of p-Tyr 307 PP2A reducing its effect in the regulation of cell proliferation.

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THE COMBINATION OF PANOBINOSTAT AND PONATINIB EXERTS SYNERGISTIC CYTOTOXICITY IN IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA CELL LINES INCLUDING BCR-ABL GENE MUTATION WITH T315I

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Background: Current first-line treatment options for chronic myeloid leukemia (CML) include imatinib (IM), nilotinib and dasatinib. Nevertheless, a small percentage of CML patients are primarily refractory or secondarily resistant to these agents. The major mechanism of drug resistance of CML is the reactivation of ABL kinase either through *BCR-ABL* gene mutation or gene amplification. A novel pan-histone deacetylase (HDAC) inhibitor, panobinostat induces the acetylation of hsp90, and inhibits its chaperone function in association with the client proteins BCR-ABL, leading to the degradation of it. Moreover it increases acetylated histone in treated cells, resulting in induction of apoptosis. A new pan-ABL tyrosine kinase inhibitor, ponatinib is a promising therapeutic option in patients with all kinds of *BCR-ABL* mutation including T315I. Thus new agents that can overcome the reactivation of ABL kinase are needed.
Aims: We hypothesized that the combination of panobinostat and ponatinib exerts synergistic cytotoxicity against IM-resistant cells through different mechanisms of action of each agent.
Results: K562/IM-R1 and Ba/F3/T315I cell lines were evaluated for the cytotoxicity of panobinostat and ponatinib *in vitro*. K562/IM-R1 cells, established in our previous study, showed BCR-ABL overexpression due to *BCR-ABL* gene amplification. Ba/F3/T315I cells showed *BCR-ABL* with a T315I mutation.The XTT proliferation assay revealed that panobinostat inhibited similarly the growth of K562, K562/IM-R1, Ba/F3, and Ba/F3/T315I cells regardless of their IM-resistance, with 50% inhibitory concentration (IC50) values of around 50 nM (40.0 - 51.0 nM). Ponatinib inhibited the growth of both K562/IM-R1 cells (IC50, 3.8 nM) and Ba/F3/T315I cells (IC50, 30 nM) as potently as their parental K562 cells (IC50, 2 nM) and Ba/F3 cells (IC50, 5 nM). Importantly, the combination of 2 agents exhibited enhanced growth inhibition effects on all cell lines. The combination index calculated using their IC50 values was less than 1.0 in all cell lines, which clearly showed synergism. In the estimation of apoptosis, when the cells were treated with panobinostat or ponatinib at 10 nM (only in Ba/F3/T315I, 30 nM) for 48 h alone or in combination, the combination led to greater-than-additive apoptotic cell death than each agent alone, evaluated by annexin V-positivity. Western blotting was used to evaluate the protein expression levels of BCR-ABL and phospho(p)-BCR-ABL and their downstream signal pathways, STAT5, AKT, ERK1/2, and their phosphorylation in all cell lines after treatment as mentioned above. Ponatinib inhibited the autophosphorylation of BCR-ABL and the phosphorylation of STAT5, AKT, ERK1/2 in both K562/IM-R1 and Ba/F3/T315I cell lines. Treatment with panobinostat mildly reduced the BCR-ABL and p-BCR-ABL expression levels in K562/IM-R1 and Ba/F3/T315I cells. Moreover, the combination of the 2 agents augmented the inhibition of the autophosphorylation of BCR-ABL in these cell lines. And especially in K562/IM-R1 and Ba/F3/T315I cell lines, it was observed that the phosphorylation of downstream signals was also more inhibited in combination than each alone. The activity of HDAC, determined using the HDAC assay Kit, was inhibited by panobinostat in all cell lines regardless of IM sensitivity. In comparison, IM did not alter cellular HDAC activity. Upon treatment with panobinostat, the protein expression levels of acetylated histone H3 and H4 were increased, suggesting the consequence of the inhibition of HDAC. Moreover, the protein levels of acetylated H3 were more enhanced in combination than each alone in all cell lines.
Summary and Conclusions: We firstly reported that panobinostat and ponatinib demonstrated synergistic cytotoxicity against IM-resistant cell lines not only due to *BCR-ABL* gene amplification but also *BCR-ABL* T315I mutation.

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GENE EXPRESSION PROFILING OF CD34+/LIN- CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA AT DIAGNOSIS IDENTIFIES NEW GENES RELATED TO THE NUMBER OF CD34+/LIN- CELLS DURING NILOTINIB TREATMENT

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Background: Chronic myeloid leukemia (CML) is a disease of stemming from genetic damage to a hematopoietic stem cell. Despite nilotinib being a very effective drug for the treatment of CML, drug resistance can emerge. Over the last few years, gene expression profiling (GEP) provides a powerful tool to predict treatment-specific response by the discovery of new biomarkers and pathways. In the contest of the REL-PhilosoPhi34 study on behalf of the Rete Ematologica Lombarda, we performed an exploratory study regarding the GEP of CD34+/lin- cells of CML patients at diagnosis before nilotinib treatment.
Aims: This study aimed to determine GEP of bone marrow (BM) CD34+/lin- cells of newly diagnosed CML patients before starting nilotinib treatment. Patients were divided into 2 groups based on the number of BM CD34+/lin- cells determined at diagnosis and after 3 and 6 months of treatment with nilotinib in order to identify genes and molecular mechanisms correlated to the nilotinib citotoxicity.
Methods: CD34+/lin- cells were isolated from BM mononuclear cells (MNCs) of 30 CML patients at diagnosis. MNCs as well as CD34+/lin- cell counts of 22 CML patients were measured at diagnosis and after 3 and 6 months of nilotinib, respectively. Microarray of BM CD34+/lin- cells of 22 CML patients at diagnosis was performed using the latest generation Affymetrix GeneChip HTA 2.0. Data were preprocessed and normalized using RMA. Selection and data analysis were performed using t-test.
Results: From the comparison between the BM CD34+/lin- cell counts from each patient at diagnosis and after 3 and 6 months of nilotinib, patients were divided into 2 groups: group 1 (*n*=18) showed highly reduced levels of CD34+/lin- cells while group 2 (*n*=4) demonstrated increasing levels of CD34+/lin- cells after 3 and 6 months of nilotinib, respectively. We identified 56 transcripts that were differently expressed between the 2 groups of CML patients. Among them, *SNORD17, SNORD105B, KIAA1324L* genes were underexpressed while *NFKBIA, SNORD58C, RNU5E-1, RNU6-82, TTN-AS1, MIR3153, HIST1H1A, SCRNA14* genes were overexpressed in group 1 compared to group 2, respectively. Very interestingly, *NFKBIA* is involved in several pathways regulating apoptosis: ERK signaling, MAPK family pathways, NF-KappaB pathways, PDGF pathway, PI3K signaling, TNF signaling. *HIST1H1A* belongs to the ProteinKinase-A signaling pathway that regulates processes as growth, development and metabolism. *RNU5E-1* and *RNU6-82* are involved in the spliceosomal splicing cycle and their functions still need to be discovered. Of note, 31/56 transcripts are located on chromosome 15 suggesting that this region could be crucial for transcriptional regulation of CML correlated to nilotinib response.
Summary and Conclusions: This is the first GEP study of CD34+/lin- cells with Affymetrix Genechip HTA 2.0 of CML patients aimed to examine the mechanisms underlying nilotinib response or resistance. The microarray study of CML patients at diagnosis highlighted 56 transcripts mostly present in chromosome 15 and differently expressed in 2 groups of patients based on the loss of cellularity induced by 3 and 6 months of nilotinib treatment. *NFKBIA* was overexpressed in patients who demonstrated a loss of CD34+/lin- cells after 3 and 6 months of nilotinib suggesting that this gene could be involved in nilotinib-response. Ongoing studies will determine the comparison of GEP of CML patients at diagnosis as well as after treatment with nilotinib to predict response or failure providing new insights into the molecular mechanisms in CML.

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EXPRESSION LEVELS OF CERAMIDE-GENERATING AND CLEARANCE GENES IN NEWLY DIAGNOSED AND TYROSINE KINASE INHIBITOR-RESISTANT CHRONIC MYELOID LEUKEMIA PATIENTS: AN ATTEMPT TO FIND NOVEL TARGETS

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Background: Ceramide, generated in response to ionizing radiation, heat stress, oxidative stress, and chemotherapeutics, is the key molecule of sphingolipid family. Increased intracellular levels of ceramide through ceramide syn-

thase genes (CerS1-6) results in growth inhibition, differentiation, apoptosis, alteration of telomerase activity and telomere length, and senescence. On the other hand, increased sphingosine 1-phosphate (S1P) and glucosylceramide (GC) levels through sphingosine kinase-1(SK1) and glucosylceramide synthase (GCS) gene overexpression, respectively, result in the induction of cell proliferation, drug resistance, transformation, angiogenesis, and mobility. In our previous *in vitro* studies, we showed for the first time that bioactive sphingolipids have essential roles in resistance to tyrosine kinase inhibitors (TKIs) and we investigated and published for the first time that targeting bioactive sphingolipids in chronic myeloid leukemia (CML) cells increased sensitivity to TKIs.
Aims: In this study, we aimed to examine the expression levels of of bioactive sphingolipid genes in newly diagnosed, TKI-treated and have demonstrated minimum hematological response or TKI-resistant CML patients.
Methods: Total RNAs were isolated from mononuclear cells obtained from bone marrow samples of CML patients and then converted to cDNA by reverse transcriptase. Expression levels of BCR/ABL, CerS1-6 genes, GCS and SK-1 genes were analyzed by real-time PCR. Forty patients with different disease profiles were involved in this study.
Results: Expression levels of CerS1, CerS2, CerS4, CerS5, and CerS6 genes were increased considerably in CML patients showing minimum hematological response to TKI treatment than newly-diagnosed and drug-resistant CML patients. On the other hand, expression levels of GCS and SK-1 genes were significantly higher in drug-resistant patients than that of the newly-diagnosed CML patients and TKI-treated patients showing minimum hematological response.
Summary and Conclusions: Our previous studies showed that overexpression of CerS1 gene or application of ceramide analogs synergistically increased cytotoxic and apoptotic effects of TKIs in CML cells whereas suppression of GCS and SK-1 resulted in synergistically increased TKI-induced cell death. We also previously showed that SK-1 regulates expression levels and protein stability of BCR/ABL. In conclusion, the results of this study confirmed our *in vitro* studies and showed a significant correlation between the expression levels of bioactive sphingolipid genes and the sensitivity or resistance of CML patients to TKIs. A correlation between expression levels of SK-1 and BCR/ABL was also determined. Taken together, all these results suggest that expression levels of bioactive sphingolipid genes can be novel markers for determination of drug resistance in CML patients. More importantly, they can be used as novel targets for more effective treatment of resistant CML patients.
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MIRNA-30E TARGETS BCR-ABL1 AND SENSITIZES K562 CELLS TO IMATINIB TREATMENT
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder of hematopoietic stem cells carrying the hallmark of the Philadelphia (Ph) chromosome and an oncogenic BCR-ABL1 fusion gene. Imatinib, a BCR-ABL1 tyrosine kinase inhibitor (TKI) is used as the first line therapy for newly diagnosed CML patients. MicroRNAs (miRNAs) are conserved small non-coding RNAs that negatively regulate gene expression. miRNA profiles are markedly altered in cancers and some of them have a causal role in cancer initiation and progression. Aberrant expression of miRNAs in CML has previously been reported however the role of miRNAs in CML is still poorly understood.
Aims: Here, we compared the miRNA expression profiles of CML cells and healthy cells, we investigated whether TKI treatment could affect the expression of these miRNAs and we focused on miRNA-30e, whose downregulated expression may be crucial in CML.
Methods: miRNA expression was profiled using Agilent miRNA microarrays and verified using Applied Biosystems TaqMan. Bioinformatics and molecular analyses were performed using: Ingenuity systems, miRNA target predictors, real-time PCR, Western blots, luciferase assays and annexin-PI.
Results: We compared the miRNA expression pattern of 2 BCR-ABL–positive CML cell lines (K562 and Meg-01) in reference to a pool of healthy blood. In addition, we looked into the expression profile of K562 cells treated with 2 different types of TKIs; imatinib and dasatinib. With the aid of unsupervised hierarchical clustering we found that healthy blood samples were clustered separately from K562 and Meg-01 cells. Untreated K562 cells were clustered separately from treated ones and imatinib treated K562 cells were clustered closely to those treated with dasatinib. The microarray results were validated by real-time PCR. The expression level of 73 miRNAs in K562 and Meg-01 cells was opposite to their expression in normal blood. Of these miRNAs, the expression of 14 was routed back to near normal levels following exposure to imatinib and/or dasatinib. Seven were downregulated in K562 and upregulated following treatment: miRNA-23b, 30e, 154, 454, 564, 671-5p and 765; and seven were upregulated in K562 and downregulated following treatment: miRNA-9, 193b, 320a, 320b, 500, 132 and 892. The reduced expression of miRNA-30e was one of the most significant changes detected between the CML cell line cells and the healthy cells. In compliance with the CML cell lines, miRNA-30e expression was downregulated in primary CML samples and was restored after imatinib treatment. Since miRNA-30e expression was low in CML we sought to assess the possibility that this miRNA targets BCR-ABL. Indeed, target predictors, TargetScan and PicTar, revealed a conserved target site for miRNA-30e in the 3'-UTR of the ABL1 gene. Overexpression of miRNA-30e led to the downregulation of BCR-ABL1 and ABL1 protein expression. Overexpression of miRNA-30e also led to reduced expression of the phosphorylated form of the BCR-ABL1 target, CrkL, signifying a decline in BCR-ABL1 activity. Additionally, based on luciferase assays, ABL1 was indeed shown to be a target gene regulated by miRNA-30e. Lastly, miRNA-30e significantly enhances imatinib-induced cytotoxicity by promoting a 2-fold induction in imatinib-mediated apoptosis.
Summary and Conclusions: Although further analyses and patient studies are required, these data suggest that miRNA-30e may function as a tumor suppressor miRNA implicated in the pathogenesis of CML and its clinical response to imatinib. Furthermore, the combination of TKI treatment and miRNA-30e present therapeutic potential for CML.

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ANTILEUKEMIC EFFECT OF PLK INHIBITOR AND IN COMBINATION WITH ABL TYROSINE KINASE INHIBITORS AGAINST BCR-ABL-POSITIVE CELLS
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Background: ABL tyrosine kinase inhibitor (TKI), imatinib and second-generation ABL TKIs, nilotinib and dasatinib have demonstrated the potency against chronic myeloid leukemia (CML) patients. However, resistance to ABL TKI can develop in CML patients due to BCR-ABL point mutations. Moreover, ABL TKIs do not eliminate the leukemia stem cells (LSCs). These leukemia cells are contained within a niche in the bone marrow and are often impervious to current treatments. Therefore, new approach against BCR-ABL mutant cells and LSCs may improve the outcome of BCR-ABL-positive leukemia patients. Polo-like kinases (PLK) are the family of serine threonine kinases and essential for mitosis. PLK is also critical regulator of cell cycle progression and DNA damage response. One of the PLK and phosphoinositide 3-kinase (PI3K) inhibitor, rigosertib (ON 01910.Na) is a novel synthetic benzyl styryl sulfone that is cytotoxic against a variety of human tumor cell lines.
Aims: We suggested that rigosertib mediated inhibition PLK and PI3K activity and in combination with ABL TKIs may abrogate the proliferation and survival of Ph-positive leukemia cells including T315I mutation and ABL TKI resistant.
Methods: In this study, we investigated the combination therapy with a rigosertib and an ABL TKIs (imatinib, nilotinib and ponatinib) by using the BCR-ABL positive cell line, K562, murine Ba/F3 BCR-ABL cell line with T315I mutant, nilotinib resistant K562 and ponatinib resistant Ba/F3 BCR-ABL cells and primary samples.
Results: 72 h treatment of rigosertib exhibits cell growth inhibition and induced apoptosis against K562 cells. The treatment of imatinib, nilotinib and ponatinib exhibits cell growth inhibition partially against K562 cells in the presence of feeder cell (HS-5) conditioned media. We found that the treatment of rigosertib abrogated the protective effects of HS-5 conditioned media in K562 cells. Phosphorylation of BCR-ABL, Crk-L was reduced and activity of caspase 3, PARP and γH2A.X phosphorylation was increased. Combined treatment of K562 cells or primary samples with imatinib and rigosertib caused significantly more cytotoxicity. Phosphorylation of BCR-ABL, Crk-L was reduced and cleaved PARP and γH2A.X phosphorylation was increased. Anti-apoptotic protein, Mcl-1 was also decreased. We also found the phosphorylation of histone H3 was increased after rigosertib treatment suggested that the cells arrested in G2/M phase. We investigated the rigosertib activity against T315I positive cells. Rigosertib potently induced cell growth inhibition and increased apoptosis. Combined treatment of Ba/F3 T315I cells with ponatinib and rigosertib caused significantly more cytotoxicity than each drug alone. We next investigated by using ponatinib resistant Ba/F3 cells and nilotinib resistant K562 cells. In the ponatinib resistant cell lines, IC50 of ponatinib was up to 200 nM. BCR-ABL triple point mutations (T315I, E255K and Y253H) were detected by direct sequence analysis. The treatment of rigosertib exhibits cell growth inhibition against Ba/F3 ponatinib resistant cells or nilotinib resistant cells and increased apoptosis. We found cleaved PARP, caspase 3 and γH2A.X phosphorylation was increased.
Summary and Conclusions: Our preclinical results indicated that administration of the PLK and PI3K inhibitor, rigosertib may be a powerful strategy against ABL TKI resistant cells and enhance cytotoxic effects of ABL TKI against those Philadelphia chromosome-positive leukemia cells.

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CYTOPLASMATYC COMPARTMENTALIZATION OF BETA CATENIN FOLLOWING CHIBBY ENFORCED EXPRESSION ACTIVATES AUTOPHAGY AS PRO-SURVIVAL MECHANISM IN CELLS EXPRESSING THE BCR-ABL1 FUSION GENE

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Background: Autophagy is a genetically regulated process of adaptation to metabolic stress. Besides its controversial role in tumor cell survival, it may promote tumor cell survival through events encompassing metabolic reprogramming. The participation of autophagy in CML cell persistence in response to tyrosine kinase inhibitors support that autophagy may be a component of BCR-ABL1 leukemogenic potential. Here we report that catenin nuclear export and inactivation driven by the enforced expression of its antagonist Chibby (Cby) activates autophagy in K562 cell line. B-Catenin translocation from nuclear into cytoplasmatic compartment was associated with a significant increase of different markers of autophagy such as Beclin 1, enhanced conversion of cytosolic-associated protein light chain 3, and presence of autophagosomes, detected by immunofluorescence microscopy. More importantly, autophagic flux activates calpain which, in turn, cleaves b-catenin into a 75 kDa fragment still owning transcriptional activity.
Aims: To identify autophagy role in CML pharmacologic resistance.
Methods: K562 cell line is a human BCR-ABL1 positive cell line. It exhibits low levels of Cby transcript and no Cby protein. A construct containing the whole wt CBY coding sequence was inserted into a commercial plasmid. The construct coding for a 14-3-3-binding defective protein (Cby S20A) has been kindly purchased by K.I. Takemaru. Both wt Cby construct and Cby S20A mutant were transfected in the BCR-ABL1+ cell line K562 by means of electroporation. Stable Cby expression was achieved after two month selection in RPMI additioned with G418. Protein and transcript expression were detected by RT-PCR and western blotting and the proliferation rate by count on a phase contrast microscopy. Autophagy was evaluated using an Autophagy Antibody Sampler Kit purchased by Cell Signaling.
Results: Our results demonstrate that b-catenin cytoplasmic relocation proceeding from Cby enforced expression or elicited by Cby induction in response to IM activates autophagy which promotes survival of K562 cells. Autophagy was neither induced in K562 cells transucing the mutant S20A conctruct not observed in K562 cells where b-catenin was abrogated by siRNA, supporting its dependence upon b-catenin relocation to the cytoplasm. Moreover, autophagy was associated with the activation of calpain, proceeding from the Ca^{2+} release from ER and caspase 12 activation, which, in turn, drives the b-catenin cleavage into a 75 kDa fragment and its nuclear import.
Summary and Conclusions: Here we confirmed the autophagy is a survival pathway likely involved in the disease persistence during therapy with IM or other TK inhibitors. Moreover, we identified a putative autophagy-associated mediator of resistance to BCR-ABL1 TK inhibition, a b-catenin 75 kDa cleaved fragment still owning transcriptional activity. Further investigation is required to elucidate the target genes of such fragment.

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TYROSINE KINASE INHIBITORS DO NOT AFFECT EXPRESSION OF DNA METHYLTRANSFERASES AND GLOBAL METHYLATION LEVEL IN CHRONIC MYELOID LEUKEMIA CELLS

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Background: Imatinib, a selective inhibitor of ABL1 kinase activity, revolutionized the treatment of BCR/ABL1-positive leukemias and paved the way for the successful development of a family of tyrosine kinase inhibitors (TKIs). Several TKIs are now approved for the treatment of different human cancers, five of them are used to treat patients with chronic myelogenous leukemia (CML). Unfortunately for a significant number of patients success of TKIs is not fully achieved by the development of resistance to the therapy or toxicities. Recent reports demonstrating serious cardiovascular side effects caused by next-generation TKIs such nilotinib and ponatinib proves that many questions regarding mechanisms of TKI activity remain unclear. Among them is the potential influence of TKI on DNA methylation. Methylation of cytosine residues within DNA is the classical epigenetic mark and is catalyzed by a group of DNA methyltransferases (DNMTs) with major members: DNMT1, DNMT3A and DNMT3B. DNMT1 is responsible for constitutive methylation while the main role of DNMT3A and DNMT3B is *de novo* methylation of unmethylated DNA. There are contradicting reports published on the role of imatinib and selected other TKIs on DNMTs expression (showing both up- an dowregulation), therefore we decided to comprehensively evaluate if, and how all TKI currently used in CML treatment (imatinib, dasatinib, nilotinib, bosutinib and ponatinib) may affect global methylation level and DNA methyltransferases expression.
Aims: Comprehensive analysis of the influence of first and next generation tyrosine kinase inhibitors (TKIs) on the expression of DNA methyltransferases in CML cell lines and in primary CML CD34+ cells from patients at various stages of the disease and on the global methylation level.
Methods: To study the expression of *DNMTs* genes in CML cells upon TKI treatment, qPCR according to MIQE guidelines was employed. Expression was analyzed in human K562 BCR-ABL1-positive cell line (and HL60 as BCR-ABL-negative control) and in CD34+ progenitor cell pool isolated from peripheral blood leukocytes of CML patients at various stages of the disease: chronic phase (CML-CP) and blastic phase (CML-BP). Blood samples were taken after informed consent. Western blotting analysis was also performed to verify the protein levels of DNMTs upon TKI treatment (imatinib, dasatinib, nilotinib, bosutinib and ponatinib) and to confirm shutdown of BCR-ABL1 kinase activity. To determine global level of 5-methylcytosine (mC) and 5-hydroxymethylcytosine, high performance liquid chromatography (HPLC) was used (according to procedure described by Wnuk *et al.*, Age (Dordr); 2014 Feb;36(1):31-48).
Results: Expression of *DNMT1, DNMT3A* and *DNMT3B* was not significantly affected at the mRNA or protein level by any of the TKIs which were used (neither in K562 cells nor in primary CML CD34+ cells from CML-CP and CML-BC patients). Cells were incubated with TKI concentrations ranging from 1nM to 1uM (relevant to concentrations observed in patients for each TKI used in this study), analysis was performed at 24, 48 and 72h timepoints. TKIs concentrations, which were employed, were effective in shutting down BCR-ABL1 kinase activity, while they were not cytotoxic yet at the time of analysis, as verified by Western blotting of phospho-CrkI and viability assays. Finally, as shown in Figure 1, level of global DNA methylation in K562 cells remained unchanged upon treatment with two selected TKIs which was determined by HPLC method. Although imatinib seemed to slightly increase proportion of methylated cytosines, this was not significant and nilotinib did not exert any effects on global methylation level.

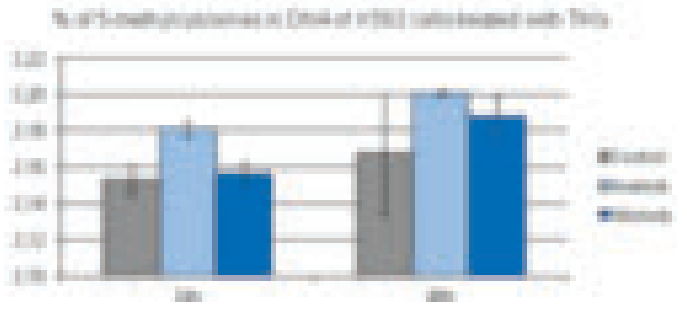

Figure 1. Global levels of methylation in K562 cells incubated for 24h or 48h with imatinib or nilotinib at 100 nM and 10 nM respectively. Global level of methylated cytosines was measured by HPLC.

Summary and Conclusions: Despite recent advances in the field of CML genetics, role of epigenetics in the pathogenesis of CML and the interplay between long-term TKI treatment and epigenetic changes remain to be fully elucidated. Although there are reports demonstrating up- or downregulation of selected *DNMT*s expression upon treatment with imatinib and other TKIs (such as dasatinib and nilotinib), in our opinion this might be caused by high concentrations and long incubation time with TKI. Our observations show that if TKIs affect epigenetic landscape of CML, this is not directly mediated through induction of aberrant DNMTs expression.

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THE EUTOS POPULATION BASED REGISTRY - INCIDENCES OF CML ACROSS EUROPE

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Background: As there are only few data available about the incidence, the stage of disease at diagnosis, the treatment and the outcome of chronic myeloid leukemia (CML) in Europe the European Treatment and Outcome Study (EUTOS) for CML collected such data in 27 European countries and promotes cooperation between hematologists and scientists.
Aims: The population-based registry was set up inside the infrastructure of the EUTOS to further explore the epidemiology, characteristics, treatment and outcomes of CML in Europe. This work focused on the estimation of incidence of CML in Europe and the single countries participating in the registry.
Methods: The population-based registry aimed to document all newly diagnosed adult patients with Ph^+ and/or BCR-ABL+ CML at any stage of disease in whole countries or specified regions of Europe. The registration period varied between 12 and 60 months in the different countries, from January 2008 to December 2012. Raw incidences were calculated for the countries and regions and adjusted to the registration period. For countries observed in whole population data from the United Nations database were used while the study groups provided the population numbers of the specified regions for countries that were observed partially.
Results: Overall Incidences could be calculated for 20 European countries: Austria, Croatia, Cyprus, Czech Republic, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, the Netherlands, Poland, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden and the United Kingdom. 2,956 patients were registered.Estimations of raw incidences per 100,000 persons per year ranged from 0.739 in the United Kingdom to 1.964 in Finland. The overall incidence for all countries was 1.029, with 0.907 in females and 1.163 in males. Incidences rose with age group (20-29 years: 0.421, 30-39 years: 0.634, 40-50 years: 0.978, 50-60 years: 1.348, 60-70 years: 1.529, 70-80 years: 1.794, 80 years and up 1.738).
Summary and Conclusions: This is the first report of the first paneuropean prospective study of the incidence of CML in Europe. The study covers 20 countries, for a total of about 80 million adult inhabitants (^{3} 18 years old), over a period of one to five years. While estimation of incidences for small countries or countries that only observed small regions may be subject to large variations due to small patient numbers, the estimation of incidence over all participating regions can serve as a robust estimation for the incidence of CML in Europe. Even though we have to assume that the calculated raw incidence of 1.029 is likely to be an underestimation as it is impossible to exclude that some new cases were not registered, solid estimates of the incidence in males and females, and particularly by age, are provided. In the very young, 18 to 40 years old, the incidence is as low as 0.498, while in people more than 70 years old the incidence is as high as 1.663. It is well known that especially older patients are underrepresented in prospective studies of treatment.

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MANAGEMENT AND OUTCOME OF CML-BLAST CRISIS: RESULTS FROM THE RANDOMIZED CML STUDY IV

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Background: Treatment of chronic myeloid leukemia (CML)-blast crisis (BC) has remained unsatisfactory in spite of the success with tyrosine kinase inhibition (TKI) of chronic phase CML. Few prospective studies of BC-treatment in the TKI-era are available.
Aims: We have used the randomized CML-Study IV to analyse treatment of BC and its outcome.
Methods: CML-Study IV is a 5-arm randomized optimization study of imatinib (IM) therapy (IM 400mg *vs.* IM 400mg+IFN *vs.* IM 400mg+AraC *vs.* IM 800mg *vs.* IM after IFN failure). Recruitment was from 7/2002 to 3/2012. Definition of BC was according to ELN criteria. No recommendations were given for the management of BC. Comparison was with 699 BC-patients from the pre-imatinib era (CML-studies I-IIIA).
Results: Median observation time was 7.2 (range:2.1-9.8) years. 78 out of 1551 patients developed BC after a median time of 15 months. 8-year cumulative incidence of BC was 5.6% (95%>confidence interval (CI): 4.5-7.7%). Cumulative incidence was higher in the IM after IFN arm (8-year cumulative incidence: 8.5%, 95%>CI:5-14%), but we did not find statistically significant differences. 21 patients from the IM 400 mg arm, 17 from the IM+IFN arm, 11 from the IM+AraC arm, 11 from the IM after IFN arm and 17 from the IM 800 mg arm developed BC. Median age at BC was 58 years, 59% were male. 23% were high-risk patients according to the EUTOS score. 32% had lymphoid and 45% myeloid BC. 23% had mixed or megakaryoblastic phenotype or no information. Median survival after diagnosis of BC was 8.9 (0.1-118+) months, compared to 4 months in the pre-imatinib era. 65 patients had received BC-specific treatment and were evaluable. Treatment consisted of TKI (group 1, n=18, 11 transplanted), intensive chemotherapy (group 2, n =18, 9 transplanted), or a combination of chemotherapy and TKI successively and / or simultaneously (group 3, n=29, 19 transplanted). 13 patients did not receive specific therapy due to early death (n=8) or other reasons (n=5). TKI comprised dasatinib (n=27), IM (n=19) and nilotinib (n=8). Intensive chemotherapy consisted of acute leukemia-type therapies. Patients with lymphoid BC showed better survival than patients with myeloid BC (median survival: 1.62 (0.01-9.8+) *vs.* 0.74 (0.02-9.6+) years, hazard ratio (HR): 0.69, 95%>CI:0.39-1.20. The differences were not statistically significant., most probably due to small sample size. 40 patients were transplanted after a median of 115 (29-309) days after diagnosis of BC. Besides age, we could not identify any prognostic factors for the success of transplantation. TKI between start of BC and transplantation appeared to have no Survival seemed to be best in patients that had been transplanted. Transplanted patients had a median survival after transplantation of 5.4 (0.05-9.6+) years in group 3, of 5,9 (0.02-9.4+)years in group 2 and of 1.4 (0.25-6.7+) years in group 1. Median survival after BC declined to 7 months after censoring at transplantation. There were hints that survival after BC was better when the patients had been originally treated at a university hospital compared to patients treated at municipal hospitals (HR: 0.44, 95%>CI: [0.23; 0.82]) or by office-based physicians (HR: 0.41, 95%>CI: [0.20; 0.83]).
Summary and Conclusions: In CML patients treated with IM the incidence of BC is comparatively low even after 8 years. BC patients show a considerable variation of survival times and transplantation may offer a superior outcome.

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DECLINING ROLE OF DISEASE TRANSORMATION AS A CAUSE OF DEATH IN CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB IN EARLY CHRONIC PHASE: A LONG-TERM ANALYSIS BY THE GIMEMA CML WP

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Background: Ten years after the introduction of tyrosine kinase inhibitors (TKIs) in the therapeutic scenario of chronic myeloid leukemia (CML), the assessment of treatment efficacy is based more and more on the early responses. However, the long term outcome remains critical. The phase 3 trials comparing 2nd generation TKIs *versus* imatinib (IM) have not clearly demonstrated an improvement in overall survival (OS) and IM still represents an important treatment option. An analysis of long-term events is extremely relevant to decide on the allocation of resources.
Aims: To assess the long-term outcome of CML patients in early chronic phase (ECP) treated frontline with IM and to analyze the causes of death.
Methods: 559 patients enrolled within 3 multicentric prospective studies conducted by the GIMEMA CML WP (NCT00514488, NCT00510926, observational trial CML023) were analyzed. Information on survival and progression were prospectively collected for all patients, including after treatment discontinuation. Definitions: failure, according to 2013 European LeukemiaNet criteria; progression, transformation to accelerated or blastic phase at any time; death (unspecified), at any time and for any reason; CML-related death, at any time and after progression or in unknown phase. All the analysis were made according to the intention-to-treat principle.

Figure 1. Estimated survival probability.

Results: Baseline demographics characteristics: median age, 52 years (18-84 years); male sex: 60%; high Sokal, high Euro and high EUTOS score: 22%, 7% and 7%; clonal chromosomal abnormalities in Ph+ cells: 4%; e13a2 BCR-ABL transcript: 36%. Median follow-up 76 months (37-99 months). Only 4% of patients were lost to follow-up and ≥95% of patients had at least 5-year observation. The reasons for IM discontinuation were: lack of efficacy (18%), toxicity or death in remission (10%), other reasons (7%). The subsequent treatment was: nilotinib or dasatinib (50%), ≥two 2nd or 3rd generation TKIs (7%), α-interferon (1%), allogeneic SCT (9%), chemotherapy (11%), none (treatment-free remission, 7%), unknown (14%). The 8-year failure-free survival (FFS), progression-free survival (PFS) and OS were 55% (95% CI: 51-60%), 66% (95% CI: 61-70%), 84% (95% CI: 78-89%) and 85% (95% CI: 79-90%), respectively. A higher age (continuous variable), a higher performance status (ECOG ≥1) and a e13a2 transcript type resulted independent poor prognostic factors on OS. Overall, sixty-five patients died. Approximately half of all the observed deaths were CML-related or due to unknown reasons; a relevant number of patients died for other causes: second malignancy (26%), infection (9%), cardiovascular event (8%), hemorrhage (3%), pulmonary embolism after surgery (1%), respiratory failure (1%). The age at death was significantly lower in patients with CML-related death. According to the time from IM start to the occurrence of death, no significant differences were observed between deaths related or unrelated to CML. Considering only CML-related deaths, patients with high Sokal, high Euro and high EUTOS scores had significantly lower survival probability with respect to low and intermediate risk patients (Figure 1).
Summary and Conclusions: In a large cohort of IM-treated CML patients in ECP, enrolled within prospective multicentric independent studies, a favourable long-term outcome was observed, especially in non-high risk patients. The age and the performance status significantly influenced the OS and the survival was almost equally dependent on CML-related or unrelated deaths. Elderly and unfit patients are often excluded from clinical trials: an effort to collect long-term data on severe and unfrequent toxicities and on cause of deaths observed in the daily practice with the different TKIs, outside of clinical trials, is strongly required to optimize medicare spending.
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NEW MOLECULAR MARKERS OF CML PROGRESSION

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Background: Chronic myeloid leukemia (CML) is prone to a progression from a chronic phase (CP) to an acceleration phase (AP) and a blast crisis (BC). Molecular mechanisms that underlie CML progression aren't clearly known and continue to be a point of investigation. It has been shown recently that some genes from a cancer-testis (CT) family may be activated and hyperexpressed during malignant tumor progression.
Aims: To analyze a cancer-testis genes (CTG) *GAGE1*, *NY-ESO-1*, *MAGEA1*, *SCP1*, *SEMG1*, *SPANXA1*, *SSX1* and *PRAME* expression profile in peripheral blood (PB) of newly diagnosed CML patients as well as in a PB and a bone marrow (BM) of CML patients, by whom a therapy was carried out, on different CML stages: CP, AP and BC.
Methods: We used RQ PCR to evaluate mRNA expression levels of CTG in a PB (n=22) and a BM (n=9) of primary CML patients; in a PB of a treated CML patients in a CP (n=45), AP (n=50) and BC (n=14), in a BM of treated CML patients in a CP (n=15), AP (n=9) and BC (n=7) and 1 patient, who had a CP after BC. A median age was 50 y.o. There were 47% of men and 53% of women. Imatinib therapy was applied for all patients. CTG expression was counted respectively to Abl expression level. PB samples of healthy donors were as a negative control (n=15).
Results: An expression of a single gene from CT-family (*PRAME*) was observed in CP of CML in 3/22 of primary patients (14%) and 7/45 of treated patients (16%) in PB samples. *PRAME* expression level was comparable in both groups (5,5% and 4,9%, respectively, p=0,004, according to Mann-Whitney test). We haven't detected any CTG expression in BM of primary patients. In BM of treated patients with CP of CML was revealed an expression of *NY-ESO-1* (1/15, 7% of cases), *SCP-1* (1/15, 7%) and *PRAME* (4/15, 27%), at the level 35%, 0,14% and 27%, respectively. A multiple CTG expression was observed in a PB of patients with AP of CML: *GAGE1* (7/50, 14% of cases), *MAGEA1* (1/50, 2%), *SEMG1* (12/50, 24%), *SPANXA1* (2/50, 4%) *SSX1* (7/50, 14%) and *PRAME* (19/50, 38%); median levels were 1,2%, 0,01%, 1,3%, 0,3%, 0,5% and 24%, respectively. In BM of patients with AP CML was observed an expression of *SEMG1* (3/9, 33% of cases), *SSX1* (1/9, 11%) and *PRAME* (7/9, 78%); median levels were 1,4%, 0,3%, and 44%, respectively. In PB of patients with BC CML was observed *GAGE1* (4/14, 29%), *SEMG1* (5/14, 36%), *SPANXA1* (1/14, 7%), *SSX1* (2/14, 14%) and *PRAME* (12/14, 86%) expression; median levels were 1,2%, 3,3%, 1,7%, 0,4% and 22%, respectively. In BM of patients with BC CML was observed an expression of *GAGE1* (1/7, 14%) and PRAME (7/7, 100%); median levels were 0,1%, and 36%, respectively. Neither of analyzed CTG was expressed in a PB of the patient who gets to CP of CML after BC.
Summary and Conclusions: It turned out that an expression activation of these eight CTG is strongly associated with CML progression from CP into AP and BP. These data suggest that at least some of CTG may be involved into evolution of CML towards terminal phases. Expression of CTG may be used as early molecular predictor of CML progression into AP and BC.

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PROGNOSIS FOR CML PATIENTS WITH >10% BCR-ABL AFTER 3 MONTHS OF IMATINIB DEPENDS ON THE INITIAL RATE OF BCR-ABL DECLINE

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Background: The molecular response to kinase inhibitors at 3 months (mo) has prognostic significance for patients (pt) with CML. BCR-ABL >10% IS (international scale) is consistently associated with significantly inferior long-term outcomes. It has been suggested that measuring BCR-ABL at 3 mo is the only requirement to predict outcome. Nevertheless, the ELN recommend caution before treatment intervention at 3 mo based on a single measurement. Indeed, many pt with values >10% ultimately achieve satisfactory outcomes. Better early discriminators of the poorest risk pt are needed.
Aims: The kinetics of response at relapse is predictive of outcome when assessed by the BCR-ABL doubling time (Lin Blood 1996, Branford Blood 2004 & 2012). We therefore examined the kinetics of molecular response at 3 mo to determine if the poorest risk pt could be identified among those with a BCR-ABL value >10%.
Methods: 528 first line imatinib treated pt were evaluated. The kinetics of response at 3 mo were determined from the number of days over which BCR-ABL halved (halving time). Halving times were calculated from the individual patient baseline BCR-ABL value (median 103%, range 2-1046). The analysis incorporated the number of days from imatinib start to the 3 mo sample collection, which varied from day 70 to 126 (median 86).

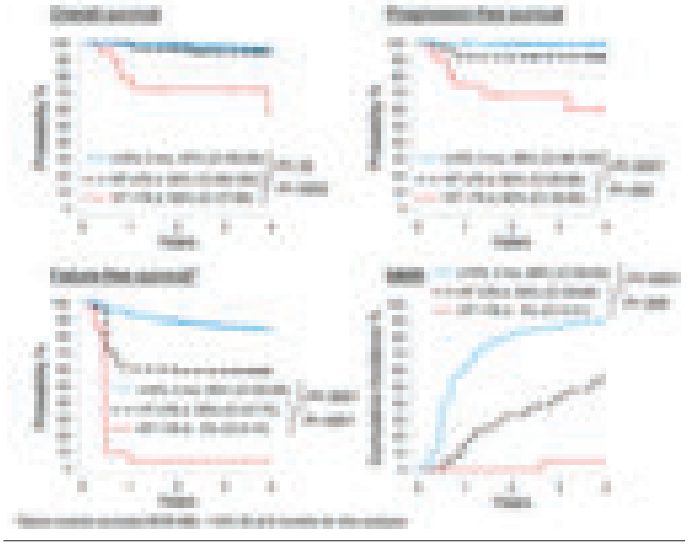

Figure 1. Outcome for patients ≤10% BCR-ABL at 3 mo compared with patients >10% divided by the 3 mo halving time. 410 patients had a BCR-ABL value ≤10% at 3 mo. Of the 97 patients with >10% at 3 mo, 95 had a baseline assessment and halving times were calculated. The results of the patients >10% at 3 mo are divided by their halving times (HT) at 3 mo: ≤76 days (d) n=74, HT >76 days n=21.

Results: 507 pt had a 3 mo assessment. Outcomes by 4 years were significantly superior for those ≤10% (n=410) compared with >10% (n=97): overall survival (OS) 97 v 87% *P*=.0001; progression-free survival (accelerated/blast phase: PFS) 99 v 86% *P*<.0001; failure-free survival (FFS) 85 v 48% *P*<.0001; MMR 88 v 41% *P*<.0001; and $MR^{4.5}$ 41 v 6% *P*<.0001. BCR-ABL halving times at 3 mo were calculated for pt >10% at 3 mo. The gradient from baseline ranged from a shallow to a steep decline, or no change or a rise in some cases, which led to variable halving times. To assess the linearity of our method outside of the IS range and hence the validity of calculating halving times, we examined 485 pt with additional measurements at 1 and 2 mo. The consistency of the logarithmic decline was assessed from the correlation coefficient *r* of the regression lines for the 356/485 pt (73%) with a decline at each measurement. Irrespective of the gradient of the decline or the baseline BCR-ABL value of these patients, BCR-ABL demonstrated a constant logarithmic decline; median *r* -0.98, quartiles -0.99, -0.97. Furthermore, there was no significant difference between these *r* values and those of 73 pt with measurements from mo 1 to 3 where all BCR-ABL values were within the recognized effective IS measurement range (≤10%), *P*=.26, indicating linearity above and below the IS range using our method. Among pt with >10% BCR-ABL at 3 mo, the kinetics were correlated with outcome measures. A halving time of 76 days was the optimal threshold for PFS by ROC analysis. Significantly inferior outcomes occurred for the 21 pt with halving times >76 days for OS, PFS, FFS and MMR, Figure 1. In a Cox proportional hazards model including Sokal, sex, age and assigned dose, the halving time at 3 mo was the only independent predictor of outcome in this poor risk group, ≤76 v >76 days: OS hazard ratio (HR) 6.6 (CI 1.5–30) *P*=.013, PFS HR 5.2 (CI 1.5–18) *P*=.009 and FFS HR 6.8 (CI 3.5–13) *P*<.0001. Only 1/410 pt with BCR-ABL <10% at 3 mo had >76 day halving time.
Summary and Conclusions: Our study has highlighted that the rate of BCR-ABL change from baseline may be a critical prognostic discriminator of the very poor prognosis pt among those who are >10% at 3 mo. This may help to further refine recommendations for treatment decisions at early timepoints.

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HIGH BCR-ABL/GUSIS LEVELS AT DIAGNOSIS ARE ASSOCIATED WITH UNFAVORABLE RESPONSES TO IMATINIB

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Background: The approval of three tyrosine kinase inhibitors (TKIs) for the first line treatment of Chronic Myeloid Leukemia (CML) has generated a need for molecular parameters predictive of unfavorable responses to Imatinib Mesylate (IM) that may identify patients that would benefit from the first line use of second generation drugs Dasatinib and Nilotinib. Growing evidence suggests that failure to achieve early molecular responses (*i.e.* BCR-ABL/ABL^{IS} levels <10% after 3 months or <1% after 6 months of TKI treatment) results in inferior rates of both overall and progression-free survival.
Aims: We wanted to establish if high *BCR-ABL* transcripts at diagnosis would be associated with unfavorable responses to IM.
Methods: Thus, we correlated quantitative determinations of *BCR-ABL* levels at diagnosis with the outcome of 230 newly diagnosed CML patients that were assigned to receive IM 400 mg/die. *BCR-ABL* transcripts were measured from peripheral blood samples drawn before exposure to any form of treatment. Real-Time Quantitative PCR (RQ-PCR) determinations were subsequently performed in triplicates using *glucuronidase-β* (*GUS*) as the reference gene, since previous evidence has demonstrated that *ABL* is not a reliable control gene in samples collected at diagnosis. Values were then reported on the International Scale employing a conversion factor obtained from the laboratory of the University of Heidelberg in Mannheim, Germany.
Results: Median follow-up of the study population was 50 months. Estimated 5-year cumulative incidences of complete hematologic response, complete cytogenetic response (CCyR) and major molecular response were 97.9%, 89.5% and 64.7%. Five-year probabilities of overall survival (OS), transformation-free survival (TFS: survival without disease transformation to the accelerated phase or blast crisis) and failure-free survival (FFS: survival without IM failure as defined by the 2009 European Leukemia Net recommendations) were 93.8%, 97.8% and 76%. Elevated BCR-ABL/GUS^{IS} correlated with inferior probabilities of optimal response (p<0.001), and lower rates of CCyR after 12 months of IM (p<0.001). Moreover, high BCR-ABL/GUS^{IS} transcripts were associated with lower probabilities of FFS (p<0.001) and TFS (p=0.01). When we employed the 2009 European Leukemia Net criteria to subdivide our patient cohort in optimal responders, suboptimal responders and individuals failing IM, we found that increasingly elevated BCR-ABL/GUS^{IS} transcripts accurately distinguished the three patient groups (optimal *vs* suboptimal p<0.001; optimal *vs* resistant p<0.001; suboptimal *vs* resistant p<0.001). Furthermore, using receiver operating characteristic curves we found that progressively higher BCR-ABL/GUS^{IS} levels at diagnosis defined quantitative transcript thresholds (15.96% for Optimal Response, 16.01% for EFS, 16.09% for FFS, 20.36% for TFS and 22.04% for OS) that separated low risk from high risk patients. Finally, we wanted to determine the concordance rates between BCR-ABL/GUS^{IS} levels at diagnosis and early molecular responses (eMRs) at 3 and 6 months. We therefore employed the 15.96% BCR-ABL/GUS^{IS} threshold to identify subjects with high (<15.96%) or low (>15.96%) probabilities of obtaining an Optimal Response and found that 69% of patients displaying <15.96% BCR-ABL/GUS^{IS} achieved <10% BCR-ABL/ABL^{IS} levels after 3 months of IM (p<0.001). Likewise, 78% of subjects presenting <15.96% BCR-ABL/GUS^{IS} at diagnosis attained <1% BCR-ABL/ABL^{IS} after 6 months of therapy (p<0.001).
Summary and Conclusions: We conclude that high *BCR-ABL* transcripts at diagnosis measured by RQ-PCR employing *GUS* as a reference gene allow the identification of CML patients unlikely to benefit from IM that should receive alternative forms of treatment.

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NGS-ASSISTED DNA-BASED DIGITAL QPCR FOR THE DETECTION AND QUANTIFICATION OF RESIDUAL DISEASE IN CML PATIENTS WITH UNDETECTABLE BCR-ABL1 TRANSCRIPTS

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Background: Recent studies indicate that 40% of CML patients who achieve complete molecular remission (CMR) on imatinib remain disease-free after discontinuation of tyrosine kinase inhibitors therapy, raising the possibility of an "operational cure" at a level that is below the threshold of detection by RT-qPCR (10^{-5}). However, the safe introduction of a TKI withdrawal policy would require a reliable and cost effective method of identifying patients with the lowest likelihood of relapse. Such event is likely to be related to the presence of residual disease, which may include transcriptionally quiescent TKI-resistant leukaemic stem cells.[3] A means of detecting these cells, (not depend on oncogene transcription) may therefore be clinically valuable. Preliminary data suggest that PCR based on genomic DNA amplification of the fusion gene may be more sensitive for the detection of residual disease than one that relies on cDNA and may therefore help to predict outcome post-withdrawal. However, the former method is labour intensive and requires a customised patient-specific assay with the use of up to 250 primers combinations.
Aims: Here we describe a DNA-based method of detecting and quantifying low levels of BCR-ABL1 positive disease that improves on previous methodologies by 1) rapid identification of *BCR-ABL1* fusion junctions using targeted next generation sequencing 2) use of a digital PCR (dPCR) platform, which provides absolute molecular quantification without the need for standard curve.
Methods: The fusion junction was mapped in disease samples from 32 CML patients using Illumina's MiSeq platform. A custom TruSeq DNA target enrichment kit (Illumina) was used to enrich for the BCR and ABL1 genes. The enriching probes were designed via the online tool Design Studio covering both genes plus 50kb upstream and downstream of *BCR* and *ABL1*, respectively. The workflow involved sample quantification, library prep, multiplexed sample pooling, enrichment-probe hybridisation, template preparation, and sequencing. Fusion junctions were predicted via a custom designed bioinformatics algorithm. Breakpoints in all 32 patients were successfully mapped. DNA-dPCR was performed using the Fluidigm BioMark Platform on 36 follow-up samples in MR^4 or better and 9 follow-up samples in MMR or higher, from 6 patients in total. DNA-dPCR was performed with and without preamplification of 14 cycles. We also compared DNA dPCR to other methods of BCR-ABL1 quantification (cDNA-dPCR, DNA-qPCR and RT-qPCR).
Results: DNA-dPCR with preamplification detected BCR-ABL1 DNA in 29/36 (80.5%) MR^4 samples. Positive samples corresponded to 6/6 patients. Without preamplification we found that only 17/36 (47.2%) samples were positive corresponding to 5/6 patients. Compared to cDNA-dPCR, we found that only 7/36 samples (19.4) were positive, corresponding to 3/6 patients. All samples were negative by cDNA-dPCR without preamplification. Compared to DNA-qPCR, 5/36 samples (13.8%) gave a positive result. The five samples corresponded to 3/6 patients. This data shows that all patients have detectable disease level by DNA-dPCR at least in one sample while in MR^4.
Summary and Conclusions: In conclusion, our investigations demonstrate that DNA based dPCR is the most sensitive available technique for detecting low levels of BCR-ABL1-positive disease, and that the introduction a preamplification step of 14 cycles prior to the dPCR amplification further increases the sensitivity of this method. NGS-based DNA-dPCR facilitates the stratification of patients with residual disease below the detection limit of RT-qPCR and may, therefore, prove useful in the in the identification of patients from whom TKI therapy could be safely reduced or withdrawn.

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VERY EARLY MOLECULAR RESPONSES IN THE FIRST TWO MONTHS OF THERAPY ARE HIGHLY PREDICTIVE OF DEEP MOLECULAR RESPONSES AT 18 MONTHS, IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED UPFRONT WITH NILOTINIB.

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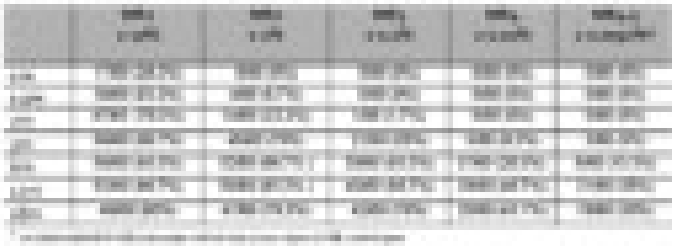
Background: In newly diagnosed CML-CP patients, BCR-ABL ratios during the first trimester of nilotinib treatment, and the kinetic of their descent, could be predictive of deep molecular response thereafter.
Aims: To analyze the molecular response during the first trimester of nilotinib therapy in newly diagnosed CML-CP patients.
Methods: Patients: ENEST1st is a study of nilotinib 300 mg BID in adults with newly diagnosed BCR-ABL+ CML-CP. In this substudy, imatinib pretreatment was not allowed. **Methods:** BCR-ABLIS ratios were measured prior to nilotinib, and subsequently every 2 weeks until the 3rd month (m), and at 6, 12 and 18 m. Sokal, Euro and Eutos scores were calculated with data at diagnosis. Differential count and BCR-ABL values were centrally measured in an ELN-EUTOS certified laboratory. Molecular response classified according to ELN2013 recommendations. Baseline ratios were not included for this analysis. Kinetics of changes were calculated using the ratio of that at a given timepoint compared to that of an earlier timepoint. Logistic regressions and ROC analysis have been used, and positive and negative predictive values (PPV and NPV, respectively) were calculated.
Results: 61 patients. Age: 51,8 y (19-81), M/F: 67%/33%. Risk: Sokal (L,I,H):60%, 28.3%, 11.7%. Euro (L, I, H): 52.5%, 42,4%, 5,1%. Eutos (L,H): 96,7%, 3,3%. 1 patient was excluded because of lack of molecular data (baseline). Ten patients abandoned during the first 18 m because of AE's. They have been classified as non-responders after the time they went off-study. Outcomes: No patient died or had transformation. Optimal responses at 3,6,12 m and 18m were obtained in 97%, 87%, 67%, and 70%, respectively. MR4.5 at 18m has been achieved in 30%. (Table 1). MMR at 12m: In multivariant analysis, only the ratio 3m/d45 was independently associated with MMR at 12m (p=0.015). MMR at 18m: Only sokal, and ratio at 2m were independently associated (p=0.024 and p=0.013, respectively]. $MR4^{\text{at 18 m:}}$ In univariant analysis, spleen size at diagnosis, ratio at 2m, and ratios 3m/d15 and 3m/d45 were associated with MR4 at 18 m. Only spleen size was significant in the multivariant analysis. ROC analysis of the ratio 2m disclosed a cut-point of 1.52. MR4.5 at 18 m: In univariant analysis, spleen size, and ratios at 1m, 1,5m and 2 m, and the ratios 3m/d15 and 3m/d45 were significantly associated with MR4.5 at 18 m. In the multivariant analysis the only independent variable was the BCR-ABL ratio at 2m. The ROC curve disclosed a cut-off of 1.52 (PPV: 90.6% NPV: 59.1%). [OR: 13.96(3.23-60.2) P=0.0004)].

Table 1.



Summary and Conclusions: Our results show that, in newly diagnosed patients, nilotinib resulted in rapid responses, and the proportions of optimal response at 3m (BCR-ABL IS ≤10%), and at 6m (BCR-ABL IS ≤1%) were 97% and 87%, respectively. Nilotinib treatment resulted in MR4 and MR4.5 at 18M in 42% and 30% of patients, respectively. The only diagnostic variable associated with MR4 and MR4.5 at 18 months has been the spleen size. Besides, our study shows the significant association of the molecular response during the first 2 months of therapy and MR4 and MR4.5 at 18 months, as demonstrated by their significant association with ratios at 1m, 1.5 m, and 2 m. The only independent variable associated with MMR, MR4, and MR4.5 at 18m was the ratio at 2m.These results support a prospective analysis of the prognostic value of the very early molecular response (before the third month of therapy) in patients treated with Nilotinib.

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THE DECLINE OF BCR-ABL/GUS RATIO IN CML PATIENTS TREATED UPFRONT WITH NILOTINIB SHOWS A RAPID DESCENT DURING THE FIRST TRIMESTER, AND THE RESPONSES ARE SIMILAR TO THOSE ACHIEVED WITH ABL AS CONTROL

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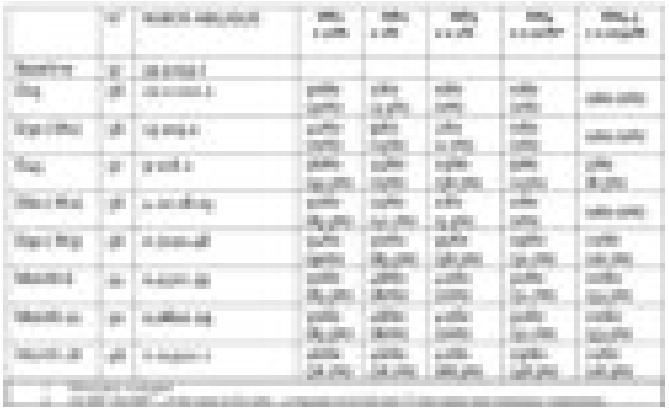
Background: The exact decline of BCR-ABL ratios during the first trimester of therapy has not been previously reported in CML patients treated upfront with Nilotinib.
Aims: To analyze the kinetic of the descent, and the molecular response, using BCR-ABL/GUS ratios during the during the first trimester of therapy, and at 6, 12 and 18 months (m).
Methods: BCR-ABL/GUS ratios were measured prior to nilotinib, and subsequently every 2 weeks until the 3rd month (m), and at 6, 12 and 18 m. Sokal scores were calculated with data at diagnosis. Kinetics of changes were calculated using the slopes of the curves of interest. Molecular response was classified according to ELN2013 recommendations.
Results: 61 patients. Age: 51,8 y (19-81), M/F: 67%/33%. Risk: Sokal (L,I,H):60%, 28.3%, 11.7%. Ten patients abandoned during the first 18 m because of AE's. They have been classified as non-responders after the time they went off-study.
Outcomes: No patient died or had transformation. The kinetic of the descent showed a fast decline of BCR-ABL/GUS ratios during the first trimester. We compared the slopes of the BCR-ABL/GUS ratio in the intervals Baseline-D90 and D90 (3m)-18m. The slopes of the values of Log(BCR-ABL/GUS) before and after the 3rd m were ignificantly different (-0,78±0,34 *vs* -0,1 ±0,11) (p<0,001). (Table 1). The responses were fast. Of note, the proportion of patients having a ratio of 10% or less at 1 month was 70%. The frequencies of optimal responses (ITT method) at 3, 6 and 12 m were 90%, 80% and 70% respectively. The frequencies of MR^4 and $MR^{4.5}$, at 18 m were 48% and 18%, respectively.

Table 1.



Summary and Conclusions: Using frequent measures during the first trimester of Nilotinib therapy, our results show that the curve of descent of BCR-ABL /GUS ratios has a biphasic shape, with a steeper descent during the first 3 months of therapy, and a significant slower decrease thereafter. The frequencies of MMR at 12m, and MR^4 and MR $^{4.5}$, at 18 m (70%, 40%, and 18%, respectively) are equivalent to those reported in the ENEST1st core study, in which ABL was used as control (68%, 36.3% and 19.8%, respectively) (Giles, F. *et al.* Blood 2013 122:4030). In summary, in CML-CP patients treated upfront with NIlotinib, the molecular response using BCR-ABL/GUS shows a rapid decline during the first trimester of therapy, and the frequency of responses are similar to those reported using ABL as gene control.

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AGE AND DIGITAL-PCR ANALYSIS PREDICT RELAPSES OF CML PATIENTS FOLLOWING PROGRAMMED IMATINIB INTERRUPTION IN Q-RT-PCR NEGATIVE CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Imatinib induces complete cytogenetic response (CCyR) in up to 80% of chronic myeloid leukemia (CML) patients (pts) and major molecular response (MMR) in 33-60% of them. These patients enjoy life expectancy similar to the general population. However even undetectable BCR-ABL may not equate to eradication of the disease because the sensitivity of the standard diagnostic method, the Q-RT-PCR, is limited. A new diagnostic method, the digital-PCR (dPCR), able to detect 1 BCR-ABL+ cell out of 10^7 cells, corresponding to a 100 times increased sensitivity as compared to conventional Q-RT-PCR, was developed (Goh HG *et al.*, Leuk Lymphoma 52(5): 896-904. 2011). Therefore dPCR, assessing with more sensitivity the presence of minimal residual disease, could potentially identify pts in whom CML is eradicated.
Aims: The Imatinib Suspension And Validation (ISAV) study is aimed at assessing the capability of dPCR to predict relapses after imatinib discontinuation in CML pts with negative Q-RT-PCR results.
Methods: This study involves 15 sites, 10 in Italy and 5 in each of the following countries: Germany, Spain, The Netherlands, Canada and Israel. In this study CML patients (Chronic Phase or Accelerated Phase) under imatinib therapy since more than 2 years and in complete molecular remission (CMR) were eligible. Patients had to be in CMR for at least 18 months, with a minimum of 3 Q-RT-PCR performed in their own sites. After signing the informed consent, pts were tested for dPCR and discontinued imatinib therapy. They are being monitored by standard Q-RT-PCR for 36 months to assess the maintenance of the molecular remission. The loss of molecular remission is defined as two consecutive positive Q-RT-PCR tests with at least one BCR-ABL/ABL value above 0.1%. Patients losing molecular remission resumed imatinib treatment at the same dosage used before interruption. Patients' quality of life during imatinib discontinuation/resumption is being evaluated through the EORTC – C30 Quality of Life questionnaire.
Results: The enrolment in the ISAV study began in November 2011 and ended in July 2013. The study enrolled 112 pts: Italy 69.6%, Berlin 21.4%, Montreal 5.3%, Zaragoza 2.6% and Tel Hashomer 0.9%. Fifty-nine percent of pts were male and 37.6% were aged 65 or older; median duration of imatinib treatment is 103.2 months with median duration of CMR of 26.94 months before imatinib discontinuation. To date, the median follow-up (FUP) time is 11.27 months [95% CI: 11.04-14.03] and 109 pts out of 112 (97.3%) had at least 1 Q-RT-PCR performed after imatinib discontinuation. All the pts have reached 6 months of FUP. Of these pts, 30 remained Q-RT-PCR negative (27.5%) and 41 pts (37.6%) relapsed and resumed imatinib. Ninety percent of pts relapsed in the first 9 months. A loss of CCyR happened in 8 pts (19.5%); no case of progression of CML was observed. After the resumption of imatinib the median time to either MMR or CMR, whichever came first, was 2.07 [95% CI: 1.15-2.53] months. Finally, 38 pts (34.9%) regained Q-RT-PCR positivity but never lost MMR. The median time to Q-RT-PCR positivity in this group was 3.68 months [95% CI: 3.02-4.99] and the range of duration of Q-RT-PCR positivity (below 0.1%) is between 2 and 19 months. No significant correlation between relapse and previous duration of imatinib treatment, time to CCyR or duration of CMR was present. Instead, an inverse relationship between pt age and risk of relapse was present: 73.9% of pts <=45 years relapsed *vs* 27.9% of >45years, P(Chisq) <0.0001. dPCR results show that 23.4% of pts were positive and 76.6% negative; however 60% (15/25) of dPCR+ and 31.7% (26/82) of dPCR- pts relapsed (see Table 1). To date, the Negative Predictive Value (NPV) of dPCR is 68%, with a significant NPV ratio (dPCR/Q-RT-PCR) of 1.11 [95% CI: 1.02-1.21].

Table 1. dPCR results.

Summary and Conclusions: After 28 months from the beginning of the study with a median follow-up of 11.27 months, 37.6% of pts relapsed; the majority of relapses happened in the first 9 months after imatinib discontinuation. Age inversely correlated with the risk of relapse. dPCR analysis preferentially identified relapsed patients. Funded by Regione Lombardia.

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EARLY RESPONSE OF RADOTINIB THERAPY MAY PREDICT LONG-TERM OUTCOMES IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO BCR-ABL1 TKIS: 24 MONTH UPDATE OF PHASE 2 TRIAL

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Background: Radotinib is an orally active, selective BCR-ABL1 tyrosine kinase inhibitor (TKI), approved for the second-line treatment of CP-CML patients in Korea. Earlier 12 month results of radotinib demonstrated that radotinib is effective and well tolerated in CP-CML patients with resistance and/or intolerance to BCR-ABL1 TKIs.
Aims: We update the latest results of radotinib treatment and evaluate early response to radotinib as a predictor of long-term outcome in failed patients to BCR-ABL 1 TKIs with a minimum follow-up of 24 months.
Methods: Ph+ CP-CML patients who failed prior TKI therapy were enrolled between July 2009 and November 2011. All patients were treated with radotinib 400 mg twice daily. Cytogenetic and molecular assays were performed at baseline, every 3 months, and at treatment failure. Safety parameters were also analyzed. Probabilities of OS and PFS were calculated using Kaplan-Meier method.
Results: A total of 77 CP CML patients (18 years of age or over) were enrolled. This analysis includes data from last enrolled patient who received at least 24 months of radotinib therapy. With a median follow-up of 34.5 months, 35 patients (45.5%) completed 24 months treatment, and 42 patients (54.5%) discontinued the treatment before 24 months. Median duration of radotinib exposure was 19.5 (0.3-48.9) months. Cumulative incidence of CCyR by 24 months was 70.0% and of patients achieving CCyR, 46.2% (18/39) achieved MMR. The drug-related safety profiles were consistent with those previously reported and new safety issues have not been observed after 12 months. Most drug-related AEs have developed within 12 months, and have shown minimal increase compared with rates at 12 months follow up. Estimated OS and PFS at 24 months were 93.3% and 90.9, respectively. In patients with BCR-ABL1 ≤10% at 3 months, higher rates of OS (100% *vs* 88.2%, *P*=0.0668) and PFS (100% *vs* 75.1%, *P*=0.0015) at 24 months were observed, compared with that of the patients with BCR-ABL1 >10%. Also, OS (100% *vs* 85.8%, *P*=0.0153) and PFS (100% *vs* 63.1%, *P*<0.0001) were higher among patients with BCR-ABL1 ≤10% at 6 months compared with those who achieved BCR-ABL1 >10%. In patients with Ph+ <65% at 3 months, higher rates of OS (100% *vs* 83.5%, *P*=0.0258) and PFS (100% *vs* 63.4%, *P*=0.0001) were observed, compared with that of the patients with Ph+ ≥65%. And, OS (100% *vs* 87.6%, *P*=0.0060) and PFS (100% *vs* 71.6%, *P*<0.0001) were higher among patients with Ph+ <35% at 6 months compared with those who achieved Ph+ ≥35% at 6 months.
Summary and Conclusions: In TKI failed CML-CP patients, the minimum 24 months follow up data shows that radotinib maintains the effective response. Furthermore, achievement of early cytogenetic and molecular responses at 3 and 6 months are predictive of long-term outcomes of second-line radotinib therapy.

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MR4.5 AT POST-TRANSPLANT 3 MONTHS WAS A PREDICTIVE FACTOR FOR LONG-TERM OUTCOMES IN THE PATIENTS UNDERWENT ALLOGENEIC SCT IN CML CP

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Background: Since the first BCR-ABL1 tyrosine kinase inhibitor (TKI), imatinib mesylate (IM), has become a first-line therapy for chronic-phase (CP)chronic myeloid leukemia (CML), more potent second-generation TKIs have used in routine practice. However, allogeneic stem cell transplantation (SCT) remains to be an important treatment of patients in advanced-phase CML at diagnosis, with a T315I mutation or progression to the accelerated phase (AP) or the blast phase (BP) at the time of TKI failure. Previous studies showed the role of TKIs as an option for salvage therapy for relapsed patients, emphasizing the importance of a post-transplant monitoring by qRT-PCR.
Aims: This study is performed to identify a predictor for post-transplant relapse and outcomes in the patients underwent allogeneic SCT in CML CP.
Methods: A total of 110 consecutive patients with CML CP underwent allogeneic SCT at Seoul St. Mary's Hospital between May 2001 and December 2013. As we intended to investigate an early predictor for post-transplant relapse in CML CP, 9 advanced patients (6 in AP and 3 in BC) at the time of transplant were excluded, and 101 CP patients were evaluated. All of the patients received grafts from either a HLA identical sibling or an unrelated donor, and 64 patients received a myeloablative regimen and 37 patients received a reduced intensity conditioning regimen. In addition, to identify a predictive role of the BCR-ABL1transcript levels after SCT, 89 and 85 patients who had available records at, respectively, 1 and 3 months after SCT were analyzed. MMR was defined as a BCR-ABL1 transcript level of 0.1% or lower on the international scale (IS). $MR^{4.5}$ was defined as a reduction in the BCR-ABL1 transcript level by 0.0032% or lower.
Results: A total of 101 patients (60 men, 41 women) with a median age of 32 years (range, 13-54 years) were accessed. Of the 101 patients, 47 were TKI-naïve at the time of transplantation, and 51 received IM as their frontline therapy; the remaining 3 received frontline 2G-TKI. Upon the failure of frontline TKI therapy, 17 patients were given other TKI as a second-line therapy, of whom 9 were administered a third-line TKI. After a median follow-up of 126.4 months, the 4-year overall survival and event-free survival were 80.6% and 57.3%, respectively. Of the 101 patients, 36 relapsed after SCT, including hematological relapse (n=11), cytogenetic relapse (n=19), and molecular relapse (n=6). The overall cumulative incidence of relapse at 4 years was 29.6%. In the analysis evaluating a predictive role of the BCR-ABL1 transcript levels after SCT, the $MR^{4.5}$, at 3 months was associated with a lower relapse rate and higher EFS. Multivariate analyses including the potential variables affecting relapse and EFS, respectively revealed that the $MR^{4.5}$, at 3 months remains a higher EFS [RR (95% CI) 2.73 (1.12-6.68), p=0.028] and had a trend for lower relapse rate [RR (95% CI) 2.46 (0.87-6.95), p=0.089].
Summary and Conclusions: Our data showed the $MR^{4.5}$, at 3 months was associated with a lower relapse rate and higher EFS in the patients underwent allogeneic SCT in CML CP. It may imply that frequent molecular monitoring and intervention are required for patients with no reduction of BCR-ABL1 transcripts to these levels after SCT. However, to evaluate the value of using a TKI in the patients with a higher risk for relapse after SCT, future studies are need.

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MOLECULAR MONITORING USING PERIPHERAL BLOOD COMBINED WITH BONE MARROW AT 3 MONTHS OR 6 MONTHS PROVIDES A BETTER PREDICTOR OF OUTCOMES FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Peripheral blood (PB) was the preferred sample of molecular monitoring by quantitative real-time RT-PCR (Q-PCR) for chronic myeloid leukemia (CML) compared with bone marrow (BM) because of a significant concordance and convenience. Prognostic significance of optimal molecular response according to European leukemiaNet (ELN) recommendations on tyrosine kinase inhibitor (TKI) therapy (Version. 2013) comes primarily from monitoring evidences using PB. However, results from Stock *et al.* and our previous prospective study questioned the concordance between PB and BM assays based on each local laboratory standard.
Aims: A comparative study was performed to determine whether early molecular response in BM had actually the same prognostic importance to that in PB and whether molecular monitoring using PB and BM were comparable based on the international scale (IS).
Methods: Between May 2006 and October 2013, 405 CML patients with Philadelphia chromosome (Ph) positive and p210 BCR-ABL positive who were treated by TKI were enrolled in our study. Among them, 137 newly diagnosed CML patients in chronic phase (CP) who received upfront imatinib or nilotinib and underwent sequential molecular detections every 3 or 6 months from the onset of TKI therapy were followed up for a median of 30 months (range, 6-78 months). All patients provided written informed consent. In total, 1148 simultaneous paired PB and BM samples from 405 CML patients and 506 paired samples from the 137 newly diagnosed CML patients on treatment were analyzed. Q-PCR sensitivity defining undetectable BCR-ABL was at least 4.5 logs below the international standard baseline. Molecular response was assessed according to the ELN recommendations (Version. 2013).
Results: Among the 137 newly diagnosed CML-CP patients on upfront TKI therapy, the median Q-PCR value of BCR-ABL in PB was lower than that in BM at 3 months or 6 months (P<0.001), comparable to that in BM at 12 months (P=0.541) and higher than that in BM 18 months later (P<0.001). There were higher proportions of patients with $BCR\text{-}ABL^{IS}$ ≤10% (82.5% *vs.* 71.5%,

P=0.031) and MR4.5 (5.8% *vs.* 0, P=0.007) at 3 months and a low tendency of patients with MR4 (25.8% *vs.* 36.3%, P=0.074) after 18 months in PB than those in BM. Patients with optimal molecular response in both PB and BM had higher rates of event-free survival (EFS) and transformation-free survival (TFS) at 3 years than those without optimal in both PB and BM at 3 months or 6 months (all P values <0.001). Patients with BCR-ABL^{IS} >10% in PB but ≤10% in BM at 3 months had higher rates of EFS and TFS at 3 years than those with BCR-ABL^{IS} >10% in both PB and BM (85.7% *vs.* 45.5%, P=0.026 and 100% *vs.* 75%, P=0.051), similar to those with BCR-ABL^{IS}, ≤10% both in PB and BM. Patients with BCR-ABL^{IS} ≤1% in PB but >1% in BM at 6 months had lower rates of EFS and TFS at 3 years than those with BCR-ABL^{IS} ≤1% both in PB and BM (75% *vs.* 96%, P<0.001 and 83.3% *vs.* 100%, P<0.001), similar to those with BCR-ABL^{IS}, >1% in PB but ≤1% in BM or BCR-ABL^{IS}, >1% in both PB and BM. Among 1148 simultaneous paired samples from 405 patients, BCR-ABL transcript level in PB was lower than that in BM where BM BCR-ABL^{IS} >1% (P<0.001), comparable with that in BM where BM BCR-ABL^{IS} 0.1% - 1% (P=0.336), and higher than that in BM where BM BCR-ABL^{IS} <0.1% (P<0.001), respectively.

Summary and Conclusions: Molecular monitoring using PB combined with BM at 3 months or 6 months provides a better predictor of long-term outcome. Our study confirmed that Q-PCR monitoring for CML using PB is not as equally effective as monitoring using BM and PB assay provides a high sensitivity than BM assay for patients with MMR or deeper molecular response.

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BCR-ABL1 RATIO LOG REDUCTION IS A BETTER PREDICTOR OF MAJOR MOLECULAR RESPONSE (MMR) THAN 10% RATIO CUT-OFF AT 3 MONTHS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS

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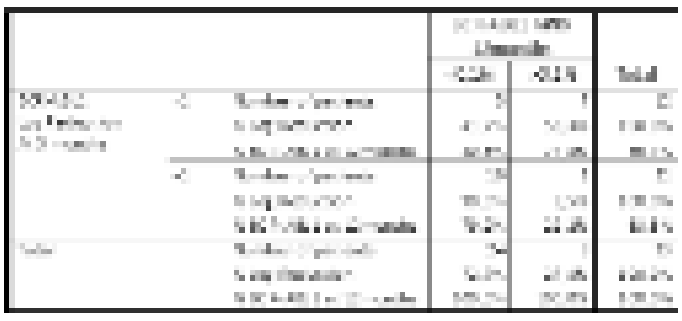
Background: Monitoring molecular response to treatment in patients diagnosed with Chronic Myeloid Leukemia (CML) has changed dramatically in recent years. The latest recommendations of the European Leukemia Net, considered an optimal response values below 10% in the ratio of BCR-ABL1 at 3 months of treatment on any Tyrosine Kinase Inhibitors (TKI) and less than 0.1% at 12 months (MMR), since it predicts better long term disease-free survival. In patients with BCR-ABL1 ratio greater than 10% at 3rd month is recommended to monitor more closely and even consider a change of treatment to another TKI. Moreover, a cutoff value of 10% BCR–ABL1 ratio at 3 months has been correlated as a predictor to achieve MMR; however the role of the kinetics of molecular response is still unclear.
Aims: To assess if BCR-ABL1 log reduction at 3rd month from diagnosis (Dx) can predict achieving MMR compared to a level below 10% of BCR–ABL1 ratio.
Methods: BCR-ABL1 levels were measured (LightCycler© 480, Roche) in a subset of 45 TKI-treated patients at Dx, 3 months and 12 months and Log reduction was calculated on basis of results at Dx and 3rd month.
Results: Of forty-five patients, 33 (73.3%) had <10% BCR–ABL1 ratio at 3rd month and 12 patients (26.7%) >10%. 29 patients (64.4%) achieved a MRR at a year. 9/33 (27.3%) of patients who were in optimal response at 3rd month (<10% BCR-ABL1) failed to achieve MMR at 12 months of treatment. BCR-ABL1 Log reduction ≥1 was observed in 19/24 (79.2%) patients who had <10% ratio BCR-ABL1 ratio at 3rd month and achieved MMR at a year; behaving as a better predictor of MMR than ratio <10% at 3rd month (odds ratio [OR] 13.3, CI 2.5-70.5, *P*=0.002). There were not significant differences in relation to age and median follow-up was 36.6 months (Table 1).

Table 1.



Summary and Conclusions: The only interpretation of the 10% BCR-ABL1 ratio at 3rd month cannot identify a subgroup of patients who fail to achieve MMR at a year, making it necessary to consider the kinetics of response, setting a cutoff log reduction ≥1 at 3 months of treatment in monitoring patients with chronic phase CML.

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GENEXPERT BCR-ABL1/ABL1 MONITOR TEST (IS) FOR ROUTINE MONITORING OF CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Molecular monitoring is an important element of therapeutic response evaluation in chronic myeloid leukemia (CML) patients treated with tyrosine-kinase inhibitors. Currently, the routinely used approach for the estimation of *BCR-ABL*1 transcripts level is a quantitative real time reverse transcription polymerase chain reaction (Q-RT-PCR). However, the method includes several steps, it is labor-intensive and time-consuming. The Cepheid's GeneXpert System is a fully automated RT-PCR based system that claims a standardized *BCR-ABL1/ABL1* measurement in less than 2 hours.
Aims: To evaluate the clinical practicability of *BCR-ABL1/ABL1* evaluation for patients' monitoring using GeneXpert BCR-ABL1/ABL1 Monitor Test (IS) in comparison to the results achieved with routine RT-PCR assays.
Methods: A total of 457 peripheral blood samples from BCR-ABL1(+) CML patients at different time points in the course of the follow up were tested using the GeneXpert *BCR-ABL1/ABL1* test including 5 patients with atypical *BCR-ABL1* mRNA (4 with e1a2; 1 with e1a3). The results from 114 samples were compared with the results, obtained by routine Q-RT-PCR testing using BCR-ABL1 Mbcr IS-MMR DX Kit CE (Ipsogen/Qiagen). Additionally, 50% of the *BCR-ABL1*(-) samples were also tested by nested RT-PCR.
Results: The automated *BCR-ABL1* testing was successful in 449 samples (98.2%): 355 samples were BCR-ABL1(+), the highest *BCR-ABL1/ABL1* ratio observed was 26% (IS), and the lowest - 0.00048% (IS), while in the remaining BCR-ABL1(-)cases the sensitivity of the detection varied from 0.011% (IS) to 0.00017% (IS). The testing failed in only 8 samples (1.8%) due to technical problems associated with high leucocytes count (n=4), other technical reasons (n=2) or unsuccessful ABL1 amplification (n=1). Besides, a weak positive reaction (*BCR-ABL1/ABL1* ratio 0.0044%) was observed in one p210(-) /p190(+) patient, who was found negative at the second testing. The comparison of the results obtained by the automated and the routine Q-RT-PCR testing revealed excellent concordance within the range of *BCR-ABL1/ABL1* ratio between 10% - 0.05%. In patients outside these ranges of *BCR-ABL1/ABL1* levels some discrepancies in the results were observed both in regard to the routine quantitative and nested RT-PCR assays, therefore the reproducibility of the results below 0.05% should be interpreted with caution and are subject for further refinement.
Summary and Conclusions: Our results clearly demonstrate that GeneXpert BCR-ABL1/ABL1 Monitor Test (IS) is a fast and convenient alternative to routine Q-RT-PCR testing, particularly in *BCR-ABL1*-positive patients with levels of *BCR-ABL1/ABL1* between 10% - 0.05%. This approach is useful in clinical practice for evaluation of early molecular response and to distinguish patients with major molecular response (MMR).
Acknowledgements: The study was partially supported by National Science Fund and Novartis Oncology Bulgaria.

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DISCONTINUATION OF DASATINIB IN PATIENTS WITH CML WHO HAVE MAINTAINED COMPLETE MOLECULAR RESPONSE FOR AT LEAST ONE YEAR: RESULTS FROM A PROSPECTIVE DISCONTINUATION (DADI) TRIAL

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Background: Imatinib can be discontinued without molecular relapse at least in some CML patients. But little is known about whether the assumption could be exploitable for the second-generation ABL-tyrosine kinase inhibitors.
Aims: We conducted a prospective, multicenter clinical trial to assess whether dasatinib can be discontinued without molecular relapse in CML patients in complete molecular response (CMR).
Methods: 88 CML patients in chronic phase were pre-registered according to

the criteria of our Dasatinib Discontinuation (DADI) trial (UMIN000005130). CMR was defined as "No detectable BCR-ABL transcript determined by international scale (IS)-based RQ-PCR at a single central laboratory. The levels of BCR-ABL transcripts were monitored every 3 months throughout the pre-registration period during the dasatinib treatment. Patients with sustained CMR for one-year duration were then enrolled for this dasatinib-discontinuation stage. After discontinuation, RQ-PCR was performed monthly for the first 6 months. Molecular relapse was defined as positivity of BCR-ABL transcript by RQ-PCR even at one analysis point. Dasatinib was immediately reintroduced in patients who showed molecular relapse. RQ-PCR monitoring was performed 1 month, 3 months, 6 months, and 12 months after the re-introduction of dasatinib. Primary endpoint of this study was "Molecular relapse-free survival (MoRFS) rate at 6 months after discontinuation of dasatinib."

Results: In total, 88 patients were pre-registered at 41 participating institutions in Japan. Among them, a total of 64 patients who maintained stable CMR for one year after pre-registration were enrolled for the dasatinib-discontinuation stage. One of 64 patients was excluded from the study because the patient's CML cells expressed both major and minor bcr-abl and minor bcr-abl was still detected even after the full enrolment. All of these 63 patients (41 male, 22 female) had been treated with imatinib before the start of the dasatinib treatments. Among these 63, 13 were imatinib-resistant, and 36 were imatinib-intolerant. Other previous treatments were; IFN-α (n=12), nilotinib (n=4), IFN-α and nilotinib (n=1). Median age was 59.4 years (range 24-84). Sokal scores were; low 70%, intermediate 15%, and high 15%. In this interim analysis with a data cut-off date of 31 Jan 2014, 62 patients out of 88 pre-registered patients were over the observation period of 6 months after the dasatinib-discontinuation. Among 62, 30 patients achieved 6 months-sustained CMR after dasatinib-discontinuation. The estimated MoRFS at 6 months determined by Kaplan-Meier method was 48.3%. Reintroduction of dasatinib to the relapsed patients showed rapid molecular responses in all of them. Among the 32 patients who lost CMR after dasatinib-discontinuation, 29 patients were available for the evaluation of reintroduction to CMR. 28 out of 29 patients (97%) returned to CMR again within 3 months (11 patients at 1 month, 15 patients at 3 months and 2 patients at 6 month) after the reintroduction, and all these patients have sustained CMR up to now. The remaining one patient out of 29 also showed a marked reduction of the BCR-ABL transcript level at 3 months.

Summary and Conclusions: Dasatinib could be safely discontinued in a proportion of CML patients with stable CMR for at least one year, provided that frequent molecular monitoring is performed. Patients who lost CMR after dasatinib-discontinuation still maintained good sensitivity to the reintroduction of dasatinib.

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DISCONTINUATION OF IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH UNDETECTABLE MOLECULAR RESIDUAL DISEASE FOR AT LEAST 1 YEAR.

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Background: The recent several reports to assess whether imatinib (IM) can be discontinued in chronic myeloid leukemia (CML) patients have shown that IM discontinuation can be employed based on clinical study in patients who had enough IM therapy and UMRD durations prior to IM discontinuation. However, further validation on precise indications for tyrosine kinase inhibitor (TKI) cessation is needed.

Aims: This study is performed to identify predictor of successful TKI discontinuation for CML patients achieving undetectable molecular residual disease (UMRD) for at least one year.

Methods: A total of 94 patients discontinued IM therapy, including 78 patients enrolled on the Korean Imatinib Discontinuation Study (KIDS) and 16 patients with IM discontinuation due to patient's request (N=15), and drug related adverse event (N=1) after achieving a UMRD. All of patients received only IM before TKI discontinuation with achieving a UMRD for at least 1 year. For the patients enrolled on KIDS, molecular response was monitored using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay every month up to 6 month follow-up, every 2 months up to 12 month follow-up, and every 3 months thereafter, whereas 16 patients who discontinued IM in real practice were monitored qRT-PCR every 3 months. The loss of MMR and UMRD were defined on 2 consecutive assessments, and if loss of MMR occurred, IM treatment was re-introduced.

Results: 49 women and 45 men were included and their median age was 48 years (range, 18-77). The percentages of patients with low, intermediate and high Sokal risk scores were 30%, 29% and 17%, respectively with unknown risk score in 24%. Prior to discontinuation, the median time from TKI therapy to UMRD was 26 months (range, 4.2 - 114.4 months) and the median TKI duration was 80.7 months (range, 32.9 - 141.3 months) including 40.2 months (range, 5.7 - 130.7 months) of sustained UMRD.

After a median follow-up of 11.6 months (range, 1.0 - 114.9), since discontinuation of IM, the 12-month probability of sustained MMR was 65.3%±5.3%.

All of 29 patients who lost MMR were re-treated with IM for a median of 9.5 months (range, 0.1 - 29.2 months). 22 of these patients re-achieved MMR at a median of 2.7 months (range, 0.9 - 14.0 months) after resuming IM therapy and 16 of these patients re-achieved UMRD at a median of 5.6 months (range, 2.8 - 17.8 months). Univariate analysis showed that IM duration and UMRD duration before treatment discontinuation had a higher 12-month probability of sustained MMR.

Summary and Conclusions: Although probability of sustained molecular response is relatively lower than those of our previous KIDS, our data suggested TKI may be discontinued in CML patients with undetectable molecular residual disease for at least 1 year, with utilizing increasingly sensitive PCR technology. To, make more concrete conclusion, further clinical investigation on a large patient population and much longer follow-up are needed.

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CHANGES OF QUALITY OF LIFE AFTER CESSATION OF IMATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH UNDETECTABLE MOLECULAR RESIDUAL DISEASE

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Background: As the number of studies of imatinib discontinuation have been increasingly conducted, the information on post-discontinuation quality of life (QOL) is becoming of considerable important for patients and health-related personnel.

Aims: The purpose of this study was to investigate whether chronic myeloid leukemia (CML) patients who maintained undetectable molecular residual disease (UMRD) with long-term imatinib therapy show different health-related profiles after cessation of imatinib.

Methods: Forty five patients who maintained UMRD after imatinib cessation were given questionnaire for 2 times (before discontinuation and at 6 month post-discontinuation). The health surveys were modified SF-36+FACT.leu composed of imatinib-related adverse events (22 parameters), physical (parameters) and mental (10 parameters) health parameters.

Results: Among 22 parameters of imatinib-related adverse events, 17 parameters significantly improved or disappeared after imatinib cessation except for weight change, skin color change to red, pruritus, skin rash, and sore throat. Three of 11 parameters of physical health (limited social activity, vulnerable to illness, and suffer from drug adverse events) and only 1 of 10 parameters of mental health (loss hope in the fight against the illness) significantly improved. Some parameters of physical health (enjoy life, well-being sense, healthy as anyone as I know, sleep well, and do for fun) and other parameters of mental health (accept the illness, satisfy to treatment, and satisfaction with family communication) worsen after cessation, but not significantly. Younger patients, female patients, and patients with low Sokal score showed a tendency of remarkable improvements in physical and mental health parameters. Interestingly some patients (approximately 25%) have experienced the aggravation of joint pain and myalgia in early period of imatinib cessation, but not related CPK elevation.

Summary and Conclusions: Regardless of significant improvement of drug related adverse events, physical and mental health related parameters were not significantly improved. In addition, older, male gender, and high Sokal risk patients should be carefully monitored after treatment discontinuation.

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DIAGNOSIS AND DISEASE MONITORING OF MDS PATIENTS WITH A DELETION 5Q USING A SIMPLE 4-PARAMETER FLOW CYTOMETRIC TEST

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Background: Patients with MDS and a del(5q) abnormality often display characteristic cytomorphologic features in the bone marrow (BM).
Aims: Whether dyspoietic abnormalities detectable by flow cytometry (FCM) differ between cytogenetic MDS subtypes is not known. Therefore, the immunophenotype in BM of MDS patients with (n=60) and without (n=160) del(5q), adding healthy BM (n=50) and non-clonal cytopenias (n=88) as further controls should be compared.
Methods: A standardized 8-color lyse-stain-wash procedure was performed. Samples were measured on a FACS Canto II. Sensitivity and specificity of the developed score were assessed using ROC analysis. Thereby, a logistic regression model allowed for a differential weighting of the single immunophenotypic features.
Results: Remarkably, a unique 4-parameter del(5q) specific immunophenotypic profile including the percentage of myeloid progenitors (myPC>2%), CD45 MFI ratio (lymphocytes and progenitors ≤7.0), sideward scatter (SSC) ratio (granulocytes and lymphocytes <6.0), as well as CD71 expression on granulocytes (≤20%) could be defined. A subsequently developed clinical score additionally including female gender and a differential weighting of all parameters allowed a distinct separation of del(5q) (score ≥15.0; maximum score=18.0) from non-del(5q) MDS by FCM with high sensitivity (95%; mean score=16.5±1.5) and specificity (85%; mean score=11.5±3.5). Notably, karyotype complexity in the context of del(5q) has no significant influence on the proposed del(5q) FCM score, arguing for del(5q) as a rather early cytogenetic abnormality which drives the cell surface protein expression. Interestingly, del(5q) MDS with a non-informative immunophenotype frequently presented with a mutation in the *TP53* gene (9/10). Furthermore, the disappearance of the del(5q) immunophenotypic profile was observed in all patients (n=13) achieving a complete cytogenetic remission during treatment with lenalidomide (median score=13.0; range: 11.5-14.5).
Summary and Conclusions: We demonstrate for the first time a strong association of a cytogenetic abnormality with a simple and reliable cell surface immunophenotypic profile in MDS, which emphasizes the role of FCM as a reliable tool for diagnostics and disease monitoring.

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LENALIDOMIDE MODIFIES THE BEHAVIOR OF MESENCHYMAL STROMAL CELLS (MSC) FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES WITH 5Q- (MDS-5Q-)

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Background: Raaijmakers *et al.* (Nature, 2010) have shown that deletion of *Dicer1* in MSC-derived osteoprogenitorsas well as its target gene *SBDS*(Shwachman-Blackfan-Diamond Syndrome gene)resulted in myelodysplasia in a murine model. We have shown that MSC from MDS patients show decreased expression of both DICER1 and SBDS, as well as an altered expression of microRNAs when compared with MSC from healthy donors, and MSC from 5q- syndromes have a different genomic profile compared to other MDS (Lopez Villar, Leukemia 2009 and Santamaría. Haematologica 2010). In the current work we have hypothesized thatlenalidomide, the standard treatment for 5q- syndrome patients, could act not only on hematopoietic progenitors but also on the BM microenvironment.
Aims: To analyze the "*in vitro*" effects of Lenalidomide onBM-MSC from MDS -5q- patients and to evaluate the characteristics of MSC from lenalidomide-treated patients before and after initiating the treatment.
Methods: MSC from BM of 9 healthy donors (HD) and 6 5q-patients were isolated and expanded up to third passage. Cells were treated with 50 mMlenalidomide or its solvent (DMSO) as control. MSC gene expression profile changes was studied with the Affymetrix platform (Human Gene S1.0) in 5q-patients (n=4) and healthy donors HD (n=3).Microarray data were confirmed by RT-PCR forANgpt1, IL32, TNFa. In addition and due to their relevance in the animal model the expression of Dicer1, Drosha, and SBDS was studied by RT-PCR. Subsequently some microRNAs involved in hematopoiesis or immune system regulation were analyzed. In order, to see whether the genetic expression could translate into protein expression,levels SBDS and ANGPT1 were analyzed byWestern Blot (WB). Once we verified the effect oflenalidomide on MSC at the molecular level,functional clonogenic assays were performed to seeif the MSC behavior was modified. For this purpose, MSC from 5q- syndromes patients and HD were pre-treated with lenalidomide, and clonogeneic assays with CD34+ HPC were performed. In addition, MSC from 5 patients before and after treatment included ina clinical trial with lenalidomide were expanded and analyzed for DICER and SBDS expression by RT-PCR and WB.
Results: Array expression analysis confirmed that 306 genesshowed significant different expression changeswhen MSC from patients and HD were compared. By RT-PCR analysis MSC from MDS-5q- showed lower expression of DICER, DROSHA, SBDS andANGPT1 when compared with those from HD. This expression increased when MSC were treated withLenalidomide. Also microRNAs expression of mir-150, mir-181, and mir-222 was increased in MSC from MDS-5q- after lenalidomide treatment. In order to know if the increased expression of molecules such as DICER or SBDS could modify the capacity of MSC for supporting hematopoiesis, MSC from patients were treated with lenalidomide and co-cultured with CD34+ HPC. In all cases the number of colonies was superior when co-culture was done with lenalidomide treated MSC. When MSC fromtreated patients were analyzed before and after therapy, we could confirm that both, gene and protein expression, were increased for SBDS in all cases showing hematological response.
Summary and Conclusions: We can conclude that Lenalidomide not onlymodifies HPC from 5q- patients but also the BMmicroenvironment by increasing the expression of*DICER-1*, *SBDS* and other genes as well as the expression of some microRNAs leading to an improvement of their capacity to support hematopoiesis.

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IMPAIRED LINEAGE PRIMING AND PROLIFERATIVE POTENTIAL OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES ARE ASSOCIATED WITH ABNORMAL WNT SIGNALING PATHWAY

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Background: We and others have previously shown that Bone Marrow (BM) Mesenchymal Stem Cells (MSCs) from patients with Myelodysplastic Syndrome (MDS) display a defective proliferative capacity associated with an impaired colony forming potential and passage recovery in long-term cultures. The underlying mechanisms remain, however, obscure
Aims: Given that WNT-signaling pathways play a key role in MSC differentiation proliferation and self-renewal, we explored the possibility that the defective proliferative potential of patient-derived MSCs might be attributed, at least in part, to deregulated WNT-signaling. Furthermore we assessed lineage priming of undifferentiated cultured MDS-derived MSCs.
Methods: We studied 30 patients with *de novo* MDS and 32 age- and sex-matched healthy individuals. BM MSCs were expanded and re-seeded for a total of 10 passages (P). Undifferentiated P2, P6 and P10 MSCs from patient and control cultures were assessed for the expression of osteogenesis- and adipogenesis -related genes by real-time RT-PCR. The expression of 84 genes related to WNT-mediated signal transduction was assessed using a PCR Array (SABiosciences, Qiagen). The fold change (FC) for each gene between the group of patients and the group of controls was calculated with the ΔΔCt method ($FC=2^{-\Delta\Delta Ct}$). In a separate set of experiments the canonical WNT pathway was pharmacologically activated in P2 patient-derived MSCs using 6-bromo-indirubin-3′-oxime (BIO) (TOCRIS, Bristol, UK)
Results: 23 out of 84 WNT-related genes were differentially expressed between patients and controls. Among the non-canonical WNT pathway signaling molecules, a significant up-regulation was observed in WNT5A (FC 3.17), WNT5B (FC 3.33), WNT9A (FC 4.14) and WNT7A (FC 2.0174). A significant increase was also identified in genes implicated in canonical WNT pathway inhibition namely KREMEN1 (FC 4.21), SENP2 (FC 4.64), AES (FC 3.2), GSK3A (FC 3.98), AXIN1 (FC 2.26), CSNK1A1 (FC 2.43), CSNK2A1 (FC 2.25), SFRP1 (FC 2.8962) and CXXC4 (FC 2.9366). To explore the connection between abnormal WNT signaling and defective proliferative potential we activated canonical WNT-pathway in patient MSCs and evaluated cell proliferation by MTT. Exposure of P2 MSCs for 20 days to 10nM BIO resulted in significantly increased cell numbers (P=.0045). Since undifferentiated MSCs are primed towards the adipogenic and osteogenic lineages among others, we examined the expression of osteogenesis and adipogenesis associated genes in undifferentiated MSC cultures, through passages. The expression of osteogenesis-related genes BSP, DLX5, RUNX2 and OSX through P2-P10 was significantly decreased in patient MSCs (P<.05 for each gene). Similarly, the adipogenesis-related genes CEBPA and PPARG were significantly decreased in P2-P10 patient MSCs (P<.05, for each gene). Activation of canonical WNT has

been shown to prime undifferentiated MSCs towards the osteoblastic lineage. Provided that in patient MSCs the canonical WNT signaling is down-regulated, we investigated whether the reduced osteoblastic lineage priming of MDS-derived MSCs might be attributed, at least partly, to the impaired canonical WNT signaling. Indeed, ttreatment with 2μM BIO for 48h resulted in significant up-regulation in the expression of RUNX2 and DLX5 (P<.05 for each gene)
Summary and Conclusions: Overall our data indicate, for the first time, abnormal WNT signaling in MSCs of MDS patients. This abnormality may be implicated in the defective proliferative potential of patient MSCs and in their reduced lineage priming towards the osteoblastic lineage. Our findings support the concept of a primary MSC defect that might have a contributory effect in MDS natural history

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INCREASED EXPRESSION OF INTERFERON RELATED GENES IN THE BONE MARROW MICROENVIRONMENT OF MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of heterogenous diseases characterized by ineffective hematopoiesis and variable degree of the increase in blasts with multiple evolutionary stages. Molecular pathogenesis of MDS has been extensively studied using BM (bone marrow) hematopoietic cells, while the molecular changes of the MDS BM microenvironment are rarely known.
Aims: We analyzed the differential gene expression of BM mesenchymal cells of non-hematopoietic origin in adult MDS patients.
Methods: Primary culture of adherent cell layers (considered as BM mesenchymal cells) derived from BM hematopoietic cells was performed obtained from 7 adult normal controls and 7 MDS patients (3 RCMD, 3 RAEB-1 and 1 RAEB-2). cDNA microarray analysis was performed using humanHT-12 expression v.4 bead array, and data were analyzed using Illumina GenomeStudio v2009.2. Differentially expressed genes (DEGs) were selected by double criteria: Student's t-test p-value <0.01 and fold change >1.5. Functional enrichment analysis with respect to Reactome pathway was performed on KOBAS web server. The significance criteria were gene count ≥3 and p-value <0.01. Functional enrichment analysis with respect to transcription factor targets was performed on TransFind web server with default options.

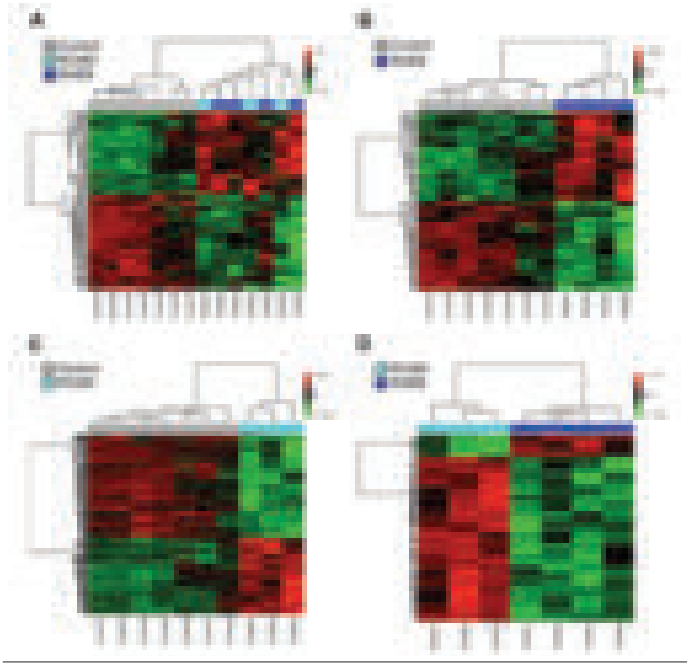

Figure 1.

Results: Differential expression was tested for all possible pairwise comparisons between sample groups, namely, MDS (=RCMD+RAEB) BM mesenchymal cells *vs.* control BM mesenchymal cells, RCMD BM mesenchymal cells *vs.* control BM mesenchymal cells, RAEB BM mesenchymal cells *vs.* control BM mesenchymal cells, and RCMD BM mesenchymal cells *vs.* RAEB BM mesenchymal cells. A total of 452 DEGs were identified: 105, 314, 68 and 51 DEGs, respectively, in each comparison. The similarity of differential expression signatures between RCMD *vs.* control and RAEB *vs.* control was significant, leading to p-value of 0 by assessing with OrderedList program. Hierarchical clustering clearly separated the two groups in every comparison (Figure 1). The functional enrichment analysis showed that both RCMD and RAEB BM mesenchymal cells showed strong up-regulation of interferon alpha/β (IFN-α/β) pathway, compared with control BM mesenchymal cells. In RCMD BM mesenchymal cells, genes involved in IFN-α/β signaling, ISG15 antiviral mechanism, immune system or IFN-γ signaling were overexpressed than in control. In RAEB BM mesenchymal cells, genes involved in IFN-α/β signaling or ISG15 (IFN stimulated genes) antiviral mechanism were overexpressed than in control. In RCMD BM mesenchymal cells, genes involved in RNA polymerase I/RNA polymerase III/mitochondrial transcription or GTP hydrolysis/joining of the 60S ribosomal subunit were overexpressed than in RAEB mesenchymal cells. The analysis for the identification of genes for transcription factor targets, RAEB BM mesenchymal cells showed overexpression of ICSBP (interferon consensus sequence-binding protein, IRF8) and IRF (interferon regulatory factor) related genes than control. RCMD BM mesenchymal cells showed overexpression of ZIC1 (Zic family member 1), STRA13 (stimulated by retinoic acid 13), USF (upstream stimulatory factor), and AhR-HIF (aryl hydrocarbon receptor - hypoxia inducible factor) related genes than RAEB BM mesenchymal cells. No specific transcription factor related genes were identified in the comparison between RCMD BM mesenchymal cells.
Summary and Conclusions: Interferon related genes were overexpressed both in adult RCMD and RAEB BM mesenchymal cells.

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IDENTIFICATION OF ACQUIRED MUTATIONS BY WHOLE-GENOME SEQUENCING IN MONOMAC SYNDROME EVOLVING INTO MYELODYSPLASIA AND ACUTE LEUKEMIA

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Background: Heterozygous *GATA-2* germline mutations are associated with overlapping clinical manifestations termed 'GATA-2 deficiency', including i) familial myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML), ii) Emberger syndrome and iii) an immunodeficiency termed monocytopenia characterised by mycobacterium avium complex (MonoMAC)/dendritic cell, monocyte, B- and NK-lymphoid deficiency (DCML) (Dickinson *et al.,* Hsu *et al.,* Ostergaard *et al.,* 2011). The disease is characterized by immunodeficiency and predisposition to MDS and AML. However, there is considerable clinical heterogeneity among patients, and the underlying molecular basis remains unknown.
Aims: We conducted whole-genome sequencing to identify acquired genome alterations attributable to the evolution of germline *GATA-2* haploinsufficiency into MDS/AML.
Methods: DNA samples were obtained from a single patient, from nails, peripheral leukocytes at immunodeficiency (MonoMAC), bone marrow (BM) mononuclear cells for MDS and BM-derived mesenchymal stem cells (BM-MSCs). To establish BM-MSCs, BM mononuclear cells from the patient were cultured in Dulbecco's modified Eagle medium containing 20% fetal bovine serum and 10 ng/mL basic fibroblast growth factor (Peprotech). The genomic DNA samples were amplified using the REPLI-g Midi Kit (QIAGEN), and sequencing libraries were subsequently prepared according to the TruSeq DNA Sample Prep Guide (Illumina). The libraries were sequenced on an Illumina HiSeq 2000. After sequencing, reads were mapped to the human reference genome (GRCh37/hg19) with decoy sequences (hs37d5) using BWA (Li and Durbin, 2009) with the default options. *GATA-2* mRNA was cloned into pBABE-puro retroviral vector, and the mutation was subsequently introduced with site-directed mutagenesis kit (Agilent). Quantitative ChIP analysis was performed using anti-GATA-2 antibody (H-116, Santa Cruz), which recognizes amino acid residues 120 – 235 of human GATA-2.
Results: We investigated a 35-year-old man with MonoMAC syndrome, who had been treated for recurrent mycobacterial and viral infections, and eventually evolved into MDS/AML. Sanger sequencing identified a germline 988 C>T heterozygous mutation. The mutation resulted in the generation of a premature stop codon at Arg330 (Arg330X), located in the N-terminal zinc finger domain. Quantitative ChIP analysis with anti-GATA-2 antibody based on K562 cells, overexpressed with the GATA-2 Arg330X, suggested that the mutation caused a loss-of-function as it was defective in DNA binding. Whole-genome sequencing was conducted with DNA samples from MonoMAC, MDS and BM-MSCs. Data from the nail sample were excluded from analysis due to a low mapping rate of the sequence on the human genome. First, we focused on the MDS-specific genome deletion based on several structural variant callers, including

BreakDancer, Pindel, and CNVnator (Suzuki *et al.,* 2011). However, we did not identify a candidate genomic deletion that may contribute to the evolution into MDS. Our next strategy was to sort the MDS-specific point mutations using the GATK Unified Genotyper (McKenna *et al.,* 2010). A total of 280 MDS-specific nonsynonymous single nucleotide variants were identified, which were subsequently narrowed down based on the single nucleotide polymorphism database, the functional missense database, and NCBI information (http://www.ncbi.nlm.nih.gov). Finally, we identified three candidate mutations, EZH2 (p.E210K), HECW2 (p.V701M) and GATA-1 (p.R293Q). Sanger sequence-based validation analysis, based on nail, MonoMAC, MDS, and BM-MSCs samples, confirmed that all three mutations were observed only in the MDS sample. We are currently performing a molecular analysis to determine how these mutations could contribute to the evolution to MDS/AML.
Summary and Conclusions: The new mutations identified in *EZH2*, *HECW2* and *GATA-1* appear to be important secondary events leading to the development of MDS/AML in the present case. Our data offer a better understanding of the pathophysiology of GATA-2 deficiency syndrome.

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CHROMOTHRIPSIS IN BONE MARROW CELLS OF ADULT PATIENTS WITH NEWLY DIAGNOSED MYELODYSPLASTIC SYNDROMES (MDS) WITH COMPLEX KARYOTYPES.

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Background: Approximately 20% of patients with newly diagnosed MDS have complex chromosomal aberrations (CCAs) which are associated with a poor prognosis and high risk of transformation to AML. The mechanisms leading to formation of CCAs in MDS remain poorly understood. It is not clear whether the complex karyotypes arise by a gradual acquisition of genetic changes during the clonal evolution or by extensive chromosome fragmentation and reorganization through a unique cellular crisis (chromothripsis).
Aims: The aim of the study was to perform detailed genome wide analyses of bone marrow cells of newly diagnosed MDS patients with CCAs in order to assess the frequency and clinical significance of chromothripsis in the high risk MDS.
Methods: A comprehensive retrospective molecular cytogenetic analysis was performed of fixed bone-marrow cells from 170 patients with CCAs (³3 aberrations) identified with conventional G-banding technique at the time of diagnosis of MDS (85 men, 85 women; median age 67 years). The CCAs and breakpoints on the affected chromosomes were studied by FISH with Vysis DNA probes (Abbott, Des Plaines, IL) and mFISH/mBAND methods, using the 24*X*Cyte and the *X*Cyte color kits (MetaSystems, Altlussheim, Germany). Genomic imbalances were identified with CytoChip Cancer SNP 180K (BlueGnome, Cambridge, UK) or with Illumina Human CytoSNP-12 arrays (Illumina, San Diego, CA).
Results: The molecular cytogenetic findings in 83 patients of this cohort corresponded to the gradual accumulation of random chromosomal changes during the clonal evolution. In the rest of the patients (87pts; 51%), mFISH/mBAND and microarray assays showed breaks with a large number of chromatin losses and gains, probably as a results of chromothripsis. The fragmentation or shattering of chromosomes into many small pieces was observed as well. Parts of the fragmented chromosomes were often translocated or inserted elsewhere in the genome, leading to the chaotically reassembled chromosomes with the most frequently shattered nos. 5, 7, 17, and 12. Surprisingly, the OS of patients with shattered chromosomes did not significantly differ from that of patients with no evidence of fragmentation (p=0.224; median OS in both groups, four months).
Summary and Conclusions: Signs of chromothripsis were observed in 51% of MDS patients with CCAs. Although initial studies have suggested that patients displaying the chromothripsis have more aggressive tumors and poor outcomes, in this cohort no significant difference in the OS of patients with and without chromothripsis was found. It can be assumed that in MDS, CCAs can occur by either mechanism, *i.e.*, as the result of chromothripsis or with gradual clonal evolution. However, under both circumstances the prognosis is very poor.
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NMR-BASED METABOLOMICS DEFINES CHARACTERISTIC METABOLOMIC SIGNATURE FOR MYELODYSPLASTIC SYNDROME (MDS)

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Background: Myelodysplastic syndrome (MDS) is one of the challenging hematological disorder from diagnostic and treatment aspects. This disease usually presents with pancytopenia and it is important to distinguish MDS from other causes of leukopenia. With aging population, the incidence of MDS patients is in a significant uptrend and it is critical to use new technologies like as metabolomics for early and definite diagnosis of this disease. Metabolomics provide a direct functional and interactive readout of overall integrated genomics, proteomics and environmental systems. By application of mass spectrometry, chromatographic techniques or NMR, metabolomics provides highly sophisticated, specific and sensitive data. Application of LC-MS in metabolomics provides major tools to analyze a large number of metabolites simultaneously.
Aims: 1. To define the metabolomic profile of hematopoietic cells of normal, myelodysplastic syndrome and Leukopenic cases. 2. To pursue the hypothesis that borderline MDS cases presenting with leukopenia may show similar metabolomic signature as MDS at early phase of the disease. This may help differentiating these cases from non-MDS leukopenia.
Methods: Hematopoietic bone marrow cells from three categories of normal, Leukopenic and MDS cases were collected and myeloid cells sorted by flow cytometry were stored at -80 C prior to analysis. Then all specimens were analysed by NMR mass spectrometry (NMR-GC-MS) to define the metabolomic profile for different types of metabolites. The data analysis was performed by biostatistical software using PCA and OPLS-DA and outcomes were plotted with no knowledge of categorical information.
Results: Total of 46 specimens were analysed; normal (21), leukopenia with diagnosis of MDS (15) and leukopenia with no history of MDS (10) respectively. Biostatiscal analysis was able to define distinct clusters of normal, Leukopenic-MDS and Leukopenic-Non-MDS cases with significant p value of 0.0001 and higher (1.23e-8). Four cases of borderline MDS were clustered with definite MDS cases on OPLS plot. A panel of metabolites with most significant differences were selected and by application of SUS-Plot (three-dimensional Shared & Unique plots) few metabolites were chosen for future studies. Heaxanic acid was identified as specific metabolite for MDS group (Figure 1).

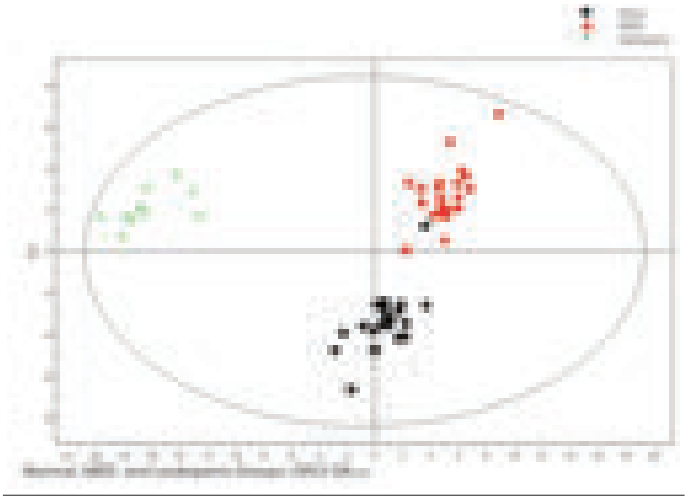

Figure 1.

Summary and Conclusions: This pilot study reveals a characteristic metabolomic signature for MDS. This finding if confirmed by future studies may provide more information for early diagnosis of MDS and distinction of borderline MDS cases form non-MDS leukopenia. In addition, the current metabolomic profile may facilitate future search for new biomarkers for diagnostic or therapeutic purposes.

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CIRCULATING SERUM MICRORNA-21 PREDICTS SURVIVAL AND RESPONSE TO HYPOMETHYLATING AGENTS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Although hypomethylating agents therapy (HMT) have considerably improved the outcomes of patients with myelodysplastic syndrome (MDS), the discovery of sensitive non-invasive biomarkers that can facilitate prediction of response to HMT and therapeutic outcomes are highly desirable to determine optimized strategies of epigenetic therapy for MDS. Circulating microRNAs have been evaluated as potential biomarkers for cancer diagnosis and prognosis prediction.
Aims: This study was designed to evaluate the significance of serum microRNA (miR)-21 as a biomarker for predicting response in MDS patients treated with HMA.

Methods: Serum miR-21 level was measured by quantitative RT-PCR in a cohort of 58 MDS patient and 14 healthy controls. We analyzed the correlation between serum miR-21 level and clinical characteristics, response to HMA and survival.
Results: Serum miR-192 was an internal control, and diagnostic performance was evaluated according to receiver operating characteristics (ROCs). ROC analysis indicated that serum miR-21 levels differentiated responders from non-responders with an area under the curve of 0.648 (95% confidence, 0.49 to 0.72). The baseline level of serum miR-21 was significantly lower in the responder group than in the non-responder group (*P*=0.041). The overall response rate (ORR) of the high miR-21 group was significantly lower than that of the low miR-21 group (41.2 *vs.* 73.2%, *P*=0.021). Progression-free survival (PFS) was significantly inferior in the high group *versus* the low group (14.0 *vs.* 44.5 months, *P*=0.001). Multivariate analyses revealed that the initial serum miR-21 level (*P*=0.001) and circulating blasts (*P*=0.007) were prognostic factors for PFS.
Summary and Conclusions: In this study, we evaluated the levels of serum miR-21 in patients with MDS, and found that Serum miR-21 level was significantly associated with ORR and PFS in MDS patients treated with HMAs. Although validation with a large prospective study is required, serum miR-21 is a potential biomarker of epigenetic therapy in MDS patients.

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ANTI-ERYTHROBLAST AUTOIMMUNITY IN EARLY MYELODYSPLASTIC SYNDROMES: ENHANCEMENT OF EPO/EPO-R SYSTEM AND PRO-APOPTOTIC EFFECT

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Background: Anti-erythroblast autoimmunity has been preliminary demonstrated in a small group of patients with early myelodysplatic syndromes (MDS), *i.e.* refractory anemia (RA) and RA with ringed sideroblasts (RARS), by mitogen-stimulated-direct antiglobulin test (MS-DAT) performed on marrow samples. This autoantibodies, rather than being cytopathic, were found to induce an hyperplastic and diserythropoietic growth *in vitro* of bone marrow (BM) progenitors.
Aims: To confirm the presence of anti-erythroblast autoantibodies in a larger cohort of patients with early MDS, and further characterise their biologic properties and target BM cell.
Methods: We evaluated 70 consecutive MDS patients (median age 78 years, range 41-93; 27 women and 43 men). Fourty-eight out of 70 patients were classified as RA and 22 as RARS, IPSS index was Low for 55/70 (78%) patients and Int-1 for 15/70 (22%). MS-DAT was performed by stimulating BM with PMA and PHA; antibodies were detected in culture supernatants by competitive solid phase ELISA. To further identify the target population of the autoimmune reaction, BM-MS-DAT positive and negative supernatants were tested on normal bone marrow and cells were labeled with Fab'anti-human IgG FITC, anti-glycophorin-A PE, anti-CD71 PerCP, anti-CD34 PECy7 and anti-CD45 APC antibodies, at 4°C in the dark for cytometric analysis. Acquisition and analysis were performed on a FACSCanto II flow cytometer, using FACSDiva 6.0 software.
Results: Thirty-eight out of 70 patients (54%) showed BM-MS-DAT positive values (cut-off value of 150 ng/mL); gender, MDS type, IPSS index, blood counts and therapy were comparable between the two groups. BM-MS-DAT positive patients showed an absolute number of reticulocytes (68±5.5 *vs* 43±4.2, mean±SE, p=0.018), unconjugated bilirubin and LDH levels higher and haptoglobin levels lower (63±6 *vs* 102.5±16.7, mean±SE, p=0.05) compared with negative patients. Moreover, the total marrow erythroblasts, as well as proerythroblasts, basophilic, polychromatic, and orthochromatic erythroblasts were higher in positive *versus* negative cases (p=0.029). Flowcytometric experiments aimed at further characterize the target BM cell of anti-erythroblast autoimmunity, showed a definite binding (20%) of BM-MS-DAT positive supernatants on $CD45^{dim}Gly\text{-}A^{dim}CD71^{bright}$, cells (erythrocyte precursors at different maturation stage), and no staining on $CD45^{-}Gly^{bright}CD71^{-}$ cells (erythrocytes); moreover we found an intermediate population $CD45^{-}Gly\text{-}A^{dim}CD71^{dim}$, with a lower IgG binding (6%). BM-MS-DAT negative supernatants gave no binding on both populations. EPO levels were reduced (13.8±3.2 *versus* 33.9±7.8 mUI/ml, mean±SE, p=0.033) and EPO-R expression increased (45.6±8.0*versus* 19.6±3.2 pg/ml, p=0.03) in bone marrow culture supernatants from BM-MS-DAT-positive patients compared with negative ones. Finally, the level of the pro-apoptotic protein Bax was higher in BM-MS-DAT positive *versus* negative patients (66.9±14.1 *versus* 21.3±3.3 pg/ml, p=0.03) and, consistently, Bcl2 level was lower in the former (10.9±2.4 *vs* 15±3.2 pg/ml), although not significantly.
Summary and Conclusions: Our results confirm the presence of autoimmunity directed against erythroblast precursors in a larger cohort of RA and RARS patients. We suggest that these autoantibodies may amplify the EPO/EPO-R system leading to a pro-apoptotic rather than a cytopathic effect.

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TET2 MUTATION IS FREQUENT AND ASSOCIATED WITH FAVORABLE OUTCOME IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic malignancy with overlap features of both myeloproliferative and myelodysplastic neoplasms and characterized by monocytosis and risk of acute myeloid leukemia transformation (sAML). Several groups have described gene mutations involving DNA methylation in CMML, but their prognostic impact remained to be defined.
Aims: We aimed to determine the frequencies of *TET2, DNMT3A, IDH1* and *IDH2* mutations and their correlation with the clinicohematological features and outcomes in CMML patients.
Methods: Bone marrow samples of 105 CMML patients were analyzed for *TET2*, *DNMT3A, IDH1* and *IDH2* mutations. *DNMT3A* mutations were detected by denaturing high performance liquid chromatography followed by direct sequencing for PCR products of exons 2-23. Mutational analysis of *TET2* gene was performed by PCR followed by direct sequencing for PCR products amplified with primer pairs covering the whole coding sequences. The hot spots of *IDH1* and *IDH2* genes on exon 4 were PCR-amplified from gDNA and subjected to direct sequencing.
Results: There were 63 CMML-1 and 42 CMML-2 patients with a median age of 70.7 (range 30.2-94.9) years. The median follow-up time was 12.0 months. Cytogenetic data was available in 78 patients. Sixty-eight patients had IPSS <2 and 24 patients had IPSS□2. Very low, low, intermediate, high and very high risk categories according to IPSS-R were found in 3, 18, 23, 19, and 15 patients, respectively. None of our patients received demethylating therapy. The overall frequency of gene mutations involving DNA methylation was 52.0%, with a frequency of 40.2% (41/102), 8.7% (9/104), 0% (0/105), and 7.6% (8/105) for *TET2*, *DNMT3A, IDH1* and *IDH2* mutations, respectively. These gene mutations were mutually exclusive except two with co-existence of *TET2* and *DNMT3A* mutations and one with coexistence of *DNMT3A* and *IDH2* mutations. *TET2* mutations were associated with higher levels of hemoglobin and lower percentage of blasts in blood and bone marrow. *DNMT3A* mutations were associated with female sex, lower levels of hemoglobin, higher percentage of blasts in blood and bone marrow, and higher cytogenetic risk. *IDH2* mutations were associated with a lower risk of AML transformation. In univariate analysis, the presence of anemia (Hb<10 g/dL) and thrombocytopenia (Platelet <$100x10^9$/L) were associated with inferior AML-free survival and overall survival, while *TET2* mutation conferred a favorable AML-free survival (median 15.2 *vs* 7.7 months, *P*=0.001) and overall survival (median 26.2 *vs* 11.8 months, *P*=0.001). In multivariate analysis, *TET2* mutations (*P*=0.001) and thrombocytopenia remained the independent prognostic factors for AML-free survival and overall survival.
Summary and Conclusions: *TET2* mutation occurred frequently and conferred a favorable outcome in CMML. The detection of *TET2* mutation is helpful in the prognostic stratification for CMML patients.
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P302

ROLE OF REACTIVE OXYGEN SPECIES PRODUCED BY MALIGNANT MONOCYTES IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Chronic myelomonocytic leukemia (CMML) is a myelodysplastic/myeloproliferative neoplasm with dismal prognosis and few efficacious treatment options. Immunotherapy with histamine dihydrochloride (HDC) and IL-2 has been shown to decrease the relapse rate in AML with monocytic differentiation by inhibiting NADPH-oxidase-derived immunosuppressive reactive oxygen species (ROS) through interaction with surface-bound histamine type 2-receptors (H_2Rs) on the malignant clone. Since monocytosis is a defining feature of CMML, we hypothesized that ROS-production by malignant monocytes may be a mechanism by which CMML escapes immune surveillance, and that HDC and IL-2 has potential as immunotherapy also in CMML
Aims: To evaluate the pre-clinical rationale of HDC-based immunotherapy in CMML
Methods: After receiving written consent, venous blood samples were collected from patients with CMML under medical surveillance at hospitals in western and southern Sweden. PBMCs were isolated by density gradient centrifugation (Lymphoprep), and subjected to immunophenotyping by flow cytometry. Monocytes were defined as being $CD33^{+}CD14^{+}$, and NK-cells as $CD56^{+}CD3^{-}$. Monocytes and NK cells were isolated by either FACS or immunomagnetic isolation. Extracellular ROS production by monocytes was assessed by isoluminol-enhanced chemiluminescense upon stimulation with fMLF in the presence or absence of the

ROS inhibiting agent HDC. To assess immunosuppressive properties of monocytes, NK cells and monocytes were co-cultures over night in presence or absence of HDC (100uM), NADPH-oxidase inhibitor DPI (3uM), ROS scavenger catalase (200U/ml) or PARP-1 inhibitor PJ34 (0.5uM) at ratios ranging from 1:1 to 16:1. NK cell exerted cytotoxicity and degranulation against malignant monocytes was studied using a FACS-based 4-hour ADCC-assay in presence of IL-2 (500U/ml). In order to achieve potent target recognition the malignant monocytes were coated with a humanized anti-CD33 antibody (SGN-33, Abbvie). Cytotoxicity, with or without ROS inhibitors, was analyzed by FACS after staining with Annexin-V and ToPro, gating on apoptotic and lysed target cells, respectively. Degranulating NK cells were defined as the $CD107a^+$ subset.

Results: We found that the $CD33^+CD14^+$ monocytic population of CMML cells express NADPH-oxidase, as measured by surface-bound $gp91^{phox}$, and H_2R. Moreover, this monocytic population produce high levels of ROS upon stimulation with fMLF. The presence of HDC decreases ROS-production significantly ($p<0.001$). Upon co-culture, malignant monocytes induced high levels of apoptosis in NK cells. This was significantly decreased by both ROS inhibitors and PARP-1 inhibitors, indicating a ROS-mediated immunosuppression. Similar suppression, albeit to a lesser extent, was seen upon co-culture with $CD4^+$ and $CD8^+$ T cells. Moreover, NK mediated cytotoxity and degranulation against ROS-producing target cells was significantly augmented in the presence of HDC.

Summary and Conclusions: Our results show that the monocytic subset of malignant cells in CMML does produce ROS, and that monocyte-derived ROS is highly suppressive to adjacent NK and T cells. Importantly, NK-cells were rescued either by preventing ROS-production via HDC or the NADPH-oxidase inhibitor DPI, or by scavenging ROS by means of catalase. In line with previous studies we show that PARP-1-inhibition rescued NK cells from succumbing to monocyte-induced death, supporting the putative notion that ROS-induced lymphocyte apoptosis is caspase independent. Our results also show that NK cell cytotoxicity can be augmentet by HDC. The results from this study reflect what is seen in monocytic AML, *i.e.* subtypes of AML that respond well to HDC/IL_2 immunotherapy, and are suggestive of a potential role for HDC as immunotherapy in CMML. HDC/IL-2 regimens are well tolerated and safe, and a clinical study of HDC-based immunotherapy in CMML thus seems highly warranted. Also, the impact of immunosuppressive malignant monocytes should be considered in all immunotherapies targeting CMML.

P303

DISTURBANCE OF EPIGENETIC STATUS IN STROMAL CELLS COULD BE INDUCED BY INTERACTION WITH MDS/AML-INITIATING CELLS

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Background: The failure of normal hematopoiesis in myeloid neoplasm could be induced by a variety of mechanism. Regarding myelodysplastic syndrome (MDS)/acute leukemia (AML), aberrant hematopoietic stem/progenitor cells with exhibiting ineffective hematopoiesis and impaired differentiation ability gradually substitute it for normal hematopoietic stem/progenitor cells during a long term as a consequent of replacement of stem cell niche. Recently, genetic deletion of Dicer gene specifically in stromal cells developing into MDS/AML, suggesting the possibility that stromal disturbance could be involved in pathogenesis of MDS/AML. However, it has not yet been clarified precise mechanism how MDS stem/progenitor cells could replace normal hematopoietic stem/progenitor cells.

Aims: To gain insight into the contribution of stromal function to develop into MDS/AML, we first examined the supporting activity of stromal cell and gene expression in MDS/AML-derived stromal cells as compared with normal stromal cells. Subsequently, underlying mechanisms involved in the pathogenesis of MDS/AML were analyzed particularly in the view of BM microenvironment.

Methods: In an attempt to analyze the supporting activity of bone marrow (BM) stromal cells, we first established the MDS/AML-derived stromal cells and healthy volunteer (HV)-derived-stromal cells. Next, MDS/AML-derived CD34+ cells or normal CD34+ cells were cocultured with established stromal cells using cytokines including stem cell factor, thrombopoietin, flt3-ligand in the presence of notch ligand (for normal CD34+ cells) or IL-3 (for AML/MDS derived cells). Subsequently, we analyzed clonogenic cells after 2 weeks coculture, 5 week cobblestone area-forming cells (CAFC) and repopulating cells in immunedeficient mice (NSG mice). Finally, we established contact and non-contact culture system between MDS/AML-initiating cells and stromal cells to determine the factors involved in the alteration of stromal function.

Results: The support of clonogenic cells after 2 weeks coculture and 5 weeks CAFCs was observed after coculture with normal CD34+ cells and HV-derived stromal cells. Furthermore, these cocultured cells engrafted into immunedeficient mice. Interestingly, the number of colony-forming units (CFU) mixed cells (MIXs) and CAFC derived from CD34+ cells was drastically reduced after coculture with MDS/AML-derived stromal cells. Nevertheless, MDS/AML-derived stromal cells support the proliferation of leukemia-initiating cells (L-ICs) and L-ICs were detected after third relating. These results indicate that MDS/AML-derived stromal cells preferentially support leukemia stem/progenitor cells, but not normal CD34+ cells. We compared the mRNA expression between (HV)-derived-stromal cells, MDS/AML-derived stromal cells and 5-aza-dC-treated stromal cells. The expression of several factors including hedgehog-interacting protein (HHIP) was reduced in MDS/AML-derived stromal cells. Moreover, promoter methylation of HHIP gene was enhanced in MDS/AML-derived stromal cells (Figure 1). 5-aza-dC treatment restored the expression in some of genes and the stromal supporting activity for normal CD34+ cells partially recovered. The contact and non-contact culture system demonstrated that alteration of stromal function could be induced by even non-contact culture, indicating that certain humoral factors may be involved in the alteration of stromal gene expression.

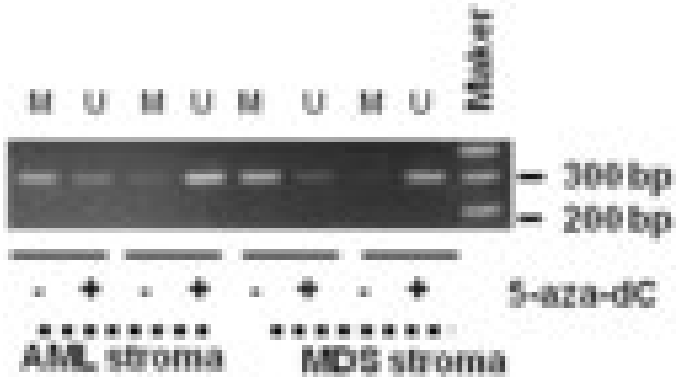

Figure 1.

Summary and Conclusions: These results suggest that reduction of several gene expressions was detected in MDS/AML stromal cells by changes of methylation status. The epigenetic alteration of stromal genome may be involved in the progression of myeloid disorders.

P304

INDEPENDENT MULTICENTRIC STUDY ON THE INTEREST OF OGATA SCORE AND OF CD5, CD7 AND CD56 IN MDS AND MDS/MPS IN REAL LIFE

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Background: Even if of interest, flow cytometry (FCM) is perceived by numerous medical biologists as difficult and expensive, requiring high level of expertise.

Aims: We performed a multicentric open real life study to evaluate the interest of the technically simple FCM score described by Ogata *et al.* for diagnosis of myelodysplastic (MDS) and myelodysplastic/myeloproliferative syndromes (MDS/MPS).

Methods: Six hundred fifty two patients were recruited prospectively in four different centers: 346 MDS, 54 MDS/MPS and 253 controls. MDS were separated in proven when there is ring sideroblast, excess of blast or clonal cytogenetic abnormalities (n=235, 12 RA, 53 RCMD, 31 ASIA, 11 $5q^-$ syndromes, 90 RAEB1 and 38 RAEB 2) and not proven low risk MDS (n=111, 38 RA and 76 RCMD). Controls (n=253) were patient with normal bone marrow (n=17) or with cytopenia due mainly to vitamin deficiency (n=45, 19%), idiopathic thrombocytopenia (n=41, 18%), idiopathic or iatrogenic cytopenia (n=38, 16%) and renal insufficiency (n=24, 10%). Markers used to assess the Ogata's FCM score were CD45, CD34, CD10 and CD19. Additional labeling of CD5 and CD7 on myeloblasts and CD56 on monocytes was evaluated on a series of 292 patients: 107 controls and 185 MDS or MDS/SMP patients (72 non proven MDS, 52 low risk proven MDS, 26 RAEB 1, 16 RAEB 2 and 19 MDS/SMP. Labeling, FCM acquisition and analysis was performed in each participating center. As described by Della Porta *et al.*, we analyzed four parameters: (1) the percentage of CD34+ myeloblasts among all acquired cells (normal range (NR) <2%); (2) the percentage B-cell progenitors, here defined as CD34+CD19+ cells, among all CD34+ cells (NR>5%); (3) the lymphocyte/myeloblast ratio (NR=4 - 7.5); (4) the granulocyte/lymphocyte SSC peak chanel ratio (NR>6). One point was given for each abnormal parameter to calculate classical Ogata score. Extended Ogata score was calculated by adding one point when CD5 or CD7 was expressed on myeloblast or when CD56 was expressed on monocytes. A score of 2 or more is considered as positive for both classical and extended Ogata's score.

Results: None of the four parameters was informative enough on its own.

Specificity of classical Ogata's score was 89% and sensitivity was 50%, 56%, 68%, 84% and 72% for not proven MDS, proven low grade MDS, RAEB1, RAEB2 and MDS/MPS respectively. Interestingly, sensitivity increased with the severity of the disease. Specificity of the extended Ogata's score was hardly changed from 87% to 85%. Sensitivity of the extended Ogata's score was increased for all categories of patients, being 63% for low risk MDS (56% for not proven and 74% for proven), 100% for RAEB 1 and 2 and 90% for MDS/MPS. We recalculated an extended Ogata's score with CD56 only (CD56-Ogata's score). Specificity was 87%, and sensitivity was 60% for low risk MDS (56% for not proven and 65% for proven), 100% for RAEB and 84% for MDS/SMP. Thus the CD56-Ogata's is almost as informative as the extended Ogata's score.

Summary and Conclusions: This study shows that Ogata's score was feasible, reproducible and robust in routine real diagnosis life of MDS. But sensitivity was poor, mainly in low risk MDS. Adding labeling of CD56 on monocytes improved the informativity of Ogata's score, being quite efficient in diagnosis MDS/MPS. Thus,CD56-Ogata's FMC score would be helpful to stratify patients with genuine myelodysplastic syndromes.

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SELECTIVE EFFECT OF LENALIDOMIDE ON CELL CYCLE AND INOSITIDE-DEPENDENT ERYTHROID SIGNALLING IN DEL(5Q) CELLS AND MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Lenalidomide is currently used in the treatment of del(5q) low-risk MDS patients, where it may suppress the del(5q) clone and restore a normal erythropoiesis. The exact molecular mechanisms underlying the effect of Lenalidomide in MDS patients are not completely understood, even though it has been demonstrated that in Lenalidomide-sensitive del(5q) cell lines, Akt phosphorylation is inhibited. Interestingly, Akt signalling is activated both in high-risk MDS, where it activates cell proliferation, and in low-risk MDS, where it is associated with response to erythropoietin.
Aims: To detect the specific effect of Lenalidomide on clonal del(5q) and normal (5q+) cells, in low-risk MDS patients and in cell lines, mainly focusing on erythropoiesis, cell cycle and signal transduction pathways involved in cell proliferation and differentiation.
Methods: This study included 6 patients diagnosed with del(5q) MDS (IPSS: Low or Int-1) treated with Lenalidomide. Given the limited number of cells, we quantified the expression of Akt in bone marrow total mononuclear cells by immunocytochemistry only. In particular, we analyzed Akt and RPS14 co-localization, in order to specifically detect the del(5q) clone. Moreover, by Real-Time PCR analyses, we also assessed the expression of Globin genes, to evaluate the effect of the drug on erythropoiesis. To better discriminate between del(5q) and normal (5q+) cells, we studied the effect of Lenalidomide on Namalwa cells, showing a del(5q) karyotype, and in U937 cells, showing a normal 5q chromosome. In both cell lines, we quantified the expression of Globin genes, cell cycle and inositide signalling molecules, by Real-Time PCR, immunocytochemistry, Western blot and flow cytometry.
Results: In our case series, 4/6 del(5q) low-risk MDS patients responded to Lenalidomide, whereas the 2 non responder patients early discontinued Lenalidomide for adverse events, and for these patients neither a clinical assessment of Lenalidomide effect, nor a molecular analysis, were possible. Responder patients showed an activation of erythropoiesis, in that Beta-Globin levels increased, as compared with baseline. Moreover, these subjects displayed a specific phosphorylation of Akt in cells not showing the 5q deletion.
As for cell lines, Lenalidomide specifically induced an accumulation of Namalwa cells in G0/G1 phase, which corresponded to a slight decrease of p-Akt, an increase of p27 and a decreased expression of cyclin B1, cyclin E and cyclin D3. On the contrary, in normal (5q+) cells, Lenalidomide did not affect cell cycle, but induced both p-Akt and Gamma-Globin.
Summary and Conclusions: Our data show that Lenalidomide can induce a selective arrest of cell cycle in G1 phase of del(5q) cells, therefore slowing the proliferation rate of this clonal cell population. On the contrary, cyclins, Akt and Gamma-Globin are specifically activated in normal (5q+) cells, possibly stimulating a normal cell proliferation and erythroid differentiation. Taken together, these findings could lead to a better comprehension of the effect of Lenalidomide in MDS and pave the way to innovative targeted therapies in low-risk MDS patients without del(5q).

P306

POSSIBLE PREVENTIVE ROLE OF CXCR4 AND ACTIVE CASPASE 3 IN LEUKEMIC EVOLUTION OF MYELODYSPLASTIC SYNDROMES

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Background: WHO guideline defines the approach to the myelodysplastic syndromes (MDS) diagnosis. Moreover, IPSS provides patient division into risk categories concerning the probability of acute myeloid leukemia (AML) transformation. Nevertheless, biological mechanisms underlying MDS evolution to AML remain not entirely understood, as well as some protective mechanisms supporting MDS clonal cells to escape from progression. Some chemokines as SDF-1 and its receptor CXCR4 are suggested to be involved in the regulation of survival-apoptosis of hematopoietic progenitor cells (HPC) in MDS.
Aims: To analyze CXCR4 expression on bone marrow myeloblasts (BM Mybl) in MDS, AML and control samples and to compare it to the intracellular levels of apoptotic proteins active Caspase-3 (aC3) and Bcl2.
Methods: Patients with MDS, AML and non-clonal cytopenia (n=89) were enrolled in the study. MDS were divided into 2 groups: MDSI [RA, RARS, RCMD] and MDSII [RAEB1, RAEB2]. CXCR4 (measured as% positive cells and MFI) was analyzed on BM Mybl by flow cytometry (FCM) using 6- or 7-color antibody combinations: CD34, CD133, CD184, CD33, CD117, HLA-DR, CD45. Additionally, the levels of aC3 and Bcl2 were measured by FCM in cell lysates from BM using a CBA method.
Results: CXCR4 showed significant difference between control cases and all MDS:%CXCR4+ Mybl (p=0.0467), CXCR4-MFI on Mybl (p=0.0004) with lower values in MDS. Moreover, both parameters can reliably distinguish reactive BM from the earliest MDS (resp. p=0.0402, p=0.0201). Additionally,%CXCR4+ Mybl revealed specific dynamics within the MDS group: considerable decrease was observed in RAEB1 compared to earlier stages followed by an increase in RAEB2. Regarding apoptotic proteins the analysis showed differences in aC3 and Bcl2 levels (UI/ml) between MDSI and MDSII with a higher increase of aC3 indicating almost 3-fold activation of apoptosis in MDSII. Additionally, Bcl2 showed difference between RAEB1 and RAEB2 (med 771.8 *vs* 1231.4, p=0.0864). Compared to the whole MDS group, the AML apoptosis protein pattern showed significant increase of Bcl2 levels (med 719.2 *vs* 3179.75) in correlation with markedly lower aC3 (med 40.5 *vs* 14.4, p=0.0087)
Summary and Conclusions: The difference in CXCR4 expression in MDS compared to reactive BM cases indicates different communication of clonal HPC in MDS with BM microenvironment (BMME). It is known that SDF-1/CXCR4 plays an important role in retaining the leukemic cells within the BMME, enhancing their survival and decreasing apoptosis. In this context, our finding of a significant decrease of the% CXCR4+ Mybl in RAEB1 preceding its subsequent increase in RAEB2 may be a sign of activated protective mechanisms against leukemic progression. We suggest that the low CXCR4 expression might aim to prevent clonal HPC from hiding in stem cell niches and thus escape apoptosis. Regarding apoptotic parameters compared to MDSI, in MDSII the increased Bcl2 is paralleled by a marked increase of aC3, while in AML further Bcl2 increase is accompanied by aC3 drop. This may indicate that C3 activation (enhanced apoptosis) might attempt to prevent clonal MDS cells from expansion and transformation. The subsequent aC3 decrease as a sign of an exhausted apoptosis and activation of proapoptotic Bcl2 can suggest leukemic evolution. Taken together, the decrease of CXCR4 expression in RAEB1 might be an additional mechanism to counteract the activated anti-apoptotic Bcl2. The pattern of subsequent CXCR4 and Bcl2 increase and aC3 level decrease may signal pending leukemic evolution and should be considered in the future risk assessment of MDS.

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NEW GERMLINE TERT GENE MUTATIONS IN APLASTIC ANEMIA / HYPOCELLULAR MYELODYSPLASTIC SYNDROMES

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Background: As linear eukaryotic chromosome end-caps telomeres serve to prevent chromosome rearrangements and genomic instability. The telomerase complex, which maintains telomere length, includes *TERT* gene encoding reverse transcriptase and *TERC* gene encoding RNA template. *TERT* and *TERC* mutations underlie diskeratosis congenita (DC) and aplastic anemia (AA) (Savage SA and Bertuch AA, Genet Med 2010). Even in patients without DC, *TERT* gene mutations may be a risk factor for Myelodysplastic syndromes (MDS) and its evolution towards acute myeloid leukemia. (Calado RT, Hematology Am Soc Hematol Educ Program, 2009).
Aims: To investigate *TERT* and *TERC* gene mutations in severe AA or hypocellular MDS (h-MDS).
Methods: 8 patients (4 males, 4 females, mean age 32 years) with AA or h-MDS were recruited. Selection criteria: clinical history, no infectious diseases, no response to immunosuppression. Mutational analysis: DNA was obtained from bone marrow or peripheral blood. *TERT* (16 exons) and *TERC* were investigated by DHPLC (Transgenomic) and Sanger (AB 3500 Genetic Analyzer). PCR:

AmpliTaq Gold Polymerase (Applied Biosystem) or Robust Start Taq KAPA2G (KapaBiosystems). Cloning: RNA extraction (Trizol, Life Technologies); reverse transcription (Thermoscript™, RT-PCR System, Life Technologies); amplification (TERT_2CF (5'-CAGCGCTACTGGCAAATGCG-3' and TERT_2543R (5'-GGCACTGGACGTAGGACTTG-3'). Sub-cloning into pGEM-T easy vector (Promega). Q-FISH: Cy3 linked telomeric (PANAGENE, Korea) and chr 2 centromeric PNA probe, (DAKOCytomation, Denmark). Data were analysed with ISIS software (MetaSystems, Altlussheim, Germany) and expressed as T/C% (Perner S *et al.*, Am J Pathol 2003). For each patient 3 age and sex-matched healthy controls were recruited.

Results: Four new *TERT* coding region mutations were found. Sibling patients nos. 3 and 4 bore the same missense mutation: c. 2093 G>A p. R698Q at exon 5, defined as damaging by *in silico* analysis (SIFT and PolyPhen-2 databases). Clinical effects were significantly different. Patient 3 (female) had severe AA, idiopathic pulmonary fibrosis and liver cirrhosis. She died 41 months after diagnosis. Patient no. 4 (male) had slight macrocytic anemia, mild pulmonary fibrosis and telomere shortening (mean T/C%: 10.9 *vs* 17.7, p<0.05). Patient no.6 with severe AA bore a new missense mutation c. 2020 G>A p. G674S at exon 5 (defined as benign by SIFT and PolyPhen-2), and had significant telomere shortening (T/C%: 18.7 *vs* 25.3, p<0.05). Matched allogeneic transplantation was successful (+20 months). The patient's mother, 1 sister and 1 brother had the same exon 5 mutation without phenotype. Patient no.8 with family background had h-MDS and an acquired 47,XX,+8 karyotype. As compound heterozygous, she bore an exon 2 nonsense mutation (c.1209C>A p.C403*), and an exon 8 missense mutation (c.2455C>T p.R819C). Both were germline, being found in CD3+ T-lymphocytes. Other phenotypes included idiopathic pulmonary fibrosis, skin hyper-pigmentation, osteoarthritis. Telomere length: mean T/C% 9.4 *vs.* 18.1 (p<0.05). Disease is stable at 136 months after diagnosis (Table 1).

Table 1.

Summary and Conclusions: We found 4 new germline *TERT* gene mutations in 8 AA/h-MDS patients. Family history was the major clue to gene mutations. Phenotypes revealed different penetrance.

P308

IS THERE A RELATIONSHIP BETWEEN BONE MARROW CYTOMORPHOLOGY AND FLOW CYTOMETRY IN MYELODYSPLASTIC SYNDROMES?

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Background: A cytomorphology score has been established by the French Group of myelodysplasia to help to morphological diagnosis of myelodysplastic syndromes (MDS). But, in cases of absence of specific markers (excess of blast, cytogenetic abnormalities, ringed sideroblasts) or obvious cytological abnormalities, diagnosis of myelodysplastic syndromes remain challenging. A recent retrospective multicenter study (Della Porta *et al.* 2009) from the European LeukemiaNet (ELN) has validated a previously published flow cytometric (FCM) score for the diagnosis of low-grade myelodysplastic syndromes published by Ogata. Based on four reproducible parameters and a threshold of two, this score has a high (92%) specificity albeit a poor sensitivity (69%), which does not allow to exclude MDS diagnosis when a score of 1 or 0 is observed.

Aims: We compared a cytomorphology score adapted from the French Group of myelodysplasia and Ogata score for FCM.

Methods: In 2011, data from 96 samples were collected. 60 MDS or MDS/MPN (myeloprolferative/myelodysplastic neoplasm) including, 29 low grade MDS, 17 high grade MDS and 14 MDS/MPN were compared to 36 controls (2 healthy subjects, 11 vitamin deficiency, 3 inflammatory, 7 renal failure, 1 excessive alcohol intake, 1 thyroid dysfunction, 4 idiopathic thrombocytopenic, 1 autoimmune cytopenia, 1 haemorrhagia, 8 transient cytopenia, 1 cancer). Bone marrow smears were analysed by two independant cytologists. The 14 discordant cases were reviewed by a third cytologist. The intensity of myelodysplasia was quantified by a cytological score from 0 to 6 for the 3 hematopoietic lineages: 0 point in absence of abnormality, 1 point for dysplasia over to 10% of cells of the lineage, and 2 points for dysplasia over 50%. By FCM, we studied the four parameters of the Ogata score: (1) the percentage of CD34+ myeloblasts among all acquired cells (threshold for normal<2%); (2) the percentage B-cell progenitors, here defined as CD34+CD19+ cells, among all CD34+ cells (threshold for normal>5%); (3) the lymphocyte/myeloblast ratio (normal range: 4- 7.5); (4) the granulocyte/lymphocyte SSC peak chanel ratio (threshold for normal>6). One point was given for each abnormal FCM parameter. Both scoring systems were compared by Cohen's kappa coefficient as well as the Chi-square test.

Results: In this series, sensitivity and specificity of the cytological score were 73% and 75% for diagnosis of MDS and was 61% and 83% for FCM. Cohen's kappa coefficient was 0.204 (cytological score *vs.* Ogata score). According to the classification of Landis and Koch, the agreement between these scores was poor. But, taking cytology as reference, Chi-square test for a cytological score greater than or equal to 2 was significant (p=0.0168). Furthermore, 36%, 50% and 78% of samples had a positive Ogata score when the cytological score was between 0 and 2, equal to 3 or between 4 and 6 respectively.

Summary and Conclusions: Cytology and FCM abnormalities both reflects dysmyelopoiesis but do not allow to assess the diagnosis of MDS on its own, being not absolutely specific. The intensity of cytomorphological dysplasia is somehow related to the number of FCM abnormalities, but the strength of this association is weak and does not reach the significance. Thus, (i) cytological evaluation of dysplasia remains the reference and (ii) further studies are needed to determine whether combination of cytomorphology and FCM would do better to diagnose MDS in case of absence of specific marker.

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MOLECULAR CYTOGENETIC CHARACTERIZATION OF T(2;5)(P16.2;Q33.1) LEADING TO PDGFRB/SPTBN1 GENE FUSION IN A CASE OF MDS RESPONSIVE TO IMATINIB MESYLATE

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Background: PDGFRB is a member of the tyrosine kinase family and PDGFRB gene rearrangements, already reported in myeloproliferative neoplasms (MPN), lead to its constitutive activation, ultimately affecting cell proliferation and migration. MPN patients with PDGFRB rearrangements have been reported to respond promptly to imatinib mesylate therapeutic regimes. The reciprocal t(2;5)(p16;q33) (PDGFRB/SPTBN1) has been reported only in one case of atypical MPN. The partner gene, SPTBN1 is a cytoskeletal protein with a role in mitotic spindles assembly.

Aims: The aim of the study was to detect the partner genes involved in the rare balanced translocation t(2;5)(p16;q33) encountered for the first time in a patient with MDS.

Methods: A 78-year-old man with a past history of surgically removed thymoma, presented in 2012 for investigation of macrocytic anemia. Bone marrow smear showed mild dysplastic features in the granulocytic and megakaryocytic series. Bone marrow biopsy showed 60% cellularity and 4% CD34+ cells. The findings were consistent with MDS diagnosis. One year later, a new biopsy showed 23% CD34+ cells and dyserythropoiesis. The patient has been recently administrated imatinib mesylate therapeutic regime.

Following karyotypic analysis on unstimulated bone marrow cells, interphase and metaphase Fluorescence *In Situ* Hybridization (FISH) analysis with specific in-house BAC probes (UCSC Genome Bioinformatics, Source Bioscience) was performed in order to refine the chromosome breakpoints and detect the partner genes. For FISH analyses, RP11-100O5/RP11-368O19 BAC probes encompassing the PDGFRB gene at 5q33.1 and RP11-423N19/RP11-1022E1 BAC probes flanking the SPTBN1 gene at 2p16.2, were fluorescently labeled by nick-translation (La Roche, Ltd) and used as dual-color break-apart probes.

Results: Chromosome analysis at diagnosis showed the karyotype: 46,XY,t(2;5)(p16;q33)[20]/46,XY[5]. PDGFRB and SPTBN1 gene rearrangements were detected in 56% of the cells. Metaphase FISH analysis revealed translocation of the 5' region of PDGFRB at 2p16.2 and fusion with the 3' region of SPTBN1 on derivative chromosome 2. Accordingly, the 5' of SPTBN1 gene was translocated at 5q33.1 and fused with the 3' of PDGFRB gene onto derivative chromosome 5. The patient has shown favorable response to imatinib treatment thus far.

Summary and Conclusions: In the current study, complete molecular cyto-

genetic characterization of the rare translocation t(2;5)(p16.2;q33), encountered for the first time in a MDS case, was carried out. FISH analyses, employing in-house developed dual-color, break-apart probes, revealed that the t(2;5)(p16;q33) initially detected in conventional karyotypic analysis, involved disruption of the genes PDGFRB and SPTBN1, located at 5q33.1 and 2p16.2, respectively. The specific chromosomal aberration led to the formation of the 3'PDGFRB/5'SPTBN1 and 5'PDGFRB/3'SPTBN1 fusion genes on derivative chromosomes 2 and 5, respectively. Importantly, based on these results underlying the rearrangement of the tyrosine kinase gene PDGFRB in the t(2;5)(p16.2;q33.1), a targeted signal transduction therapy (imatinib mesylate) has been administrated.

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THE INTERPLAY BETWEEN OXIDATIVE STRESS AND EPIGENETIC PROFILE IN MYELOID NEOPLASIAS

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Background: The pathogenesis of myeloid neoplasias (MN), such as Myelodysplastic Syndrome (MDS) and Myeloproliferative Neoplasias (MPN), is complex and involve multiple genetic and epigenetic events. Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell proliferation and damage, apoptosis and dysfunctional hematopoiesis. Furthermore, aberrant methylation patterns are other mechanisms common in hematopoietic neoplasias. Although the cause of altered patterns of DNA methylation in cancer remains unknown, oxidative stress might influence DNA methylation profile.
Aims: In this context, we evaluate the interplay between oxidative stress and epigenetic profile in MN patients, and analyzed their possible role as a risk factor and prognostic marker in these hematological neoplasias.
Methods: This study enrolled 73 MN patients (60 MDS and 13 MPN) and 20 controls (CTL), after informed consent. The oxidative stress levels were analyzed by the quantifications of erythrocyte activity of glutathione peroxidase (GL-PX) and glutathione redutase (GL-Red), erythrocyte levels of reduced (GSH), oxidized (GSSH) and total glutathione (totalGS), and plasma levels of vitamin A (vitA) and E (vitE), uric acid, total antioxidant status (TAS) and nitrate ($NO3^-$) plus nitrite ($NO2^-$), using standard methods. The oxidative damage was analyzed through malondialdehyde (MDA) levels and 8-hydroxy-2'-deoxyguanosine (8-OHdG) plasma levels by ELISA assays. The promoter methylation profile of cancer related genes, namely *p15, p16, p53, MGMT and DAPK*, were assessed by MS-PCR and global methylation were analyzed by 5-methyl-cytosine (5mC) and 5-hydroxymethyl-cytosine (5hmC) levels and methylation status of LINE1 sequences. The statistical analysis was carried out by variance analysis and c^2 test (p<0.05).
Results: Our results shown a decrease in enzymatic and non-enzymatic antioxidant defenses (TAS: 1,09±0,16 mM MN *vs* 1,22±0,23 mM CTL, p=0,039; totalGS: 10,75±3,53 µmol/gHb MN *vs* 11,86±2,37 µmol/gHb CTL, p=0,047) and an increase in erythrocyte lipid peroxidation (58,05±21,36 nmol/gHb MN *vs* 49,47± 24,61 nmol/gHb CTL, p=0,042) and DNA damage (8-OHdG: 37,67±5,18 pg/mL MN *vs* 31,36±4,03 pg/mL CTL, p=1,45x10^{-5}). Moreover, 81,5% of these patients presented at least one methylated gene and 58% presented two or more methylated genes. Besides that, MN patients show increased levels of 5mC and 5hmC, comparatively to controls (5mC: 8,27±4,23% *vs* 4,34±2,18%, p=0,022; 5hmC: 3,72±2,98% *vs* 1,82±0,47%, p=0,049). Moreover, taken together our results, we observe that MN patient's with the highest oxidative stress levels, namely the high DNA damage levels (8-OHdG), presents elevated levels of 5mC and 5hmC. Since we have observed a decrease in LINE1 methylation and an increased in the frequency of promoter methylation of cancer related genes, these results suggest that oxidative stress influence the methylation status inducing global hypomethylation but also gene specific methylation. Finally, we also observe that methylation status and oxidative stress are MDS subtype dependent and those patients we the high levels of 8-OHdG and with two or more methylated genes seem to have lower survival.
Summary and Conclusions: In summary, oxidative stress and aberrant methylation status may contribute to the development of MN, such as MDS and NMP, and could be a risk factor and prognostic marker in these neoplasias.
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IMMUNE SURVEILLANCE IMPACTS PROGNOSIS: REGULATORY T CELLS AND PROGENITOR B CELLS ARE INDEPENDENT PREDICTORS OF SURVIVAL IN LOW AND INTERMEDIATE RISK MDS

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Background: Research has provided evidence for active immune surveillance in patients with myelodysplastic syndromes (MDS). Immune surveillance is characterized by an activated, mostly adaptive, immune system. While the presence of active immune surveillance in MDS has been observed, little is known about its prognostic implications.
Aims: To identify the prognostic implications of immune surveillance in patients with low and intermediate risk MDS.
Methods: 47 patients with low and intermediate risk MDS were included in the study. All patients had signed informed consent forms. Patients received standard Epo-G-CSF therapy according to Dutch guidelines. Bone marrow and peripheral blood aspirates were taken and analysed with flow cytometry. Our focus lay on immune cells possibly involved in active immune surveillance, including progenitor B cells ($CD10^+CD34^+$), effector and naïve T cells ($CD8^+CD45RA^{++}CD27^+$, $CD8^+CD45RA^{++}CD27^-$, $CD4^+CD45RA^{++}CD27^+$, $CD4^+CD45RA^{++}CD27^-$), regulatory T cells ($CD4^+CD25^{hi}FoxP3^+$) and NK cells ($CD3^-CD16^+CD56^{dim}$). Overall survival (OS) and progression free survival (PFS) were calculated for all our patients. OS was defined as the time between inclusion in our study and death or date of last follow-us, PFS was defined as time between inclusion in our study until progression to RAEB-2 or AML, death or last follow-up. Statistical analyses were performed using the Cox proportional hazards regression model. The predictive power of our survival model and markers was calculated with Harrold's c-concordance. In all analyses, a p-value <5% was considered statistically significant.
Results: The median OS of our patient group was 35 months [range: 8–62 months] with a similar median PFS of 35 months [range: 22-46 months]. We found the percentage of regulatory T cells to be predictive of PFS and OS, with a hazard ratio of respectively 1.30 (95% CI 1.07 -1.58, p<0.01) and 1.29 (95% CI 1.03 – 1.60, p<0.05). In addition, we found the percentage of progenitor B cells to be a predictor of PFS and OS with a hazard ratio of 0.87 (95% CI 0.80-0.96, p<0.01) and 0.87 (95% CI 0.79 – 0.96, p<0.01). We did not find any of the other markers to be predictors of survival. Based on multivariate analyses, the percentage of regulatory T cells and progenitor B cells impacted survival independently of known prognostic factors, including the newly revised international prognostic scoring system (IPSS-R). Using Harrol's c concordance, we demonstrate an increase in the correct prediction of PFS from 73% to 83% when adding our newly identified prognostic markers to the universally used IPSS-R.
Summary and Conclusions: This study provides evidence for a prognostic impact of immune surveillance in patients with low and intermediate risk MDS. Our data show that an immune regulatory environment, characterized by a high number of regulatory T cells and a low numbers of progenitor B cells, is associated with poorer survival. The number of regulatory T cells and progenitor B cells impacted survival independently from known prognostic factors such as the IPSS-R. They can therefore be considered novel, independent prognostic factors in low- and intermediate risk MDS, underlining the importance of immune surveillance in the prognosis of MDS.

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DEFERASIROX CHELATION THERAPY IN TRANSFUSION DEPENDENT MDS PATIENTS. A "REAL WORLD" REPORT FROM TWO REGIONAL ITALIAN REGISTRIES: GRUPPO ROMANO MIELODISPLASIE AND REGISTRO BASILICATA

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Background: Deferasirox (DFX) has been approved for iron chelation therapy (ICT) in transfusion dependent MDS patients. Most of the available data

comes from selected MDS patients. Few data are available regarding the use of DFX outside clinical trials.
Aims: to retrospectively evaluate safety, compliance, efficacy and effect on hematopoiesis of DFX in a large "real world" MDS population
Methods: one hundred and eighteen MDS transfusion dependent patients were submitted to DFX in 11 hematological centres (9 from Lazio and 2 from Basilicata). Patients were followed-up for 24 months and data were collected at baseline (soon before starting DFX) and at 3, 6, 12, 24 months from starting ICT. DFX dose was established at discretion of the prescribing clinician. Adverse events (AEs) were defined according to CTCAE.3 (2003) definition. Efficacy of the treatment was measured by monitoring serum ferritin levels at the abovementioned time-points. Hematological improvement (HI) was defined according to International Working Group criteria 2006. Friedman test was performed to evaluate differences in serum ferritin levels over the time.
Results: Median age was 72 yrs (range 35-90) and 61% of the population aged more than 70 yrs. There were 66 males and 52 female. Fifty-five (47%), 41 (35%), 12 (10%) and 3 (2%) patients qualified as low, intermediate-1, intermediate-2 and high IPSS, respectively; IPSS was unknown in 7 (6%). Median time between diagnosis and DFX initiation was 30 months (3–153). Patients have been receiving regular blood transfusions for a median of 14 months (3–156) with a median number of units received of 26 (10–362). Median serum ferritin level was 1773 ng/ml (range 443-7339 ng/ml). Fifty five (47%) patients received DFX at the starting dose of 10 mg/kg and 63 (53%) of 20 mg/kg. Seventy-seven (65%) completed the planned treatment (12 months). Forty-one (35%) discontinued ICT, 21 (19%) due to DFX toxicities: 12 (54%) gastrointestinal, 4 (18%) hepatic, 3 (14%) renal, 2 (9%) cutaneous and 1 (5%) arthralgias. ICT discontinuation was not significantly associated with concomitant treatment (lenalidomide/azacitidine) nor with any clinical feature. Overall, 64 drug related AEs were reported in 56 (47%) distinct patients. AEs were: in 31 (48%) gastrointestinal, in 15 (23%) renal, in 10 (17%) cutaneous, in 4 (6%) hepatic and in 4 (6%) arthralgias. Of these 64 AEs, 27 (42%) were >grade 2. From a median starting value of 1773 ng/ml (range 443-7339) serum ferritin decreased to a median value of 1300 ng/ml (101–4623) and 1180 ng/ml (100-4500), at 12 and 24 months, respectively (P<0.0001). We also evaluated the effect of DFX on hematopoiesis function. Patients who were contemporarily receiving DFX plus azacitidine or lenalidomide were excluded from this analysis; therefore the analyzed cohort accounted for 85 patients. HI was observed in 19 (22%) and the type of improvement was as follows: HI-E in 16 (19%) with 5 converting to transfusion independency, HI-N and HI-P in 7 (8%) and in 4 (5%), respectively. Responses were observed, after at least 6 months of ICT. No clinical feature significantly associated with HI was found.
Summary and Conclusions: 65% of the patients on ICT was able to complete the planned treatment despite the advanced age or the assumption of concomitant medications such as lenalidomide and azacitidine. Discontinuation due to toxicity was observed in a limited number of patients. DFX was effective in reducing serum ferritin levels. HI was associated in some instances with reduced transfusion need or transfusion independency.

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THE INCIDENCE OF COMORBIDITIES AT THE TIME OF DIAGNOSIS IN PATIENTS AFFECTED BY MYELODYSPLASTIC SYNDROMES: OVERVIEW THROUGH THE "REL" ("RETE EMATOLOGICA LOMBARDA") DISEASE REGISTRY

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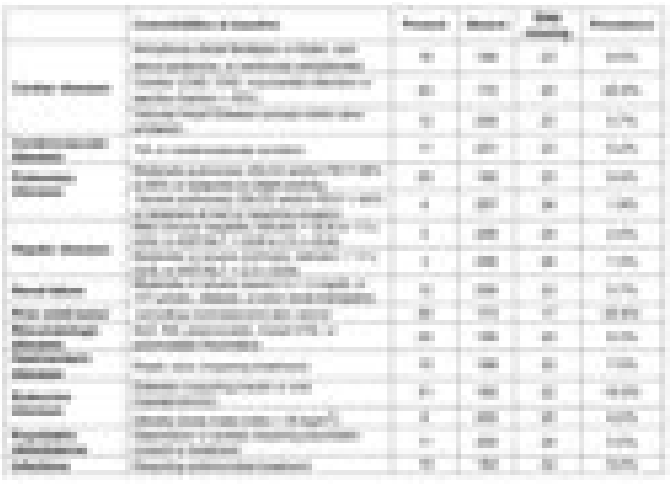
Background: The importance of evaluation of comorbidities in patients (pts) affected by myelodysplastic syndromes (MDS) was recently highlighted, as it may help tailoring treatment strategies and improving the ability of therapeutic decisions. A prognostic score, called MDS-CI, was recently validated to provide information about overall survival and risk of non-leukemic mortality, identifying three groups of risk.
Aims: To assess the incidence of comorbidities at the time of diagnosis in MDS pts in the "REL" Disease Registry.
Methods: The "REL" MDS Disease Registry was consulted after 31 months of activity (July 2011 – February 2014) and the recorded data were analyzed. Comorbidities, according to an adapted "Hematopoietic Cell Transplantation-specific Comorbidity Index" (HCT-CI) were assessed primarily at the time of diagnosis. The MDS-CI was calculated at baseline. Comorbidities subsequently arisen and recorded were then also reported.
Results: The Registry, at time of extraction, contained data of 255 pts from 11 centers in Lombardia region.
Data about the presence of comorbidities were available in 235 cases. Of them: 179 were primary MDS, 24 t-related MDS and 32 MDS/MPN cases. Concerning primary MDS pts: the RCUD were 25, the RCMD were 62, the MDS 5q-associated were 6, the RARS were 27, the AREB-1 were 35, the AREB-2 were 23, the MDS-U was 1. The IPSS was distributed as follow: "low" in 43 cases, "intermediate-1" in 85, "intermediate-2" in 25, "high" in 5, "not valuable" in 21. According to WPSS the pts were stratified in: "very low" risk 35 cases, "low" risk 54, "intermediate" risk 31, "high" risk 30, "very high" risk 7 and "not valuable" 22. The prevalence of comorbidities at baseline, as explicated in the Table 1, was found as follows: cardiac diseases 35.1%, cerebrovascular disease 5.2%, pulmonary diseases 11.3%, hepatic diseases 3.8%, renal failures 5.7%, prior solid tumor 20.6%, rheumatologic diseases 9.3%, gastroenteric diseases 7.0%, endocrine diseases 18.5%, psychiatric disturbances 5.5%, infections 9.4%. The MDS-CI was evaluable in 207 cases. Of them, 131 pts had a "low" risk, 66 an "intermediate" risk and 10 a "high" risk. The comorbidities arisen at least 30 days after the diagnosis time were the following: cardiac disease in 13 more cases, cerebrovascular disease in 2 more cases, pulmonary disease in 3 more cases, hepatic disease in 5 more cases, renal failure in 1 more case, solid tumor in 5 more cases, rheumatologic disease in 2 more cases, gastroenteric diseases in 2 more cases, endocrine disease in 4 more cases, psychiatric disturbance in 1 more case, infections in 9 more cases.

Table 1.



Summary and Conclusions: These data, coming from a Disease Registry, allow to obtain an insight into the real life of the targeted disease. Explicitly, among the comorbidities, cardiac disease, diabetes and prior solid tumor are widely recurrent in MSD pts. Considering the disease specific MDS-CI at baseline, the most patients have a low comorbidity risk.

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MYOCARDIAL TISSUE CHARACTERIZATION BY CARDIAC MR IMAGING IN MYELODYSPLASTIC SYNDROMES

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Background: Magnetic Resonance Imaging (MRI) provides unique insight regarding tissue characterization in the heart.
Aims: We reported the baseline MRI findings at the end of the recruitment in the MIOMED (Myocardial Iron Overload in MyElodysplastic Diseases) study. In particular, we evaluated the distribution of iron overload in the whole left ventricle (LV) and he presence of myocardial fibrosis in patients with myelodysplastic syndromes (MDS); the association with LV function was also investigated. No data are available in the literature about this issue.
Methods: MIOMED is an observational, MRI multicentre study in low and inter-

mediate-1 risk MDS patients who have not received regular iron chelation therapy. Out of the 51 MDS patients enrolled, 48 underwent the baseline MRI exam. Mean age was 71.7±8.5 years and 17 patients were females. MIO was assessed using a multislice multiecho T2* approach. Biventricular function parameters were quantified by cine sequences. Myocardial fibrosis was evaluated by late gadolinium enhancement acquisitions.

Results: We found 27 (56.3%) patients with no MIO (all 16 segmental T2* values >20 ms). The remaining patients showed an heterogeneous MIO (some segments with T2* values >20 ms and other segments with T2* values <20 ms) and of them 2 (9.5%) showed a global T2* value <20 ms, indicating significant MIO. A reduced LV ejection fraction (EF) was found in the 29.5% of cases and a reduced RV EF in the 23.3%. There was not a significant association between heart T2* values and LV EF. Myocardial fibrosis was detected in the 35.9% of the patients. Three patients showed an ischemic pattern and one of them had a transmural fibrosis in the LV apical region. Out of the 3 patients with an ischemic pattern, only one patient had a positive history for a previous myocardial infarction. The majority of the patients had two or more foci of myocardial fibrosis, involving more frequently the septal segments. Patients with myocardial fibrosis were significantly older (75.4±7.9 *vs* 68.9±7.6 yrs; P=0.019). Global heart T2* and LV volumes were not significantly different between patients with and without fibrosis. The LV EF was lower in fibrotic patients but the statistical significance was not reached (58.4±11.7 *vs* 64.8±8.9%; P=0.067).

Summary and Conclusions: Although a significant heart iron was found only in two cases, nearly half the patients had abnormal T2* values in at least one myocardial segment. This finding underlines the importance to use a multislice approach in order to perform an early diagnosis and prevent a more diffuse iron distribution by chelation therapy. This goal could be critical in patients with myocardial fibrosis that seems to be a relative common findings in the old MDS patients. In fact, an underlying heart damage as represented by fibrosis could make the hearts of the old MDS patients more sensitive to lower levels of accumulated iron.

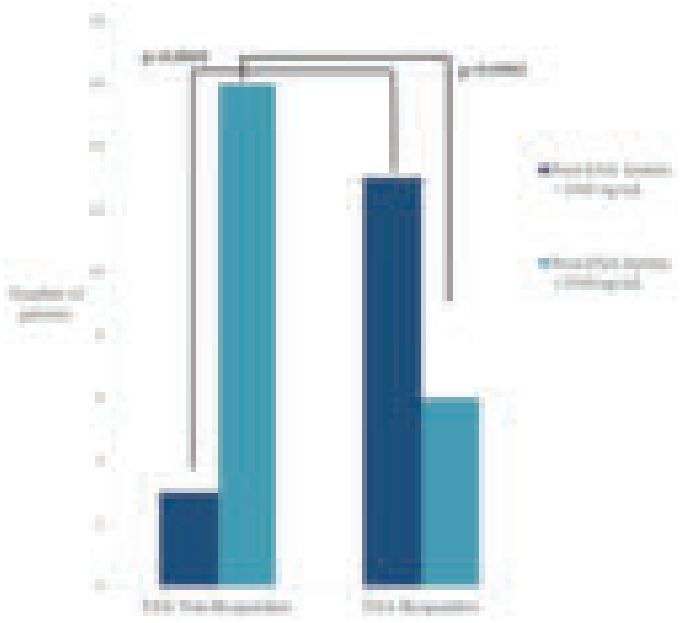

Figure 1. Number of patients with post-ESA ferritin level < or ≥ 1000 ng/mL by response to ESA.

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PRE AND POST-TREATMENT SERUM FERRITIN LEVELS IN PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES RECEIVING ERYTHROPOIESIS STIMULATING AGENTS

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Background: Multiple analyses in lower risk MDS document inferior survival in patients with transfusional iron overload (IOL) indicated by increased ferritin levels. Reducing IOL with iron chelation therapy appears to result in clinical benefit in at least some MDS patients. Erythropoiesis stimulating agents (ESA) may reduce red blood cell (RBC) transfusion requirements (TR). Restoration of effective erythropoiesis should reduce IOL by incorporation of excess iron into newly formed RBC, however, few data are available on IOL status with ESA.

Aims: We aimed to examine IOL status in MDS patients receiving ESA.

Methods: We performed an analysis of MDS patients treated with ESA. Patients were identified from the database and charts reviewed. Data extracted included baseline characteristics and ferritin levels prior to (pre-ESA) and following (post-ESA) treatment, and response by IWG 2006 criteria was determined.

Results: Of 349 MDS patients, 49 received an ESA for at least 1.5 months, had ferritin levels available and were not receiving concomitant iron chelation or other MDS therapy. The median age was 70 (range 32-89) years and 26 (53%) were male. MDS diagnosis by FAB or WHO criteria was: RA, n=3; RARS, n=20; RCMD, n=16; other, n=10. IPSS scores were: low, n=23; intermediate (int)-1, n=23; ≤int-1, n=4; and int-2 risk, n=4. Median marrow blast count was 2 (0-7)%. Median erythropoietin (EPO; n=23) level was 50 (18-1459) IU/mL and median pre-ESA ferritin level 509 (76-3285) ng/mL. Median pre-ESA hemoglobin (HGB) was 89 (56-120)G/L and 26 patients were RBC transfusion dependent. Prognostic score for ESA response was: 0, n=13; 1, n=5; and 2, n=2. Baseline characteristics were similar in ESA responders (ER) and ESA non-responders (ENR) as was time from ESA start date to post-ESA ferritin measurement (P=NS for all). 46 patients received EPO and 3 darbopoietin in standard doses and schedules. Dose escalations were given in 12 patients and GCSF added in 5. Median ESA duration was 6.7 (1.5-85.9) months. 21 (43%) patients had an erythroid response: HGB improvement, n=21 (43%); transfusion response, n=4 (8%) with 3 (6%) becoming transfusion independent. The median TR in ER was stable at 4 (2-5) U/8wk (P=NS) but increased in ENR from 4 (1.2-10.6) to 5 (1-16) U/8wk (P=0.0001). Median ferritin level in ER was: pre-ESA, 473 (91-2727); post-ESA, 801 (130-11,164) ng/mL (P=0.01). In ENR median ferritin was: pre-ESA, 672 (76-3285); post-ESA, 1423 (431-6593)ng/mL (P<0.0001). Patients with post-ESA ferritin ≥1000ng/mL were: ER, 6/19 (32%); ENR, 16/19 (84%). Conversely, post-ESA ferritin <1000ng/mL was: ER, 13/19 (68%); ENR, 3/19 (16%; P=0.003; Figure 1). Similarly, though there was no association between pre-ESA HGB level and post-ESA HGB ≥100g/L (P=NS), of patients with post-ESA HGB ≥100g/L, the ferritin was <1000ng/mL in 13/20 (65%) and ≥1000ng/mL in 7/20 (35%). Conversely, with post-ESA HGB <100g/L, ferritin was <1000ng/mL in 1/7 (14%) and ≥1000ng/mL in 6/7 (35%; P=0.03). At a median follow up of 28 (4-226) months, the two year OS for ER and ENR, respectively, was 80% and 86% (P=NS).

Summary and Conclusions: In MDS patients responding to ESA, ferritin levels increased less than in ESA non-responders. Both response to ESA and achieving a post-ESA HGB ≥100g/L were associated with post-ESA ferritin <1000ng/mL (P<0.0001 and P=0.03, respectively). This is to our knowledge the first analysis of serial ferritin levels in patients receiving ESA. Whether iron overload may be significantly reduced by ESA will require analysis of larger numbers of patients with measurement of IOL parameters at regular intervals. An analysis from the Canadian MDS database of iron status in non- transfusion dependent patients with lower risk MDS receiving ESA is in progress.

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VALIDATION OF LOW RISK PROGNOSTIC SCORING SYSTEM (LR-PSS) IN 318 PATIENTS WITH LOWER RISK IPSS MYELODYSPLASTIC SYNDROME FROM A SINGLE CENTER.

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Background: Myelodysplastic syndrome (MDS) treatment decision is currently based on the International Prognostic Scoring System (IPSS) and IPSS-R (Greenberg *et al.*, 2012), which have been recently validated in our series of patients (P-195. Stockholm. EHA 2013). Nearly 90% of patients from our series were low and intermediated-1 IPSS. Current prognostic models for MDS do not allow the identification of patients with lower risk IPSS disease and poor prognosis. A prognostic scoring system specifically for this cohort of patients (LR-PSS) was developed based on age ≥60 years, hemoglobin <10g/dl, platelet count <50k/uL or 50-200k/uL, bone marrow blast ≥4% and unfavorable cytogenetic (non-del(5q), non–diploid) (Garcia-Manero. *Leukemia* 2008) which divided patients into 3 risk categories.

Aims: The purpose of this study was to validate the prognostic value of Low Risk Prognostic Scoring System (LR-PSS) in our cohort of 318 lower risk IPSS (Low and intermediate-1 IPSS) MDS patients.

Methods: Between 1987 and 2013, 744 patients with MDS were diagnosed at the Catalan Institute of Oncology in Barcelona. Among them, IPSS could be assessed in 360 (48.3%) patients with cytogenetic analysis available. We evaluated in lower IPSS patients the prognostic power of LR-PSS analyzing as endpoints overall survival (OS) and leukemia free survival (LFS).

Results: 318 (88%) of patients with IPSS available, had lower risk MDS. 200 (63%) had low risk disease and 118 (37%) had intermediate-1 risk. Median age at diagnosis was 71 years (range 29-101). 219 (69%) were male. Median follow up was 102 months (range 79-125). WHO 2008 was: 1% CRDU, 9%RA, 43% RCMD, 19% RAEB-1, 2% RAEB-2, 23% CMML, the remaining 3% were MDS-U and isolated 5q deletion. At diagnosis: Median hemoglobin, platelet and bone marrow blast were 11.7 g/dL (5.5-16), 156 x10^9/L (1-1492) and 3% (0-10). 46 (14,5%) patients have unfavorable LR-PSS cytogenetic at diagnosis. At the time of last follow up 137 (43.1%) patients had died and among patients with PFS data available (n=210) 37 (17.6%) had progressed to AML.

Classification of lower risk IPSS MDS patients (n=318) according to LR-PSS were: category 1 (score 0-2), category 2 (score 3-4) and category 3 (score 5-7) in 70 (22%), 211 (66.4%) and 37 (11,6%), respectively. Categories based on the LR-PSS system had different median survival rates: 113 months (95% CI 79-145) in category 1; 78 months (95% CI 63-93) in category 2 and 31 months (95% CI 26-37) in category 3 (p<0.001). Rate of progression to acute myeloid leukemia in category 1 and 2 of LR-PSS was 13% (6/47) and 18% (23/126) respectively while 30% (8/27) of lower risk IPSS patients who were reclassified as category 3 LR-PSS progressed to acute myeloid leukemia (Figure 1).

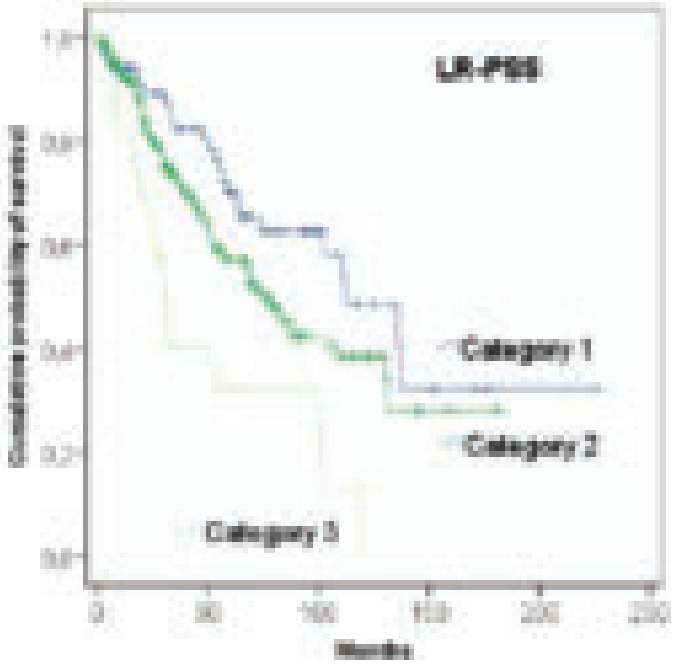

Figure 1. Survival of lower risk IPSS MDS patients based on LR-PSS categories.

Summary and Conclusions: The implementation of LR-PSS in lower IPSS MDS patients could allow us to identify patients with lower risk MDS and poor prognosis who could benefit from an earlier intervention.

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MRI SURVEY IN TRANSFUSION-DEPENDENT AND NON-TRANSFUSION-DEPENDENT MDS PATIENTS

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Background: Several studies have shown cardiac diseases as causes of death in myelodisplastic (MDS) patients receiving transfusions. So iron overload may be considered an independent negative prognostic factor. There are few and rather contradictory studies using Magnetic Resonance Imaging (MRI) in the evaluation of MDS syndromes.
Aims: We report the baseline MRI findings at the end of the recruitment in the MIOMED (Myocardial Iron Overload in MyElodysplastic Diseases) study. We investigated hepatic, pancreatic and cardiac iron overload and biventricular functional parameters in MDS patients, outlying the differences between transfusion dependent (TD) and non transfusion dependent (non-TD) patients.
Methods: MIOMED is an observational, MRI multicentre study in low and intermediate-1 risk MDS patients who have not received regular iron chelation therapy. Forty-eight patients (71.7±8.5 years, 17 F) underwent the baseline MRI exam. Hepatic T2* values were converted into liver iron concentration (LIC). T2* measurements were performed in pancreatic head, body and tail. Myocardial iron overload (MIO) was assessed using a multislice multiecho T2* approach. Biventricular function parameters were quantified by cine sequences.
Results: The mean LIC was 7.6±8.8 mg/g/dw, the mean global pancreas T2*, assessed in 20 patients, was 24.0±13.46 ms and the mean global heart T2* was 38.7±8.3 ms. Global heart T2* values were not significantly correlated with LIC or serum ferritin levels. Thirty-two (66.6%) patients were non-TD while 16 patients were TD. The two groups were homogeneous for age, sex and hemoglobin levels but TD patients had significantly higher serum ferritin levels (1612±864 *vs* 711±430; P<0.0001). The percentage of patients with detectable hepatic iron (LIC≥3 mg/g/dw) was significantly higher in the TD group (Figure 1, left). Mean LIC was 14.4±11.1 mg/g/dw in the TD group and 4.2±4.6 mg/g/dw in the non-TD group (P<0.0001). The percentage of patients with detectable pancreatic iron (T2*<26 ms) was comparable between the two groups (Figure 1, center). A significant heart iron (T2*<20 ms) was found in two patients; both with an heterogeneous pattern (some segments with T2*>20 ms and others with T2*<20 ms). Out of the two patients with significant heart iron, one patient was not transfused and he did not show significant hepatic iron (LIC=2.12 mg/g/dw) while the other one was regularly transfused and he received sporadically (less than two weeks/month) chelation treatment with deferoxamine in the 2 years before the MRI. Both patients had pancreatic iron overload (T2*=9.79 ms and T2*=2.45 ms, respectively). The global heart T2* (Figure 1, right), the pattern of iron burden and the number of segments with T2*<20 ms were comparable between the two groups. Biventricular volumes and ejection fraction and left ventricular mass index were comparable between the two groups.

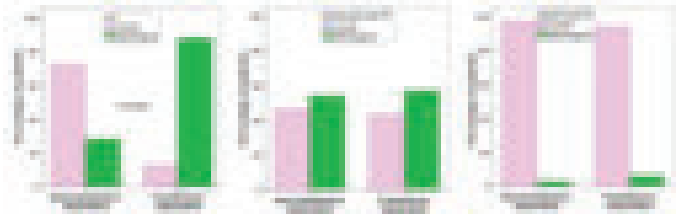

Figure 1.

Summary and Conclusions: As expected, regularly transfused MDS patients showed significantly higher levels of hepatic iron overload, that, however, was present in almost the 30% of non-TD patients, mainly due to increased intestinal iron and augmented erythropoiesis. MIO is not frequent in MDS patients but it can be present also in non-TD patients and in absence of detectable hepatic iron. Conversely, no patients without pancreatic iron overload had cardiac iron overload. Our data remark the importance to check directly for heart iron with a more sensitive segmental approach avoiding to estimate heart iron burden from indirect indicators such as LIC, serum ferritin or transfusion state.

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THE IMPACT OF TRANSFUSION DEPENDENCY ON THE OUTCOME OF PATIENTS WITH VERY LOW AND LOW RISK MYELODYSPLASTIC SYNDROME ACCORDING TO THE IPSS-R.

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Background: The recent revision of the International Prognostic Scoring System (IPSS-R) (Greenberg P *et al.* Blood 2012) has provided us an updated tool to better define prognosis and life expectancy of patients with myelodysplastic syndrome (MDS). Although transfusion dependency (TD) has been described as an independent prognostic factor for survival n MDS patients (Malcovati L *et al.* JCO 2007), it was not included in IPSS-R analysis mainly due to limited availability of transfusion data on patients used for deriving the IPSS-R.
Aims: We retrospectively analysed the outcome of 744 patients with the novo MDS diagnosed between 1994 and June 2013 at the Catalan Institute of Oncology in Barcelona and we analysed the prognostic value of TD and the impact on survival in IPSS-R subgroups.
Results: A total of 744 patients were included in this analysis. Their median age was 72 years (27-101 years) and 64% patients were male. IPSS-R could be assessed in 360 patients, of whom 267 (74%) were in the Very Low or Low risk groups (determined as 'LOWER RISK'). The median OS of IPSS-R Very Low, Low, Intermediate, High and Very High risk groups was 8.5, 7.3, 4.4, 1.3 and 0.6 years, respectively. (Figure 1. p<0.0001). Among the 360 patients in whom IPSS-R could be assessed, 34% of patients were RBC TD (defined as having at least 1 RBC transfusion every 8 weeks for at least 4 months) and 9% of patients presented IO (defined as serum ferritin ≥1000 ng/ml). TD and IO are more common in the Very High (76%/8%), High (56%/20%) and Intermediate (53%/17%) risk groups as compared to the lower risk IPSS-R groups: Low (29%/8%) and Very Low (20%/5%). In multivariate analysis, RBC-transfusion dependency (HR 3.18; P<0.0001) was associated with poor survival, inde-

pendent of the IPSS-R category, age at diagnosis and IO. The median OS of TD patients (n=116) was significantly lower (4.1 *vs.* 10.7 years; p<0.0001) than that of Non-TD (n=227). (Figure 2. p<0.0001). As TD is more common in the IPSS-R higher risk patients, but the majority of patients of our series were of lower risk, we restricted further analysis to assess the specific impact of TD in IPSS-R lower risk groups. The median OS between Low and Very Low risk group was not significantly different (8.1 *vs* 9.5 years; p=0.4), consequently we grouped both together. The median OS of TD lower risk IPSS-R patients (n=65) was significantly shorter than that of transfusion-independent (n=190) patients (5 *vs* 11 years; p<0.0001).

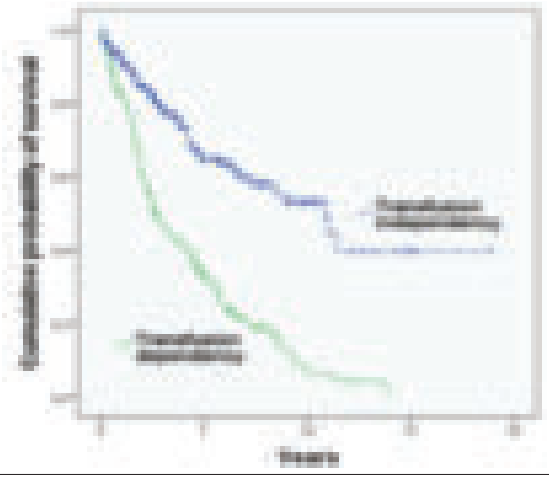

Figure 1. Survival of MDS patients based on development of transfusion requirements.

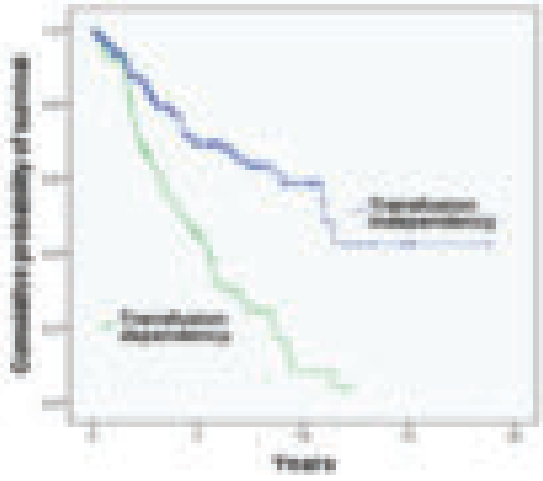

Figure 2. Survival of lower risk IPSS-R MDS patients based on development of transfusion requirements.

Summary and Conclusions: The inclusion of transfusion dependency in risk stratification of patients with lower risk IPSS-R, could allow us to identify a subset of patients with lower risk disease disease and poor prognosis who could benefit from an earlier intervention.

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CALRETICULIN MUTATION WAS RARELY DETECTED IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Recently, somatic mutations in exon 9 of *calreticulin* (*CALR*) were detected in patients with myeloproliferative neoplasms (MPN) who had nonmutated *JAK2* and *MPL*. Similar genetic alterations, such as mutations of *ASXL1, IDH,* and *TET2,* etc, have been identified in both myelodysplastic syndrome (MDS) and MPN, raising the possibility that *CALR* mutations may also occur in patients with MDS.
Aims: The study was aimed to investigate the incidence and clinical implication of *CALR* mutations in MDS patients.
Methods: To further characterize *CALR* mutations in MDS, we performed Sanger sequencing of *CALR* exon 9 in 453 patients with *de novo* MDS, according to the WHO classification, as well as 98 patients with CMML and acute myeloid leukemia (AML) with blast percentage of 20% to 30%, that is refractory anemia with excess blasts in transformation (RAEBT) by the FAB classification. Mutational analysis was performed by genomic DNA polymerase chain reaction followed by direct sequencing. The primer sequences were as follows: human *CALR* exon 9 sense primer 5'-GGCAACGAGACGTGGGGCGT-3', and antisense primer 5'-CAGAGACATT ATTTGGCGCGGCC-3'. Abnormal sequencing results were confirmed by at least two repeated analyses.
Results: Totally, two (0.56%) of 355 WHO-defined MDS patients had *CALR* mutations at diagnosis. Patient 1, who had initial presentation of anemia and thrombocytopenia, was diagnosed as having RARS. He had intermediate-1 risk MDS based on the International Prognostic Scoring System (IPSS) and low risk MDS by the revised IPSS (IPSS-R). He also harbored *DNMT3A* and *SF3B1* mutations in addition to *CALR* mutation at diagnosis. He was still alive without disease progression at the time of this study 57 months after diagnosis. Patient 2, diagnosed as having RA, had low risk characteristics according to both the IPSS and the IPSS-R. She remained transfusion dependent during the follow-up period of 27.4 months. We did not find any *CALR* mutation in 98 patients with CMML or RAEBT at diagnosis. Intriguingly, among 107 *CALR*-wild patients who were sequentially studied, one (patient 3) acquired *CALR* mutation 33.5 months after diagnosis when the disease progressed from RAEBT to AML by the FAB classification. A novel *RUNX1* mutation was also acquired at the same time in this patient. The *CALR* of other 106 patients remained in germline during follow-ups. Because direct sequencing might not be sensitive enough to detect low level of *CALR* mutant, we therefore did TA cloning followed by direct sequencing of the three *CALR*-wild samples obtained at diagnosis and subsequent follow-ups from patient 3. We could not find any *CALR* mutation in the 65, 43 and 54 clones analyzed using cloning technique in this patient, respectively. Nevertheless, we could not exclude the possibility that minor population of cells with *CALR* mutant existed initially but escaped the detection of direct sequencing or TA cloning technique.
Summary and Conclusions: We showed that *CALR* mutations were rarely detected in patients with MDS both at diagnosis and disease progression.

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THE ROLE OF TP53 MUTATIONS IN LOW-RISK MDS PATIENTS

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Background: A negative prognostic impact of *TP53* gene point mutations in the myelodysplastic syndrome (MDS) patients has not been intensively studied yet.
Aims: In this study we performed an analysis of: the incidence of *TP53* gene mutations in low-risk MDS patients, the effect of *TP53* mutations on disease progression, the impact of treatment on mutated clones, the size of mutated clones with regard to the type of cell population and the effect of *TP53* mutations on mRNA levels of *TP53*, *MDM2, MYC,* and *p21* genes.
Methods: *TP53* mutations were analyzed in DNA from CD34+ or CD34- cells isolated from bone marrow of 103 patients included into cohort according to the International Prognostic Scoring System low risk or intermediate-1 risk using primers designed within the IRON-II study research consortium (Roche Applied Science) on the GS Junior system. If mutations were detected, we searched for mutations in the various cell populations (CD34+, CD34-, CD14+, CD3+, granulocytes) and at different time-points of the disease. TaqMan Gene Expression Assays were used to determine relative expression levels of mRNA of *TP53, p21, MYC* and *MDM2* genes in CD34+ cells between patients with and without mutation.
Results: The overall incidence of *TP53* mutations was 11.7% (12/103), 3 patients had two mutated clones. The mutation frequency in low-risk MDS patients with and without del(5q) were 20.4% (11/54) and 2.0% (2/49) respectively. The allele burden of mutation was in the range of 2.1 – 76.8%. Out of the detected mutations were 14 missense and 1 nonsense. We found significant differences in the size of the mutated clone cells between bone marrow and peripheral blood. So far, 9 of 12 (75%) patients carrying mutations showed progression towards acute leukemia. Patients with *TP53* mutation showed 1.6-fold lower mRNA expression of *TP53* (p=0.05) in CD34+ cells than those with wild type *TP53*. Non-significant change of mRNA expression of *p21, MYC* and *MDM2* genes were detected between groups of patients with and without mutation.
Summary and Conclusions: Using sensitive next generation sequencing technology, that is able to detect mutations with low abundance, we confirmed in a large cohort of patients a significantly higher frequency of *TP53* mutations in low-risk MDS patients with del(5q) in comparison to those without del(5q). Furthermore, we found the association between presence of *TP53* mutations and increased risk of leukemic transformation. We were not able to detect a half of identified mutations by Sanger sequencing (<20% mutation burden).
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IDENTIFICATION OF DYSPLASIA IN PERIPHERAL BLOOD USING FLOW CYTOMETRY

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Background: For the diagnostic work-up of patients with suspected myelodysplastic syndrome (MDS) flow cytometry (FC) of bone marrow (BM) is recommended by ELN guidelines.
Aims: Identify dysplastic features by FC in peripheral blood (PB) and assess their significance by follow-up BM analysis.
Methods: From 157 patients (pts) with suspected MDS PB was analyzed by FC applying defined ELN criteria. In addition, samples were analyzed for the presence of CD71-positive erythroid cells. In all pts at least one BM sample was analyzed during follow-up by morphology and cytogenetics to confirm MDS according to WHO 2008 criteria.
Results: BM assessment at follow-up resulted in 1) proven MDS (n=96), 2) no MDS (n=32), and 3) MPN, MDS/MPN, or "MDS possible" (dysplasia by morphology, not sufficient to diagnose MDS) (n=29). Median time to MDS confirmation was 0.9 months (range, 0.1-53.0), median time to last BM assessment without MDS confirmation was 0.8 months (0.2-23.0). All 34 pts with myeloid progenitor cells (MPC) by FC in PB had finally proven MDS. However, in addition 62/94 (66.0%) of those without MPC (p<0.0001) also had proven MDS. 17 of these 34 cases in addition displayed an aberrant antigen expression. Focusing on granulocytes side-scatter (SSC) signals were analyzed as ratio of mean SSC signals granulocytes/lymphocytes (G/L). We found a significantly lower SSC ratio G/L in pts with proven MDS as compared to those without (mean±SD 5.7±1.1 *vs.* 6.3±1.0, p=0.015). A mean SSC ratio G/L of 3.9 was found to most specifically identify pts with MDS: all 6 cases with a ratio <3.9 had MDS. Regarding aberrant antigen expression in granulocytes, MDS was more frequently diagnosed among cases with *vs.* without the following features: aberrant CD11b/CD16 expression pattern (43/46 investigated, 93.5% *vs.* 53/82, 64.6%; p=0.0002), lack of CD10 expression (37/43, 86.0% *vs.* 59/85, 69.4%; p=0.052), CD56 expression (19/21, 90.5% *vs.* 77/107, 72.0%; p=0.098). Cumulating this data, ≥2 aberrantly expressed antigens on granulocytes were found indicative of MDS: 42/45 (93.3%) of investigated pts with aberrant expression of ≥2 antigens had MDS while only 54/83 (65.1%) of those with <2 aberrantly expressed antigen had finally proven MDS (p=0.0003). Regarding aberrant antigen expression in monocytes, pts with the following features more frequently had MDS as compared to those without: reduced expression of HLA-DR, CD13, CD11b, or CD15, aberrant expression of CD2 or CD34 (as single makers all n.s.). Cumulating this data also resulted in a significant relation to a diagnosis of MDS during follow-up: 31/36 (86.1%) of pts with aberrant expression of ≥2 antigens on monocytes were diagnosed MDS *vs.* 65/92 (70.7%) of those without (p=0.052). Regarding erythroid cells, MDS was diagnosed in more cases with CD71-positivity (33/37, 89.2%) than in those without (63/91, 65.5%; p=0.023). Integrating the data for the different cell compartments, pts were separated according to the presence of the following 5 criteria: 1) presence of MPC in PB by FC, 2) aberrant expression of ≥1 antigen in MPC in PB, 3) aberrant expression of ≥2 antigens in granulocytes in PB, 4) aberrant expression of ≥2 antigens in monocytes in PB, and 5) presence of CD71-positive erythroid cells in PB: 44/46 (95.7%) cases with ≥2 of the criteria had MDS which was true for 52/82 (63.4%) of those with ≤1 criterion (p<0.0001). Applying these criteria to the remaining 29 pts with MPN, MDS/MPN, or possible MDS, 11 (37.9%) of them fulfilled ≥2 criteria which was true for 2/32 (6.3%) of pts not diagnosed MDS (p=0.004).
Summary and Conclusions: FC reveals MDS-related features in PB which may be used to identify pts with a high probability of MDS.

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DEVELOPMENT OF A HIGH RESOLUTION MELTING CURVE ANALYSIS SCREENING TEST FOR SRSF2 SPLICING FACTOR GENE MUTATIONS

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Background: Recently, somatic mutations of the spliceosome machinery have been identified in hematological malignancies and solid tumors. SRSF2 (*Serine-Arginine Splicing Factor 2*), is a member of the serine/arginine (S/A) rich family pre-mRNA splicing factors and plays a role in mRNA export from the nucleus and translation. SRSF2 mutations are present in about 12% myelodysplastic syndromes (MDS) without ringed sideroblasts (RS), 5.5% refractory anemia with ringed sideroblasts (RARS)/refractory cytopenia with multilineage dysplasia (RCMD) with RS(n: 73), 28% chronic myelomonocytic leukemia (CMML) (n: 88), 6.5% acute myeloblastic leukemia (AML)/MDS (n: 62), 0.7% in *de novo* AML (n: 151) and 1.9% in myeloproliferative disease (MPN) (n: 53). Subsequent studies have shown similar results regarding the "hot spot" localization in which 95% of the mutations were located at the aminoacid position P95, corresponding to the exon 2 of the SRSF2 gene. In MDS SRSF2 mutations have shown a significant prognostic impact, associated to higher AML transformation rates, shorter overall survival and shorter time to AML progression compared to non mutated cases.
Aims: The aim of this study was the development of a high resolution melting curve analysis (HRM) to screen for SRSF2 "hot spot" mutations in a fast, sensitive and reliable way.
Methods: 42 bone marrow samples were selected from patients who underwent cytogenetic analysis as a part of the laboratory workup to establish the diagnosis of MDS. Positive controls from known mutated cases were included. DNA was obtained using silica columns. All samples analyzed by HRM were confirmed by direct sequencing. HRM was carried out in a 50 µL reaction and the following reagent concentrations were used: high resolution mastermix 2X, $MgCl_2$ 3.5 mM, primers 0.5 µM and DNA 50 ng/µL. PCR protocol was composed by a single hot start cycle with a temperature of 95°C for 10 minutes then 55 cycles of 95°C for 10s, 61°C for 10s and 72°C for 10s. A melting temperature form 65-95°C with a ramp of 0.02°C/s was used. The real time platform and the software used for analysis were operated according to manufacturer's instructions.
Results: HRM gene scanning test was able to identify 4 melting patterns corresponding to a negative (wild type) group, and 3 different mutated groups (Figure 1). Each mutated group was identified accordingly to the positive control used; P95H, P95L and P95R respectively. From the 42 selected patients, 4 samples were identified as mutated (9.5%). Positive and negative results from HRM were compared with direct sequencing results with a concordance of 100%. Sensitivity analysis was performed using a mutated DNA sample subjected to dilutions with a wild type (WT) sample (Figure 1). The test showed detection sensitivity up to 1:9 (mutated:WT) dilutions, indicating high analytical sensitivity. Melting genotype analysis was not included in this study.

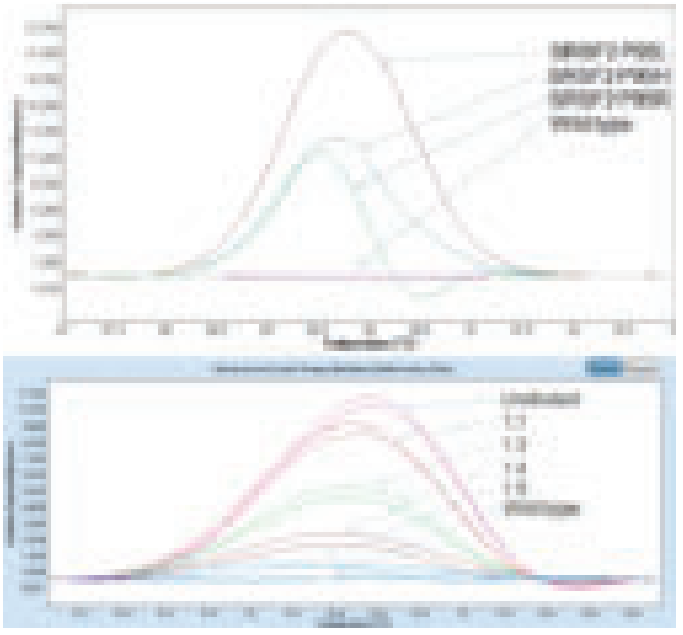

Figure 1. Normalized and Temp-Shifted Difference Plot.

Summary and Conclusions: In the present study, SRSF2 "hot spot" mutations were identified in a fast and reliable fashion using a HRM screening test. This test might be helpful to save and optimize laboratory resources by selecting cases to be confirmed thus, reducing turn-around time. Given the clinical implications of SRSF2 mutations, this rapid screening method may provide useful Information for clinical decision making.

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THE USE OF MEDICAL CLAIMS TO ASSESS INCIDENCE, DIAGNOSTIC PROCEDURES AND INITIAL TREATMENT AMONG PATIENTS WITH MYELODYSPLASTIC SYNDROMES IN THE NETHERLANDS

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Background: Recent evidence from a medical claims-based study suggested that elderly patients (≥65 years) with myelodysplastic syndromes (MDS) were underreported in population-based cancer registries from the US (Cogle, *Blood* 2011). This may also hold true for the nationwide population-based Netherlands Cancer Registry (NCR). All residents of the Netherlands are legally obliged to take out a Dutch medical insurance for the standard package. Analyses of Dutch medical claims might therefore complement NCR data on MDS.
Aims: We conducted a nationwide medical claims-based study to assess incidence, diagnostic procedures and initial treatment among adult MDS patients (≥18 years) in the Netherlands from 2008 to 2010.
Methods: We identified 3,681 adult MDS patients (median age 77 years) from the nationwide *Diagnose Behandeling Combinatie* (DBC; Diagnosis Treatment Combination) Information System (DIS) from 2008 to 2010. A DBC medical claim is initiated when a diagnosis is made and a particular treatment is started. We exclusively selected patients with an initial DBC medical claim for MDS to omit prevalent cases for measuring the disease incidence. Age-standardized incidence rates (ASRs) of MDS were calculated per 100,000 person-years. Information regarding diagnostic procedures and initial treatment was available in the DIS for 3,628 patients (99%).
Results: The overall ASR of MDS was 5.4/100,000 in 2008-2010, and was higher in males (7.0/100,000) than in females (3.9/100,000). The age-specific incidence increased in parallel with older age, with the highest incidence among those aged ≥80 years (84.4/100,000). In 45% of the patients no bone marrow (BM) examinations were performed. Patients that did not undergo a BM examination were older compared with those who underwent a BM examination (median age 79 *vs.* 74; *P*<.001). The performance of BM examinations decreased in parallel with older age (Figure 1; *P* for trend <.001). The overall ASR of MDS in the cohort of patients that underwent BM examinations was 3.0/100,000 in 2008-2010. A comparison of the DBC cohort with recent population-based data from the NCR (Dinmohamed, *Eur J Cancer* 2013) revealed that the ASR in the entire DBC cohort was almost 2-fold higher than the NCR (5.4 *vs.* 2.8/100,000 in 2008-2010). However, rates were almost equal if only cases with BM examinations were selected in the DBC cohort (3.0 *vs.* 2.8/100,000 in 2008-2010). Regarding initial treatment in the BM (*n*=1,969) and non-BM (*n*=1,659) cohort, 66% and 66% received no therapy, 22% and 28% received supportive care, 6% and 1% were started on intensive therapy (chemotherapy alone+allogeneic stem cell transplantation), and 6% and 5% received other therapy, respectively.

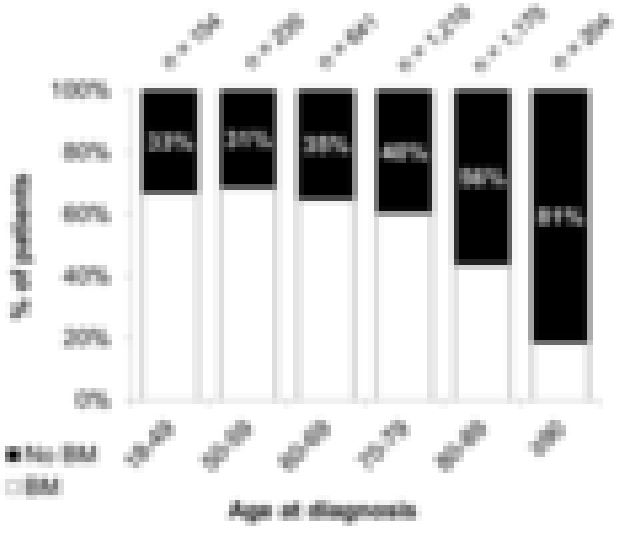

Figure 1. The performance of bone marrow (BM) examinations in MDS patients with initial DBC claims according to age category at diagnosis. Data are shown for 3,628 patients with available data regarding the BM performance status.

Summary and Conclusions: The discrepancy in incidence rates between the population-based NCR and this medical-claims based study is mostly due to the large proportion of diagnoses without BM examinations in the DBC cohort. Incidence rates were comparable with NCR data if cases with only BM examinations were selected in the DBC cohort. We cannot assess whether the non-BM cases in the DBC cohort were truly MDS, though it is unlikely that all non-BM cases reflect true MDS cases. As expected, initial treatment was conservative as MDS is primarily a disorder of the elderly. Elderly MDS patients are by definition not suitable candidates for intensive therapy. BM examination is an essential diagnostic procedure in MDS as MDS might be underdiagnosed or misdiagnosed without BM confirmation. Therefore, guidelines on diagnosis of MDS should be followed more stringently to ensure accurate prognostication and appropriate risk-adapted treatment (Malcovati, *Blood* 2013).

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GLUTATHIONE S-TRANSFERASE P1 (GSTP1) PROMOTER HYPERMETHYLATION IN MDS

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Background: GSTP1 gene expression has been linked with cancer incidence and drug resistance. Recently, there is an ongoing preclinical and clinical platform of drug discovery and development around GSTP1 linked to the activity of GSTP1 inhibitors in stimulating multilineage differentiation of hematopoietic progenitors. It is well-established that gene silencing by epigenetic alterations are common events in carcinogenesis. The GSTP1 gene has been reported to be the target of somatic CpG island promoter hypermethylation in various types of neoplasia, such as prostate, breast and liver cancer.
Aims: The aim of the study was to investigate the possible contribution of epigenetic inactivation of the GSTP1 gene in MDS and AML, through DNA promoter hypermethylation. We also assessed possible associations of GSTP1 methylation pattern with the presence of the $A^{313}G$ GSTP1 inactivating polymorphism and specific chromosomal aberrations.
Methods: Our case-control study enrolled 76 patients [(22 MDS, 29 *de novo* AML and 25 AML cases preceded by MDS (MDS-AML)] and 13 healthy donors. Cytogenetic analysis of bone marrow samples was successfully performed for all patients at diagnosis. Total genomic DNA was extracted from patients' and controls' samples using the QIAamp DNA-extraction midi kit (Qiagen, Hilden, Germany). GSTP1 promoter methylation status was studied by methylation-specific PCR (MSP) using the CpG WIZ® GSTpi Amplification Kit (Chemicon, Canada, USA). $A^{313}G$ GSTP1 genotyping was performed by Real-Time PCR on the Biorad CFX96 (Biorad, California, USA) using GoTaq® qPCR Master Mix (Promega, USA).
Results: Our results showed that GSTP1 promoter was hypermethylated in 32 out of 76 patients (42.1%). All control samples were found to be completely unmethylated. Hypermethylation of GSTP1 promoter was found in 14 out of 22 MDS (63.6%), 6 out of 29 AML (20.7%) and 12 out of 25 MDS-AML cases (48.0%). A significantly increased frequency was observed in MDS ($p<0.001$) and MDS-AML patients ($p<0.001$). The GSTP1 promoter hypermethylation was found not to be associated with the GSTP1 polymorphic gene status. Stratification of patients according to their karyotype revealed an increased frequency of methylated cases in the group of patients showing a normal karyotype, as compared to those with an abnormal karyotype (53.3% *vs* 39.3%, respectively); however, this trend did not reach statistical significance. Moreover, among patients with an abnormal karyotype, the higher frequency of methylated cases was found in cases with -7/del(7q) (60.0%).
Summary and Conclusions: Our results indicate an important role of the GSTP1 epigenetic silencing in MDS pathogenesis, extending the knowledge on epigenetic alterations of the GSTP1 gene in hematological malignancies. Further, these findings might suggest that hypermethylated GSTP1 gene could be a potential epigenetic biomarker for MDS response to treatment, mainly related to administration of GSTP1 inhibitor.

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THE IMPORTANCE OF CYTOGENETIC SCORING SYSTEM IN MYELODYSPLASTIC SYNDROME

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Background: The myelodysplastic syndromes (MDS) are a group of hematopoietic disorders characterized by clonal hematopoiesis, impaired differentiation, peripheral-blood cytopenias, and a risk of progression to acute myeloid leukemia. Conventional cytogenetic analysis (CCA) is one of many prognostic factors included in International Prognostic Scoring System (IPSS).
Aims: Compare our data with New Comprehensive Cytogenetic Scoring System defined by Schanz *et al.* 2012.
Methods: We investigated 728 patients (346 men, 382 woman) with primary MDS in years 2003-2013. The CCA was performed on bone marrow samples using short time cultivation (24-48 hours). Interphase FISH (I-FISH) was carried out using DNA probes designed to detect 5q, 7q, 11q, 12p, 17p and 20q deletions, trisomy 8, loss Y (MetaSystems Germany, Abbott Molecular).
Results: We cultivated 94% of samples succesfully. Chromosomal aberrations were detected in 22% of patients analyzed using CCA and I-FISH. CCA revealed chromosomal aberrations in 8% of patients with negative I-FISH results. For statistical analysis of our data we followed The New Comprehensive Cytogenetic Scoring System of chromosomal aberrations in MDS defined by Schanz (Schanz J, *et al.* J Clin Oncol 2012) and we classified chromosomal

abnormalities into five prognostic subgroups: *very good* -20 patients(3%), *good* – 556 patients (81%), *intermediate* -41 patients (6%), *poor* – 22 patients (3%) and *very poor* – 43 patients (6%).
Summary and Conclusions: We compared our data with New Comprehensive Cytogenetic Scoring System defined by Schanz *et al.* 2012 and our results are consistent with it. We were able to classify 686 of our patients into this scoring system. According to our results it is important to combined both cytogenetic methods (CCA and I-FISH) to correct classification of patients into prognostic subgroups and suggest appropriate therapy.

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DAPK AND TRAIL RECEPTORS METHYLATION STATUS IN MYELODYSPLASTIC SYNDROMES PATIENTS
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Background: MDS are a heterogeneous group of clonal hematopoietic stem-cell disorders characterized by ineffective hematopoiesis, peripheral-blood cytopenias, and increased probability of leukemic transformation. Although MDS pathogenesis is not completely understood, deregulated epigenetic mechanisms are likely involved. DNA methylation is one of the epigenetics processes more frequently studied and several genes have been shown to be transcriptionally silenced in association with promoter methylation. Our previous studies, according with others, show that aberrant methylation of *p15* and *p16* gene promoter region could be associated with MDS initiation and progression. However, the role of methylation status of *DAPK* gene and particularly of *TRAIL-Rs* (TNF Related Apoptotic Induced Ligand, receptors) genes is not yet clarified. Furthermore, methylation profile could be influenced by Vitamin B12 and Folic acid levels as they are co-factors in 1-carbon metabolism enzymes related with SAM (S-Adenosyl Methionine) formation, the major endogenous methyl donor.
Aims: In this context, our aims were to investigate the DNA methylation status of *DAPK, and TRAIL-Rs, TRAIL DcR2* and *TRAIL DR4,* genes in Myelodysplastic Syndrome (MDS) patients at diagnosis, and correlated it with clinical data and vitamin levels.
Methods: The methylation status of *DAPK and TRAIL-Rs (namely, the anti-apoptotic TRAIL DcR2* and *the pro-apoptotic, TRAIL DR4)* genes were analyzed in bone marrow cells of 82 patients with *de novo* MDS, collected at diagnosis and in 14 non-neoplastic patients. Genomic DNA was isolated by standard protocols and modified using sodium bisulfite. The MS-PCR for gene promoter methylation was performed using two sets of primers, one for methylated DNA and other for unmethylated DNA. The MDS patient group median age was 76 years (22-89), gender M/F=40/42, WHO subtypes: 10 RCUD, 5 RA-RS, 41 RCMD, 3 RCMD-RS, 5 RAEB-1, 9 RAEB-2, 8 CMML and 1 5q-. χ^2 and Kruskal-Wallis testes were used to analysed results, that were considered statistically significant when $p<0.05$.
Results: Overall, 81,5% of MDS patients presented at least one methylated gene in BM samples and 58% presented two or more methylated genes. Moreover, about 71% of the MDS patients, presented the *DAPK* gene methylated (distributed according MDS subtype: RCUD:66%; RARS:100%, RCMD:65,8%, RAEB-1:100%, RAEB-2:0%, CMML:100%), 23% presented the TRAIL *DR4* gene methylated (RCUD:33%, RARS:67%,RCDM:23%, RAEB-1:0%, RAEB-2:0, CMML:0%) and 18% presented the TRAIL *DcR2* gene methylated (RCUD:11%, RARS:13,4%, RCMD:13,8%, RAEB-1:33%, RAEB-2:20%, CMML:22%) Moreover, we observed a significant increase in TRAIL DR4 methylation in MDS patients relatively to controls (p=0,041). Besides that, in MDS patients, methylation status seems to be independent of cytogenetic abnormalities, ferritin, erythropoietin and ß-2 microglobulin levels. However, we observed a decrease of DAPK and all TRAIL receptors methylation in patients with high folate levels. We also observed an absence of methylation in TRAIL DcR1 genes in patients with high vitamin B12 levels.
Summary and Conclusions: Our results show that DAPK and TRAIL-DR4 aberrant methylation seems to be a common event in MDS patients and could be related with MDS subtypes and group risk. Moreover, folate and vitamin B12 levels could modulate, at least in part, the methylation gene status in MDS patients.
This work was supported by CIMAGO (Project 24/09).

Myeloma and other monoclonal gammopathies - Biology 1

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IDENTIFICATION OF NOVEL ALTERNATIVE SPLICE VARIANTS OF SIRTUINS IN MULTIPLE MYELOMA: A BIOLOGICALLY RELEVANT INDICATOR OF POOR OUTCOME
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Background: Alternative splicing (AS) is a normal epigenetic event with a critical role in the regulation of gene expression. Previous studies showed increased AS in Multiple Myeloma (MM) cells, suggesting the need to assess both expression level of genes and post translational modifications, mediating overall gene function. The NAD-dependent deacetylases Sirtuins (SIRTs), mammalian homologues of the yeast Sir2, modulate various biological processes including metabolism, cell survival, development, chromatin dynamics, or DNA repair. Recent microarray profiling data using newly diagnosed patients with MM, suggests clinical relevance of such deacetylases since their level predicts for both progression free and overall survival. Among SIRTs family members SIRT-5 and SIRT-7 transcript levels positively correlated with disease progression (from MGUS to active MM).
Aims: These studies provide the rationale for further examining the biological processes including epigenetic changes, mutations, or AS events that contribute to aberrant expression of SIRTs in MM.
Methods: Purified RNA from MM cell lines, newly diagnosed MM patient cells, as well as peripheral blood mononuclear cells (PBMCs) from normal healthy donors was subjected to SIRT expression analysis. Specifically, SIRTs-specific primers were developed and optimized using standard RT-PCR conditions. RNA integrity was confirmed using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control. Aberrant splicing of SIRT-5 and SIRT-7 was confirmed by cloning and sequencing, followed by analysis of their expression patterns in MM patients *versus* normal PBMCs. High throughput GeneChip Human Exon 1.0 ST Arrays in MM patients (n=170) confirmed the clinical relevance of our findings
Results: We found that SIRTs genes undergo aberrant splicing in MM patients. To our knowledge, this is the first report showing SIRTs miss-splicing event in MM. Through cloning and sequencing analysis we identified novel spliced isoforms of SIRT-5 and SIRT-7; these occurred as a result of aberrant AS within exon-3 and exon-2, respectively. Specifically, exon skipping was noted in SIRT-7 variants, via cryptic 5 prime or 3 prime splice sites on exon 3 and/or through partial retention of an intron (500bp) created SIRT-5 variants. The novel spliced forms were widely expressed in MM cell lines as well as in patients with MM or monoclonal gammopathy of undetermined significance (MGUS), without significance occurrence in normal PBMCs. Preliminary data show that even though these novel isoforms exhibit reduced deacetylase activity *versus* full-length variants, this characteristic may impart distinct functional outcome. Finally, longitudinal analyses suggested that aberrant splicing of SIRT5 and SIRT7 correlated with disease status. Correlation analyses between splice-variants of these genes and clinical features of patients showed an association between SIRT5 splice-variants and overall survival of MM patients
Summary and Conclusions: In the current study, we have identified novel transcript variants of SIRT-5 and SIRT-7 in MM cells. These aberrant isoforms allow for generating transcripts that encode for dysfunctional proteins, which in turn, may contribute to the genetic heterogeneity in MM. Overall, our results suggest that SIRT5 and SIRT7 mis-splicing are a common characteristic of MM and has the potential to generate transcripts encoding proteins with altered function. Thus, splice-variants of these genes might provide disease markers and targets for novel therapeutics in MM.

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BONE MARROW CD14+ CELLS SHOW DIFFERENT TRANSCRIPTIONAL PROFILES IN MULTIPLE MYELOMA (MM) AS COMPARED TO SMOLDERING MM AND MGUS: OVEREXPRESSION OF IL-21R AND ITS INVOLVEMENT IN OSTEOCLASTOGENESIS.
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Background: Bone marrow (BM) microenvironment alterations characterize patients with multiple myeloma (MM), as compared to smoldering MM (SMM) and monoclonal gammopathy of uncertain significance (MGUS). Studies focusing on the presence of potential molecular alterations in the BM microenvironment cells are ongoing.
Aims: This study was aimed at analyzing the transcriptional profile of the BM

CD14+ cells across different types of monoclonal gammopathies, based on their primarily involvement in osteoclastogenesis that is typically increased in MM patients.
Methods: CD14+ monocytes were purified from a total cohort of 36 patients including 21 patients with symptomatic MM, 8 patients with SMM and 7 patients with MGUS. CD14+, cells were isolated from the CD138 negative fraction of BM samples of patients by immunomagnetic method with anti-CD14 monoclonal antibody conjugated with microbeads. The presence of potential hemopoietic and CD138+ contaminating cells was excluded by FACS analysis. Only samples with CD14 purity greater than 95% were profiled on GeneChip® HG-U133Plus 2.0 arrays (Affymetrix®). Selected genes were then validated by Real-Time quantitative PCR.
Results: A multiclass analysis identified 14 differentially expressed genes in MGUS, SMM and symptomatic MM. The comparison of symptomatic MM with both SMM and MGUS samples identified 101 genes differentially expressed in CD14+ (58 up-regulated and 43 down-regulated genes in MM). Interestingly, among the differentially expressed genes we found cytokine receptors (*IL21R* and *IL-15R),* chemokines with pro-osteoclastogenic properties *(CXCL10* and *CXCL11),* interferon-inducible proteins (*IFI27* and *IFI44*) and *SLAM7* that were up-regulated in CD14+ of MM patients as compared to SMM and MGUS.
Because recent data indicate that IL-21 is a growth factor for MM cells and may promote osteoclastogenesis in pathophysiological conditions such as rheumatoid arthritis, we further investigate the potential role of *IL-21R* over-expression in CD14+, cells. Firstly, by western blot analysis we confirmed that IL-21 receptor was up-regulated at protein level in CD14+, of MM patients as compared to both SMM and MGUS; whereas the BM IL-21 levels detected by ELISA in a proprietary larger cohort of 77 newly diagnosed MM, 42 SMM and 41 MGUS patients did not showed any statistically significant difference across the three groups of patients (IL-21 median levels: 34, 30.6, and 33.71 pg/ml, respectively). On the other hand, the treatment with rhIL-21 at the concentration levels detected into the BM (30 pg/ml) stimulated BM CD14+-derived *in vitro* osteoclastogenesis and increased the number and the size of osteoclasts, in the presence of RANKL (20-30 ng/ml), in BM samples of MM patients; conversely, this was not observed in samples obtained from SMM and MGUS. Finally, we showed that rhIL-21 stimulated the expression of the RANKL receptor RANK in CD14+, indicating that IL-21 increased the sensitivity of CD14+ cells to the pro-osteoclastogenic effect of RANKL.
Summary and Conclusions: Overall, our results indicate that a different transcriptional signature may be identified in BM CD14+ cells of patients with MM as compared to those with SMM and MGUS, including the overexpression of *IL-21R.* Consequently the involvement of IL-21/IL-21R axis has been demonstrated in the increased CD14+-derived osteoclastogenesis that characterizes MM patients.

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HOW DOES LENALIDOMIDE/DEXAMETHASONE REDUCES RISK OF TRANSFORMATION IN HIGH-RISK SMOLDERING MULTIPLE MYELOMA? LONGITUDINAL IMMUNOPHENOTYPIC PROFILING OF IMMUNE EFFECTOR CELLS FROM THE QUIREDEX TRIAL

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Background: Recently reported from the QUIREDEX trial showed a significantly longer time-to progression and overall survival for high-risk smoldering multiple myeloma (SMM) patients receiving the combination of lenalidomide with low-dose dexamethasone (LEN-LoDEX) *versus* observation (Mateos, NEJM 2013). In addition to the anti-myeloma effect of lenalidomide, its immunomodulatory properties over effector T- and NK-cells may also be playing a role delaying tumor progression; however, since lenalidomide was combined with the immunosuppressant dexamethasone, the direct possible *in vivo* synergistic effects of the combination on SMM patients' immune system remains unknown.
Aims: To evaluate the immunophenotypic profiles of T- and NK-cells from high-risk SMM patients treated according to the QUIREDEX trial.
Methods: In this pre-planned exploratory analysis, we evaluated by multiparameter flow cytometry a total of 30 different markers distributed across 4-color MoAb combinations on longitudinally collected peripheral blood (PB) samples, and analyzing the numbers and immunophenotypic profiles of T- and NK-cells from high-risk SMM patients (N=31; 104 samples) treated according to the QUIREDEX trial (NCT 00480363): an induction phase of 9 four-week cycles of LenDex followed by maintenance with lenalidomide until disease progression. 10 healthy adults (HA) aged over 60 years were also studied and used as normal reference.
Results: To evaluate the immune status of T and NK cells of SMM patients, we compared them at baseline *vs* HA. Overall, the absolute number T and NK cells was similar between the two groups, except for γδ T cells which were significantly increased in SMM patients (median of 37 *vs* 27 cells/μL; P=0.02) However, when a detailed immunophenotypic profiling was performed, CD4 and/or CD8 T cells from high-risk SMM patients showed decreased expression of activation markers (CD25, P≤.004; CD54, P<0.001 and CD154, P=0.002), as well as decreased production of the Th1 related cytokines (IFNγ, P=0.03; TNFα, P≤0.003; and IL-2, P=0.02). To assess the combined effect of LEN-LoDEX on T- and NK-cells, we compared baseline samples *vs* those after 3 and 9 cycles of LEN-LoDEX. Accordingly, CD4 and/or CD8 T cells showed up-regulation of Th1 related chemokines (CCR5; p<.001) and cytokine production (IFNγ, P=.03; TNFα, P=.03 and IL-2, P=.02), as well as increased expression of activation markers (CD69, P≤.005; CD25, P<.001; CD28, P≤.04; CD54, P<.001 and HLA-DR, P<.001). Similarly, CD56dim and CD56bright NK cells showed up-regulation of HLA-DR (P<.001), the ADCC associated receptor CD16 (p≤0.005), and the adhesion molecules CD11a (p≤0.001) and CD11b (p≤0.005). Cell cycle analysis unraveled that the percentage of cells in S-phase was significantly increased from baseline *vs.* 3 *vs.* 9 cycles of LEN-LoDEX for T CD4 (0.04 *vs.* 0.13 *vs.* 0.13; p<0.001), CD8 (0.05 *vs* 0.13 *vs* 0.18; p<0.001) and NK cells (0.07 *vs.* 0.16 *vs.* 0.15; p<0.001), suggesting that PB analyses may reflect increased proliferation observed on other sites. To address the question whether dexamethasone antagonizes the immunomodulatory properties of LEN, we compared in 11 patients' T- and NK-cells immunophenotypic profiles at the end of induction *vs* maintenance (LEN alone and at least 3 months after DEX discontinuation). Overall, there were no significant differences in cell numbers nor immunophenotypic expression profiles of all T- and NK- subsets under study, with the exception for CD40L (CD154) which was slightly higher at the end of induction (P=0.048).
Summary and Conclusions: We showed that high-risk SMM patients present increased absolute numbers of γδ T cells but an overall T-cell impaired activation profile. Treatment with LEN-LoDEX induces immune activation and proliferation of T- and NK-cells which may contribute to the clinically observed disease control. Finally, our results show that DEX does not significantly alter the immunomodulatory effects of LEN.

P330

LONG-TERM RESULTS OF THE GIMEMA VEL-03-096 TRIAL IN MULTIPLE MYELOMA PATIENTS RECEIVING VTD CONSOLIDATION AFTER ASCT: IMPACT OF MRD KINETICS ON DURATION OF RESPONSE AND OVERALL SURVIVAL

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Background: New drugs allowed the achievement of remarkable levels of cytoreduction and improved survival rates in multiple myeloma (MM), though relapse is still the rule even in case of optimal response. This led to a growing interest in minimal residual disease (MRD) monitoring for early identification of patients at high relapse risk. Polimerase-chain reaction (PCR)-based approaches proved highly predictive for outcome discrimination, including in autologous (ASCT) transplantation and new drugs settings. Our group reported some MRD responses following a consolidation program with bortezomib, thalidomide and dexamethasone in 39 patients obtaining at least very good partial remission after ASCT: moreover, patients achieving a low post-consolidation tumor burden by Real-time Quantitative (RQ)-PCR had lower relapse risk.[Ladetto JCO2010]
Aims: Based on the long-term (93 months median follow-up, mFU) results of the GIMEMA VEL-03-096 trial (EudraCTN200400053128), our present work addresses additional relevant issues, such as: 1) the long-term outcome of patients achieving MRD responses in the absence of further treatment and particularly the impact on overall survival (OS); 2) the prognostic role of MRD kinetics during post-consolidation phases and in particular the impact of MRD reappearance; 3) the time lag between MRD reappearance and clinical relapse.
Methods: Inclusion criteria and treatment schedule have been already reported. MRD was assessed on bone marrow at diagnosis and at specific timepoints up to clinical relapse using both qualitative nested-PCR and RQ-PCR and employing immunoglobulin heavy chain-derived patient specific primers, as described [Voena *et al.*, Leukemia 1997; Ladetto *et al.*, Biol Bone Marrow Transpl 2000; van der Velden *et al.*, Leukemia 2007]. "Major MRD response" was defined as the occurrence of two consecutive MRD results scoring less than 1.00E-04 by RQ-PCR; "MRD reappearance" as a confirmed positive MRD

conversion by nested-PCR or an increase of MRD levels of at least one log by RQ-PCR; "MRD persistence" as not attaining MRD response at all.
Results: Among the 39 patients 27 serological progressions, 22 clinical relapses and 12 deaths were observed. Median duration of response (DOR) was 38 months and median OS was not reached (nr), OS at mFU was 64%. 270 of the planned samples (86%) were received by our lab. Overall, 26 patients reached major MRD response (67%) at a median time of 19 months (range: 9-42) from therapy start. Major MRD response was strongly associated with better outcome in terms of median DOR (62 *vs* 9 months, p<0.001) and OS (72% *vs* 48% at mFU, p=0.041, Figure 1A-B). Interestingly, patients with ongoing major MRD response, MRD reappearance or MRD persistence showed progressive increase in relapse risk (median DOR nr *vs* 38 *vs* 9 months, Figure 1C). Of the 26 patients obtaining major MRD response 11 (42%) received a retreatment at a median time of 42 months from major MRD response achievement (range: 22-87). Of these 11 clinical relapses, 7 were anticipated by MRD reappearance (64%), occurring at a median time of 9 months before the start of salvage treatment (range: 2-39). The 4 relapses not anticipated by a documented MRD reappearance always occurred in cases with inadequate follow-up sampling. Interestingly, disease kinetics from MRD reappearance was comparable to that of MRD persistence (time to next treatment, TNT 9 *vs* 10 months, p=0.706), indicating that the time spent in MRD response represents the measure of the benefit obtained by achieving a deeper level of cytoreduction.

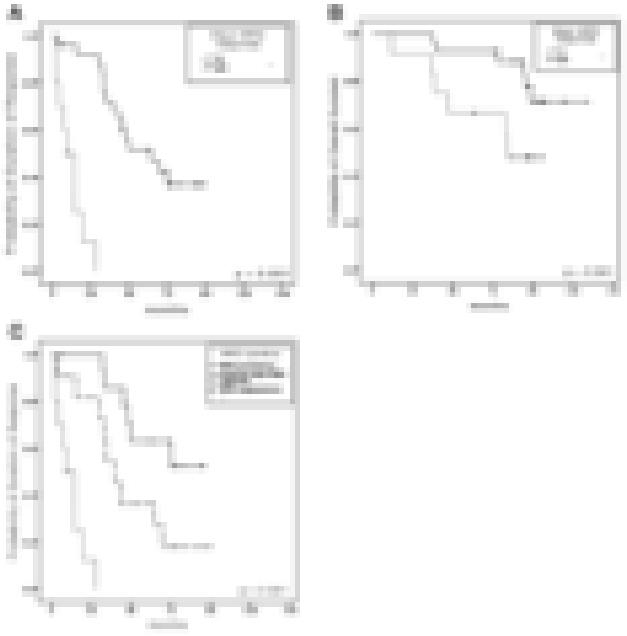

Figure 1. DOR (A) and OS (B) by major MRD response achievement. DOR (C) by MRD kynetics.

Summary and Conclusions: Our long-term data suggest that MM treatment should pursue two main objectives: 1) inducing maximal cytoreduction and possibly MRD response with induction and consolidation treatments; 2) avoiding MRD reappearances, through long-term maintenance or by testing preemptive therapy in the context of future prospective trials. These observations will have increasing relevance considering that high-throughput sequencing will allow MRD monitoring in the vast majority of MM patients.

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THE EXPRESSION OF HIGH LEVELS OF CRBN ISOFORM LACKING IMIDS BINDING DOMAIN PREDICTS FOR A LOWER PROBABILITY OF HIGH-QUALITY RESPONSE TO IMID-BASED UPFRONT THERAPY IN NEWLY DIAGNOSED MYELOMA PATIENTS

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Background: In recent years, the immunomodulator derivatives (IMiDs) have been extensively used for the treatment of multiple myeloma (MM). The protein Cereblon, which is encoded by *CRBN* located on chromosome 3, has been proposed as a primary target of IMiDs. In particular, in several studies the down-regulation of CRBN was linked to IMiDs resistance. More recently, attempts have been done, to validate *CRBN* as a molecular biomarker of response to IMiDs. However, development of a reproducible assay has been hampered by the recognition that splicing events ultimately result in the expression of different CRBN isoforms. Of interest, the loss of exon 10 results in a truncated CRBN isoform, which lack the IMiDs binding domain.
Aims: To set up and validate a consistent Real-time assay for the quantification of *CRBN* isoforms and to explore its role as biomarker for the response to IMiDs-based therapy.
Methods: The study included a cohort of 100 patients with newly diagnosed MM who were homogeneously treated with IMiD-based induction therapy prior to autologous stem cell transplantation. Response to therapy was evaluated according to the International Myeloma Working Group criteria. Gene expression profiling was performed according to standardized procedures (Affymetrix), using the HG-U133 Plus 2.0 array. The study was performed on the enriched CD138+ cell fraction obtained from patients' bone marrow samples collected at diagnosis. A Real-time assay was set up to get an absolute quantification of both the *CRBN* full-length isoform and the truncated one, lacking exons 8 and 10, which code for the IMiDs binding domain. The GAPDH gene was used to normalize expression data and final results were expressed as the percentage of the truncated isoform out of the whole amount of detected isoforms.
Results: The *CRBN*-assay was tested on 100 newly diagnosed MM patients who were clustered in two subgroups according to the percentage of spliced *CRBN (e.g.* exceeding or not the median threshold value of 4.49%). Group A included 34 patients who expressed >4.49% of spliced *CRBN*, while in group B were included 66 patients with a lower percentage of spliced *CRBN*. The two subgroups of patients were homogeneous for baseline clinical characteristics, except for a slightly higher frequency of t(4;14) in group B. After induction therapy, 23/34 (67.6%) patients in group A failed at least a VGPR (very good partial response), whereas the remaining 10 achieved this objective (p<0,01, Fisher exact test). Conversely, in group B patients the frequencies of patients achieving or failing at least VGPR were almost similar (46% and 54%, respectively). The differential expression of 1352 probe sets characterized the transcriptome profile of patients with higher amount of spliced *CRBN*: *IRF4* resulted significantly down regulated in this subgroup of patients (Fold Change, FC=-1,304, p=0,004); the DNA damage control pathway resulted significantly de-regulated, as a consequence of the over-expression of *CHK1* (FC=3,083, p=0,01), *14-3-3* (FC=2,266, p=0,02), *CDC25* (FC=1,531, p=0,01) and the down-regulation of RAD9 (FC=-1,057, p=0,01).
Summary and Conclusions: We set up and validate a consistent Real-time assay for the quantification of actually available *CRBN* isoforms. By applying this assay, we found that a higher expression of spliced *CRBN* predicts for a lower probability to achieve a high-quality response to IMiD-based upfront therapy.

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ACTIVATED AND EXPANDED NATURAL KILLER (NKAE) CELLS KILL MULTIPLE MYELOMA (MM) CLONOGENIC PLASMA CELLS THROUGH NKG2D AND NKP30 RECEPTORS AND THEIR LIGANDS

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Background: MM remains an incurable disease, nowadays most of the available drugs do not destroy clonogenic tumor cell what leads to disease progression. MM precursor display stem cell properties that mediate drug resistance and could be analyzed by side population detection. Natural Killer (NK) cells produce death of MM cells, however they preserve CD34+ hematopoietic progenitor cell. To date NK cell specific activity on MM clonogenic tumor cell is still unknown.
Aims: This study compares cytotoxic activity of autologous NKAE cells on MM clonogenic tumor cells with baseline effect of autologous NK cells. On the other hand, MM clonogenic cells were phenotypically characterized for NK cell ligands to see specific sensibility to NK cell activity.
Methods: We studied NK cells from MM patients' (n=36) or healthy subject's (HS, n=14) peripheral blood. MM patients' NK cells were co-cultured with K562-mb15-41BBL (kindly gave by Baylor College of Medicine, Houston, Texas) cells for NKAEs obtaining. Time resolved fluorescence was employed to detect anti-tumor cytotoxic activity after facing NK/NKAEs cells to MM cells. Flow cytometry analysis was performed to determine NK and MM phenotypic membrane receptor expression profile. For side population studies DyeCycle Violet efflux was detected. NK cell specific activity on clonogenic tumor cells was studied by methylcellulose clonogenic assay. Specific receptor blocking was achieved by NK cells incubation with monoclonal antibodies. Results are presented as mean±SEM.
Results: NK cells from patients (n=20) produce 28,8±2,8% lysis of bulk MM cells, nevertheless NKAEs killed 68±0,4% of bulk MM cells (n=3) at 8:1 ratio. Colony formation showed that NK cells from HS (n=5) decreased MM colony growth in a NK cell dose dependent manner when MM cells are exposed to NK cells, maximum effect was 87,6±3,8% colonies growth inhibition at 32:1 ratio

(NK:MM) compared to autonomous growth of human MM cells (RPMI-8226, NCI-H929 and OPM-2 cell lines). Patients NK cells (n=8) reduced 57,9±12,1% (32:1) colonies generation of MM cells without evidence of NK cell dose relationship. In contrast, patient NKAEs (n=6) reduced 93,6±2,7% (32:1) colonies growth, showing a strong dose-dependent relationship as expected (Figure 1A). At 8:1 ratio MM cell destruction by NK cell was higher on clonogenic MM cells (48,2±6,9% in MM patients and 58,3±4% in HS) than corresponding bulk MM cells (28,8±2,8% in MM patients and 21,1±4,5% in HS). Then NK cell (n=18) and NKAEs (n=5) phenotype were compared. NKAEs showed overexpression of NKG2D and NKp30 receptors. Blocking NKAEs NKp30 receptor (n=2) reduced 38% MM cells lysis, while NKG2D blockade decreased 12% lysis. NKAEs methylcellulose cultures (n=3) showed 15% and 20% increased colonies growth when NKp30 or NKG2D receptors were previously blocked respectively. Flow cytometry analysis of MM cells exhibited that side population have same expression profile of NKG2D ligands (ULBP-1, ULBP-2, ULBP-3, MICA, MICB) when compare to no side population cells in MM cell lines (U-266, RPMI-8226, NCI-H929, OPM-2, L-363, JJN-3, MM.1S), however they have down regulated apoptosis receptors (FAS, TRAIL-DR4, TRAIL-DR5) and DNAM-1 ligands (CD112 and CD155) expression (Figure 1B).

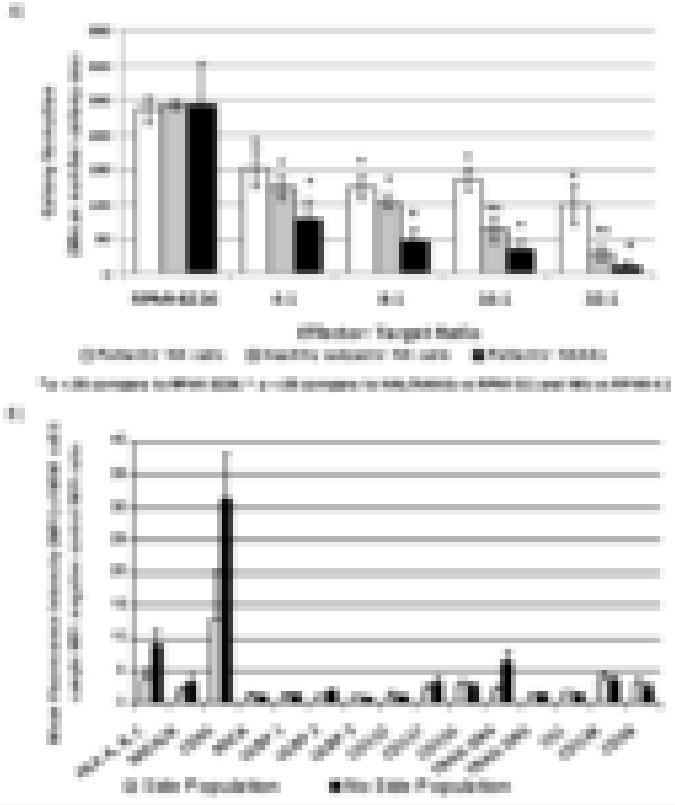

Figure 1. A) Clonogenic assay of MM cell line R PMI-8226 after exposure to MM patients' NK cells, healthy subjects' NK cells and MM patients' N KAEs. B) Membrane receptor expression profile analysis by flow cytometry of side population and no side population from MM cell lines (n=7).

Summary and Conclusions: NK cells are effective against MM clonogenic tumor cell. Patient NK cell stimulation produces NKAEs that have enhanced cytotoxic activity against MM clonogenic tumor cells. Integrity of NKp30 and NKG2D receptors is critical in NK cell activity against MM clonogenic cells. NKG2D ligands of MM side population could be a therapeutic target for MM treatment.

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CIRCULATING MIR-34A AND MIR-130A AS BIOMARKERS OF EXTRAMEDULLARY DISEASE

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Background: MicroRNAs (miRNA), short non-coding regulatory RNAs, are implicated in deregulation of critical pathways involved in multiple myeloma (MM) and extramedullary form of MM (EM). Circulating miRNAs are highly stable and are both detectable and quantifiable in a range of accessible body fluids; thus, they have the potential to be useful diagnostic biomarkers, as was shown in our previous study on MM. Here, we have identified a specific serum miRNA profile in patients with extramedullary disease and correlated it with clinically important parameters.
Aims: The goal of this study was to identify circulating miRNA signature using Taqman Low Density Arrays and assay specific quantitative PCR (qPCR) on a cohort of patients with extramedullary disease, MM patients and healthy controls, and to compare miRNA levels with clinical parameters.
Methods: One hundred serum samples obtained from relapsed EM patients, newly diagnosed MM patients and healthy donors (HD) were evaluated for this study. Screening analysis of 667 miRNAs was performed on 5 EM samples, 5 MM samples and 6 HD samples using TaqMan Low Density Arrays (TLDA). Levels of 4 differentially expressed miRNAs from TLDA (p<0.05) between EM *vs* MM, and EM *vs* HD were confirmed by qPCR using absolute quantification approach on 35 EM, 35 MM and 30 HD serum samples. Receiver Operating Characteristic (ROC) analysis was used to calculate specificity and sensitivity of each miRNA and their combination. Biochemical characteristics were also available for EM and MM patients. P values <0.05 were considered as significant.
Results: MiRNA TLDA profiling revealed 14 deregulated miRNAs (all p<0.05, adjusted p<0.41) between MM patients and EM. Further, 20 miRNAs were on the top of the list of deregulated miRNAs between EM and HD serum samples (all p<0.05, adjusted p<0.40). MiR-222, miR-130a, miR-34a and miR-195 were further verified on a bigger cohort of EM, MM and HD samples. MiR-130a was significantly down-regulated, miR-222 and miR-34a were up-regulated in EM samples when compared with HD (all p<0.005); moreover, miR-130a was down-regulated and miR-34a up-regulated also in EM when compared with MM sera (p<0.06). To discriminate EM from other groups, ROC curve was calculated. To distinguish EM from HD, it revealed highest sensitivity of 74.3%, specificity of 90.0% and area under the curve (AUC)=0.879 for the combination of miR-130a and miR-34a. Further, when EM *vs* MM were compared, this combination of miRNA revealed sensitivity of 54.3% and specificity of 80% with AUC=0.675. In the cohort of EM patients, miR-130a significantly correlated with most of clinically relevant parameters; there was a positive correlation with level of hemoglobin and thrombocytes count (r_s=0.397 and 0.439, all p<0.05) and a negative correlation with levels of monoclonal immunoglobulin, β_2-microglobulin and C-reactive protein (r_s=-0.398, -0.427 and -0.488, all p<0.05). This miRNA was also associated with higher ISS stage (p=0.017). Further, miR-222 correlated positively with lactate dehydrogenase (r_s=0.417, p<0.05); miR-222 and miR-34a were associated positively with percentage of plasma cell infiltration in the bone marrow (r_s=0.435 and 0.562, p<0.05).
Summary and Conclusions: Altogether, our first observations demonstrate that circulating miR-130a and miR-34a may be promising biomarkers for patients with extramedullary disease and prompt further studies in this field.
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THE EFFECT OF BORTEZOMIB ON DIFFERENT CELL SUBSETS: AN APPROACH FOR AN INDIVIDUAL PERSONALIZED MEDICINE

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Background: Our automated flow cytometry-based Exvitech© platform allows the measurement of bortezomib induced depletion of different bone marrow cell populations. We can evaluate in the same myeloma bone marrow sample, the efficacy of bortezomib in depleting myeloma plasma cells as well as the toxic-

ity on myeloid precursors, B-precursors, and mature lymphocytes. Sensitivity is measured by standard pharmacology with 8 concentration dose responses in each of these cell subpopulations.
Aims: To compare bortezomib drug efficacy in the target myeloma population with its expected hematotoxicity from depleting myeloid precursors. This data enables an *ex vivo* therapeutic index for each patient.
Methods: 10 bone marrow (BM) samples from patients diagnosed with MM were sent to Vivia from different hospitals across Spain within 24h. The whole sample in heparin tubes was diluted with 20% SBF retaining the erythrocyte population and serum proteins, and was plated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 12-hours, and then prepared for analysis by our flow cytometry-based Exvitech© platform. A multiple staining (CD45v450/Anexin-FITC/CD117-PE/CD34PerCP/CD38-APC/CD19APC.Cy7) was performed to identify and distinguish the following BM populations: plasma cells ($CD45^{-/+}/CD38^{+}/CD19^{-}$), myeloid precursors ($CD34^{+}/CD45^{-/+}CD117^{+}/CD19^{-}$), B-lymphocyte precursors ($CD34^{+}/CD45^{-/+}CD117^{-}/CD19^{+}$), and normal lymphocytes ($CD45^{+}/SSC^{lo}$). Drug response was evaluated as depletion of each cell population relative to the average of 6 control wells without drug in each plate. All processes have been automated increasing the accuracy of the analysis.
Results: Overall, the effect of bortezomib was clearly higher in the tumor population, since the toxicity to residual BM normal populations was low, demonstrating its selectivity for the pathologic plasma cells. However, there is a high inter-patient variability in the bortezomib's effect inside the plasma cell population and in the healthy cell populations that could correspond to the range of responses seen in the clinical outcome of the patients. Interestingly, we observed the opposite effect as expected in one patient sample, shown in the Figure 1; bortezomib has a non-selective action, with a similar effect in the plasma cells than in all the precursor populations with the exception of the normal residual lymphocytes. We would interpret this data as suggestive that the probability of hematological toxicity in this case could be especially high, and hence the patient may not be a good candidate for bortezomib based therapies.

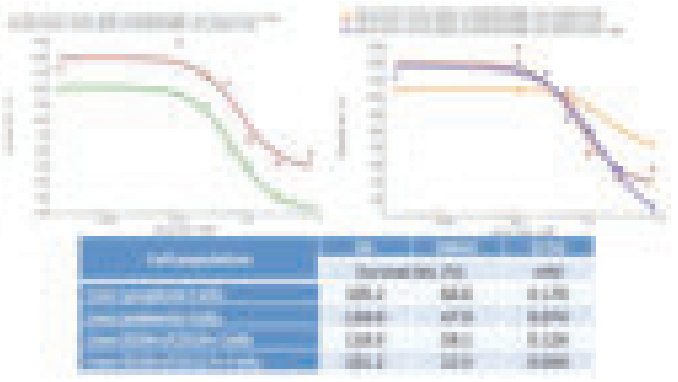

Figure 1.

Summary and Conclusions: These preliminary results show that Vivia Exvitech© platform is able to measure within the same sample the efficacy and toxicity of bortezomib on different BM populations. This platform enables measuring the effect simultaneously in the clonal plasma cells and in a putative stem cell precursor, myeloid precursors or mature lymphocytes. The example shown here for bortezomib is being extended to evaluate full drug combination treatments. This simultaneous analysis for bortezomib based treatments at the different cell levels might be able to predict the clinical response, the clinical follow up and possible hematological toxicities associated with these treatments. We pretend to establish for each individual patient an *ex vivo* hematologic therapeutic index based on the responses of each cell population to a given treatment.

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DOES CHARACTERISTIC PHENOTYPE FOR PLASMA CELL LEUKAEMIA EXIST?

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Background: Plasma cell leukaemia (PCL) is characterized by a presence of circulating plasma cells (PCs) in peripheral blood. Primary PCL (pPCL) occurs in patients with no evidence of multiple myeloma (MM) while secondary PCL (sPCL) is end-stage of relapsed and/or refractory MM. Detection of circulating PCs by flow cytometry is important for diagnosis determination and for discrimination of PCL from reactive plasmacytosis as well. Identification of phenotype profile characteristic for PCL could help in early treatment intervention.
Aims: Analyses of pPCL and sPCL to identify phenotype profile in comparison with MM.
Methods: Total of 86 patients was analysed: 12 patients with pPCL, 10 patients with sPCL and 64 newly diagnosed MM patients. Whole peripheral blood (PB) and/or bone marrow (BM) $CD38^{+}CD138^{+}$ PCs were analysed. Expression of surface antigens (CD19, CD20, CD27, CD28, CD44, CD56, and CD117) together with intracellular nestin was studied by flow cytometry. PCs were considered positive for given marker when its expression exceeds 20%.
Results: There were found similar relative number of PCs in peripheral blood of pPCL (26.7%) and sPCL (26.8%), on the other hand, infiltration of bone marrow was the highest in sPCL (55.6%) when compared to pPCL (39.2%) and MM (5.8%). No presence of $CD19^{+}$ and/or $CD20^{+}$ PCs was found in sPCL, but slightly increased number of positive cases was found in pPCL (16.7% for CD19 and/or CD20 in PB; 18.2% for CD19 and 27.3% for CD20 in BM) when compared to MM (4.7% for CD19 and 11.7% for CD20 in BM). Number of $CD56^{+}$ positive cases was higher in BM of MM (87.5%) then in pPCL (54.5%) and sPCL (66.7%), similar expression was found in PB (58.3% for pPCL and 50.0% for sPCL). Number of CD27 positive cases was the highest in BM of MM (MM 51.6%; pPCL 9.1%; sPCL 28.6%); the same positivity of CD27 was found in PB of both PCLs (25.0%). No big differences were detected in expression of CD28 in BM (30.0% in pPCL, 42.9% in sPCL and 22.6% in MM) and /or PB (16.7% in pPCL *vs.* 28.6% in sPCL). Surprisingly both PCLs expressed CD44 in 100% of BM samples, while MM in 70.7%; PB expression was lower in pPCL (85.7%) then in sPCL (100.0%). CD117 was mostly expressed in BM of sPCL (50.0%) and MM (43.5%) when compared to pPCL (10.0%), similar expression were found in PB of both PCLs (8.3% in pPCL *vs.* 14.3% in sPCL). Nestin, a marker of stem cell, was highly expressed in sPCL (80.0%) and pPCL (75.0%), but decreased in MM (36.1%); PB expression in PCLs was not so different (57.1% in pPCL *vs.* 66.7% in sPCL).
Summary and Conclusions: Phenotype profile of pPCL and sPCL did not differ so much in peripheral blood and/or bone marrow, except for a disappearance of CD19 and CD20 in sPCL and decrease of CD117 in pPCL. Lower expression of CD56, CD27 and overexpression of CD44 with nestin was characteristic for both PCLs when compared to MM.
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GENE EXPRESSION PROFILING AND COPY NUMBER ALTERATIONS OF CIRCULATING CLONOTYPIC B CELLS OF MULTIPLE MYELOMA NEWLY DIAGNOSED PATIENTS REVEALS PATHWAYS POTENTIALLY INVOLVED IN THE DISEASE PERSISTENCE

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Background: In recent years, increasing efforts have been devoted to adjust the Multiple Myeloma (MM) therapeutic approach, in order to get patients' "real cure", instead of "functional cure". Indeed, even if a significant fraction of MM patients are able to achieve complete responses, nevertheless they ultimately relapse. The persistence of Myeloma Propagating Cells (MPCs) has been supposed to be one of the major causes of MM drug-resistance. However, very little is known about the molecular background of MPCs, even if several studies suggested that they display phenotypic characteristics resembling the memory B cells that reside in the CD138- compartment.
Aims: To evaluate the genomic and genetic background MM CD138-B+ cells, located both in bone marrow (BM) and in peripheral blood (PBL) as compared to the CD138+ neoplastic clone.
Methods: We collected the CD138+ and CD138- cell fractions from 50 newly diagnosed MM patients. We isolated the B cell population and, whenever possible, the memory B cell clone. Clonogenic assays were performed using cell fractions obtained from RPMI-8226 and NCI-H929. For each cell fraction, we performed a sequencing of the IgH VDJ rearrangement. The complete set of genomic aberrations and the gene expression profile were performed according to standardized procedures (Affymetrix), using the SNPs array 6.0 and the HG-U133 Plus 2.0 array.
Results: The clonogenic potential, tested by plating cells in conditioned media, resulted higher for CD138- as compared to CD138+ cells. By VDJ rearrangement analyses, a clonal relationship between the CD138+ clone and the memory B cells clone was confirmed. SNPs arrays showed that both BM and PBL CD138+ cell fractions displayed exactly the same genomic macro-alterations. On the contrary, in the BM and PBL CD138-19+27+ memory B cell fractions any macro-alteration was detected, whereas several micro-alterations were highlighted. These micro-alterations were located out of any genomic variant regions and were presumably associated to MM pathogenesis, since, among others, *KRAS*, *WWOX* and *XIAP* genes are located in the amplified regions. The memory B cells were also characterized by the presence of several LOH

regions, which cover at least 106 tumor suppressor genes (among which are *TP53*, *CDKN2C* and *RASSF1A*), already known to be involved in MM and other hematological malignancies. To get insight into the biology of the clonotypic B cell population, the gene expression profiles of 5 donors B cells and 11 MM B cells samples were compared. Unsupervised analysis, by hierarchical clustering was able discriminate the differential expression of 11480 probes (Fold Change: <-2;>2; FDR: 0,05; p <0,05). An overall de-regulation of pathways involved in self-renewal mechanisms was observed (down-regulation of Hedgehog pathway; over-expression of Notch and Wnt signaling). In addition, the down-regulation of genes related to the unfolded protein response (such as *IRE1*α and *XBP1*: -18.0; -19.96. p<0,05) supports the hypothesis of a phenotypic expression related to proteasome inhibitor resistance.
Summary and Conclusions: Data suggest that the MM CD138+ clone might resume the end of the complex process of tumorigenesis, proven by the presence of numerous macro-alterations, possibly due to an already established genomic instability. In contrast, MM B cells, while lacking macro-alterations, display several micro-alterations and a peculiar transcriptional program, thus supporting the idea that these post germinal center cells might be involved in the transforming event, which originate and sustain the neoplastic clone.

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CAN GENOME ARRAY SCREENING REPLACE FISH AS A FRONT-LINE TEST IN MULTIPLE MYELOMA?

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Background: Multiple myeloma (MM) is a malignant disorder characterized by neoplastic transformation of mature B cells in the bone marrow, accompanied by complex genetic changes. The disease is heterogeneous at both clinical and genomic levels. Molecular genetics and genomic investigations have demonstrated that disease evolution is associated with accumulation of specific aberrations, mostly genome imbalances that not only shed light on the disease pathogenesis but also allow risk assessment and treatment monitoring.
Aims: The aim of our study was to evaluate the role of whole genome profiling in identification of multiple changes in bone marrow samples from MM patients.
Methods: Here we present results of FISH and whole genome screening by array comparative genomic hybridization (aCGH) of CD138(+) cell enriched bone marrow samples from 50 patients with MM.
Results: Genome copy number changes were detected in all samples. One of the two genome imbalances clinically significant for high-risk myeloma, *i.e.* 1q gain and 17p13 loss, as per the guidelines of the International Myeloma Working Group (Chng *et al.*, Leukemia 2013), were detected in 30/50 samples (60%). Six of the samples tested by FISH from this group were also *IGH/FGFR3* fusion carriers. The remaining 20 samples (40%) have complex molecular karyotypes of which 7 cases had 12p13 deletions, also associated with poor overall survival (Avet-Loiseau H *et al.*, JCO 2009). Only one of these 20 samples was found to be positive for *IGH/FGFR3* fusion, which was also harboring the 12p13 loss. These data, albeit limited, indicate that the *IGH/FGFR3* fusion gene appears to be a secondary event in MM genomes.
Summary and Conclusions: Employing array platforms routinely used in constitutional genetic studies we demonstrate the ability of aCGH to detect clonal imbalances to a level well below established clinically significant thresholds. By combining target enrichment and complemented with tests for IGH rearrangements aCGH offers a cost-neutral alternative to multiprobe FISH screening. The application of aCGH as a first tier test in the diagnostic workup of myeloma enables high-risk disease stratification with the added benefit of providing whole genome data to assist in establishing clinically relevant predicative markers.

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COMPARATIVE STUDY OF GENE EXPRESSION PROFILING BETWEEN ASYMPTOMATIC AND SYMPTOMATIC WALDENSTRÖM'S MACROGLOBULINEMIA PATIENTS

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Background: Waldenström's macroglobulinemia (WM) is a B-cell malignancy characterized by the accumulation in the bone marrow of clonal B lymphocytes with lymphoplasmacytic differentiation. The recently identified somatic mutations in *MYD88* and *CXCR4* genes have provided new insights into the molecular mechanisms that may contribute to the pathogenesis of the disease. However, these genetic alterations do not allow either to differentiate indolent from symptomatic forms, or to predict progression.
Aims: To identify differently expressed genes among asymptomatic and symptomatic WM. To correlate the gene expression pattern with clinical and biological characteristics of the patients in order to establish risk factors and molecular mechanisms associated with progression.
Methods: Gene expression profile (GEP) by quantitative PCR (Taqman probes) was performed in bone marrow CD19+ cells of 19 asymptomatic WM and 18 symptomatic WM. Data were analyzed with ExpressionSuite Software v1.0.3 (Life Technologies software) and statistical analysis was performed with SPSS v15.0 (SPSS Inc., Armonk, New York) using the Mann-Whitney U-test to identify statistically significant differences (p<0.05) between groups.
Results: The comparison between asymptomatic and symptomatic WM CD19+ cells highlighted differently expressed genes; in particular, ADARB1 (alternative splicing) and GPSM2 (G-protein signaling modulator) were upregulated in asymptomatic cases, whereas CD79A (BCR related), MEKK and P38 (MAPKs pathway) and MYD88 were overexpressed in symptomatic WM, thus suggesting perhaps a more intense activity of the signaling pathways responsible for WM cell growth and survival. Regarding the other relevant pathway in the pathogenesis of WM (which involves CXCR4 receptor), our study showed different gene expression profiling signature in patients with CXCR4 mutation (n=10) *versus* unmutated patients (n=27). Interestingly, among these genes are included ARID1A (which was recently found to be mutated in 17% WM), BTK (which is activated by CXCR4), CD79A (also reported to have mutations in 5.5% WM), IRAK4, PI3K and NF-KB3 (activated by MYD88 pathway), MEKK, MAP3K, ERK and JNK (belonging to MAPKs pathway), PAX5 (gene involved in plasma cell differentiation that had already been described as deregulated in WM) and OBF1 (required for the formation of germinal centers). Then, we correlated clinical and biological characteristics of symptomatic WM patients with GEP. We identified some slight differences in patients with poor prognosis features (*i.e.* albumin <3.5g/dl) and concerned oncogenes (SRC), genes involved in cell cycle (cyclin D1 and D2) or genes that define the molecular signature of WM (CXCR4). It is worth pointing out that the different gene expression pattern also affected parameters included in the International prognostic scoring system for WM such as β2-microglobulin, monoclonal component and hemoglobin. However, due to the low number of patients included, further validation is required. Finally, regarding response to treatment, in contrast to asymptomatic WM, GPSM2 was downregulated in patients with complete or partial response.
Summary and Conclusions: We have identified significant differences in GEP among indolent *versus* symptomatic WM patients, concerning genes involved in WM pathogenic mechanisms. Besides, the results suggest that BCR and BTK signaling pathways (together with the already known MYD88 and CXCR4) may play an important role in the biology and pathogenesis of the disease.

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TRANSCRIPTIONAL REGULATION OF ALTERNATE SPLICING BY DP1 AND THEIR FUNCTIONAL IMPACT IN MULTIPLE MYELOMA

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Background: With cumulative acquisition of new genetic and epigenetic abnormalities there is growing interest in targeting transcription factors (TFs) as they represent common oncogenic regulators in malignant cell growth and survival. Our gene expression profile analysis of 172 uniformly treated MM patients identified significant association of TFDP1 overexpression with shorter overall and event-free survival in MM. DP1 is a binding partners for E2F transcription factors and the heterodimer complex enhances both the DNA binding affinity and the transcriptional activity of the complex.
Aims: We evaluated functional and molecular role of Dp1 in MM and comprehensively characterized its impact on transcriptome and allelic variants in MM.
Methods: Specific shRNA constructs were utilized to evaluate functional and molecular effect of Dp1 silencing in MM. An integrated analysis incorporating ChIP-seq and expression data following gene silencing was performed in MM cell lines in order to identify DP1 response program in MM. Impact of Dp1 on alternate splicing was evaluated by genome-wide analysis of alternate splicing in total RNA from Dp1 silenced MM1S cells using Human Exon1 ST array.
Results: We have observed inhibition of MM growth after DP1 knock-down, independently of the genetic background of myeloma cells. Based on these results suggesting a potential role of DP1 as candidate oncogene in MM, we performed an integrated analysis incorporating genomewide DNA-binding regions and RNA expression following Dp1 and E2F1 gene silencing. Functional annotation of the overlapping target genes derived from both genome-wide studies revealed a strong enrichment for pathways involved in DNA replication, metabolism and repair. Interestingly, the most significant association was observed with ''regulation of RNA metabolic processes'' (40 target genes), ''RNA processing'' (93 target genes) and ''RNA splicing'' (95 genes), suggesting role of Dp1 in RNA splicing. This results prompted us to evaluate the impact of Dp1 on alternate splicing (AS). Splicing profiles showed that Dp1 knock down causes widespread changes in AS. We have identified 1213 alternatively spliced exons that mapped to 818 genes in shDP1 compared to control pLKO.1-transduced MM1S cells,

suggesting impact of Dp1 silencing on alternate splicing through its transcriptional regulation of splicing factor (SF) expression. Further analysis of Dp1 modulated SFs and alternatively spliced genes identified significant interrelation between the two suggesting that Dp1 controls SFs expression which in turn lead to changes in alternate splicing pattern to have subsequent molecular and biological impact. We next evaluated the expression of Dp1-modulated splicing factors in our clinically annotated cohort of MM patients and identified 23 SFs upregulated in MM compared to normal plasma cells. Importantly, the Dp1 was associated with increased expression of the SR protein splicing factor SRSF1 and its high expression was observed to predict poor prognosis in 2 different datasets. Our data for the first time shows that SFs are upregulated in myeloma and link to clinical outcome. Moreover, we have observed significant coexpression of DP1 and SRSF1 in MM patients; and that SRSF1 knockdown reduces DP1-mediated cell proliferation. These results suggest a mechanism for SRSF1 upregulation in tumors with elevated DP1 and identify SRSF1 as a critical DP1 target that contributes to its oncogenic potential by enabling expression of specific protein isoforms through alternative splicing.
Summary and Conclusions: We observed that the DP1/E2F signaling pathway plays a significant role in myeloma by inducing alternate splicing through their transcriptional control of specific splicing factors with potential functional, clinical and therapeutic implications in myeloma.

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DOWNREGULATION OF TELOMERASE ACTIVITY IN RESPONSE TO NEW GENERATION OF PROTEASOME INHIBITORS (EPOXOMICIN AND MG132) IN MULTIPLE MYELOMA CELLS

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Background: Proteasome inhibitors are effective drugs against multiple myeloma (MM). The mechanism of action of these pleiotropic agents has not been yet fully elucidated. The importance of telomerase activity (TA) in MM has been repeatedly demonstrated. Previously we showed that the first generation proteasome inhibitor bortezomib (B) inhibits TA in MM cells by both transcriptional and post-translational mechanisms and has a potential clinical significance.
Aims: In the current study we evaluated the anti telomerase activity of the new generation of proteasome inhibitors, epoxomicin (EP) and MG132 (MG) in order to clarify whether telomerase inhibition represents a class effect.
Methods: MM cell lines, ARP-1, CAG, RPMI 8226 and U266 were exposed to EP or MG for 24-48 hours. Viability was assessed by the WST-1 and Trypan Blue exclusion assays, TA by the TRAP assay, hTERT expression by real time PCR, transcription factors (TF) binding by the ChIP assay and post-translational modifications were evaluated by IP and Western blot.
Results: EP and MG differentially downregulated the proliferation and TA in all MM cell lines. The downregulation of TA and hTERT (the gene encoding for telomerase) expression was faster in CAG than ARP-1 cells. EP was more potent than MG and therefore further mechanistic studies were performed on this compound. The inhibition of TA was mainly transcriptionally regulated and the phosphorylation of the enzyme was not changed. The binding of three positive regulator transcription factors (TF): SP1, c-Myc and NFkB to the hTERT promoter was decreased by EP in CAG cells as well as their total cellular concentrations. In ARP-1 cells the SP1 and c-MYC binding and content were similarly affected by EP while NFkB was not affected. Interestingly the transcription factor WT-1 (Wilms' tumor-1) exhibited an increased binding to the hTERT promoter while its total cellular amount remained unchanged.
Summary and Conclusions: Our results demonstrate the effects of EP and MG on TA in MM. These results combined with our previous study of bortezomib define telomerase as a general target for proteasome inhibitors. The inhibitory effect on TA is exerted on several regulatory levels, transcriptional and post translational. SP1, C-Myc and NFkB were involved in mediating these effects. A novel finding of this study is the role of WT-1. WT-1 appears as a negative regulator of hTERT expression. The results of this study may contribute to future development of telomerase inhibition as a therapeutic modality in MM.

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HSP90 INHIBITOR-DRUG CONJUGATES (HDC) WITH ANTITUMOR AGENTS TARGETING HEMATOLOGICAL MALIGNANCES

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Background: Chemotherapeutic drugs have been the mainstay of cancer therapy for many decades however their effectiveness is often hampered by systemic toxicities due to off-target effects on normal tissues. Targeted delivery strategies such as Antibody Drug-Conjugates (ADCs) offer a means to overcome this limitation, by delivering cytotoxic payloads directly to tumors. We have developed a novel small molecule-drug conjugate platform technology (Hsp90 Inhibitor-Drug Conjugate; HDC) that exploits the unique properties of Hsp90 (heat shock protein 90), a ubiquitously expressed molecular chaperone protein that regulates the maturation and functional stability of multiple cellular client proteins. Hsp90 is overexpressed in many human cancers and inhibitors of Hsp90 have shown preferential tumor retention in preclinical models, compared to normal tissues.
Aims: HDCs consist of three key components: the Hsp90 inhibitor targeting moiety (ligand), the payload (chemotherapeutic agent), and the linker. We have made conjugates with payloads targeting hematological malignancies including lenalidomide, proteasome inhibitors and DNA alkylating agents.
Results: Using a lenalidomide-containing HDC we have demonstrated preferential uptake and retention in myeloma cell lines compared with normal PBMC *in vitro*. Moreover, robust tumor uptake was observed in xenograft tumors *in vivo*, with retention of both the HDC and the lenalidomide payload for more than 24 hours following a single administration. In contrast, dosing with lenalidomide alone resulted in drug clearance from tumors within 6 hours. HDC-induced extended tumor retention of lenalidomide produced xenograft tumor growth inhibition equivalent to using lenalidomide at 7 times higher dose. At the molecular level, both lenalidomide and the lenalidomide HDC exhibit antitumor effects by down regulating IRF4 and inducing degradation of Ikaros and Aiolos, two transcription factors responsible for production of factors promoting the expansion of normal and malignant B and plasma cell lineages. Like lenalidomide, they also induce phosphorylation of CD28 and increase CD56 expression on NKT cells. Proteasome inhibitors such as bortezomib and carfilzomib have been also been approved for treatment of multiple myeloma. But side effects such as neuropathy have been noted. HDC conjugates carrying bortezomib, carfilzomib and delanzomib with demonstrated proteasome inhibition activity have been produced. Several of the most potently cytotoxic conjugates have both proteasome and Hsp90 inhibitory activity, revealing the feasibility of developing a tunable HDC with various degrees of intrinsic proteasome and Hsp90 inhibitory activity.
Summary and Conclusions: The HDC technology presented here can be applied to other chemotherapeutic payloads in order to improve antitumor activity and work on HDC-alkylating agents is underway. Thus HDC represents a promising platform technology for generating diverse anticancer agents for evaluation in patients with hematological malignances.

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TOLL-LIKE RECEPTOR ACTIVATION PROMOTES MULTIPLE MYELOMA CELL GROWTH AND SURVIVAL BY SUPPRESSION OF ENDOPLASMIC RETICULUM STRESS FACTOR CHOP

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Background: Toll-like receptor family (TLR) of receptors is an essential part of innate immunity. During infection, human immune cells sense the presence of invading pathogens through the TLRs which then activate transcriptional programs that orchestrate adaptive responses and induce the endoplasmic reticulum (ER) unfolded protein response (UPR) to accomodate essential protein translation. If the UPR fails to resolve the protein-folding defect, due to severe and prolonged ER stress, apoptosis is activated. However, the timing sequence of the prolonged ER stress has probably imlications for ER stress-induced apoptosis that might help cells to adapt under these conditions rather than driving them to apoptosis. The prolonged stress, possibly arising from a massive increase in protein synthesis, has shown to suppress CHOP, an apoptosis mediator, in ER-stressed macrophages, while low levels of CHOP expression promote B cell survival.
Aims: The aim of our study was to investigate TLR4 signaling in myeloma cells and to explore possible implications with ER UPR as a potential mechanism of drug resistance.
Results: First we found that TLR-4 mRNA is expressed at increased levels (2-10 fold) both in HMCLs and primary cells. To test the whether TLR-4 signaling may suppress CHOP expression during sustained UPR response, two HMCLs, H929 and U266, were pre-treated with low dose LPS (1 ng/mL) and then subjected to ER stress conditions with tunicamycin (TM).LPS pre-treatment significantly decreased CHOP mRNA expression but did not suppress ATF4 mRNA levels which also were not altered by TM treatment. Furthermore, LPS pre-treatment did not suppress XBP-1 splicing compared to the control ER-stressed cells. To test the specificity of these effects, similar experiments where performed on other cancer tissues such as ovarian cancer cell lines. Although LPS pre-treatment increased TLR-4 mRNA expression in SKOV3 ovarian cell line, CHOP mRNA levels remained unchanged prior and after treatment while TM treatment did not make any difference in CHOP mRNA expression. These results indicate the relevance of this pathway in MM cells which are highly dependent on the UPR as a repair mechanism. Apoptosis of pre-treated LPS myeloma cells which were

exposed to TM was reduced by 30% compared to the TM-stressed only cells as observed with Annexin-PI staining. Thus, downregulation of CHOP by TLR4 ligands may confer resistance to apoptotic stimuli and enhance survival of MM cells. Consequently we evaluated the activity of bortezomib in LPS pre-treated cell lines (U266 and JJN3). LPS pre-treatment partially abrogated the efficacy of bortezomib in these cell lines by decreasing its anti-proliferative effects compared to the non-LPS-pretreated cell lines as tested by the WST1 assay.Additional data on the mechanisms involved in these effects will be presented at the meeting. Then, we examined the impact bortezomib therapy on TLR4 and CHOP mRNA expression in primary tumors cells, collected before and at day 7 after bortezomib-based therapy from 6 myeloma patients. In 5 out of 6 cases TLR-4 expression was significantly up-regulated and was accompanied with a coupled down-regulation of CHOP mRNA expression.

Summary and Conclusions: In conclusion, our data suggest that the TLR-4 signaling pathway might provide a translational control pathway which enables cells to carry out essential protein synthesis and avoid CHOP-induced apoptosis. Further exploration of this pathway is needed to establish its role as a potential mechanism of drug resistance of MM cells.

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ENDOPLASMIC RETICULUM STRESS-RELATED GENE EXPRESSION CAN PREDICT RESPONSE TO BORTEZOMIB IN MYELOMA

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Background: Bortezomib (BTZ), a proteasome inhibitor, is widely used in the treatment of Multiple Myeloma (MM). However, a fraction of MM patients shows poor response to the treatment.

Aims: To explore predictive biomarkers in terms of the efficacy of the BTZ, expression of proteasome and endoplasmic reticulum (ER) stress-related genes was evaluated in primary samples obtained from MM patients who received combination treatment of BTZ and Dexamethasone (BD) after informed consent being obtained in our institute.

Methods: Fifty-seven MM samples were collected prior to BD therapy, and subjected to mRNA analysis using real-time PCR. We then analyzed if their progression free survival (PFS) correlated with specific gene expression profiles. We artificially modulated the specific gene expression in MM cell lines to make sure its role in the sensitivity to BTZ.

Results: Fifty-seven patients were divided into two groups, *i.e.*, short PFS group (PFS=<6mos, n=32) and long PFS group (PFS>6mos, n=25). Among 15 genes analyzed, *ATF3* and *ATF4* expression levels were significantly lower in short responders (p=0.0157 and p=0.0085). When either *ATF3* or *ATF4* expression was silenced in KMS-11 cells, these cells became resistant to BTZ accompanied with lower induction levels of Noxa, CHOP and DR5 (Figure 1).

Figure 1.

Summary and Conclusions: Basal expression levels of *ATF3* and *ATF4* may be used as predictive biomarkers for the efficacy of BTZ in the patients with MM, although large-scale replication studies are still needed.

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MODIFYING DNA REPAIR PATHWAYS IN CHEMOTHERAPY BY GENOTOXIC DRUGS – TOWARDS PERSONALIZED MYELOMA THERAPY

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Background: Melphalan is an alkylating agent producing N-alkylpurine-monoadducts, transformed in part into DNA interstrand crosslinks (ICL) and considered to be the critical cytotoxic lesions. ICLs are repaired by the coordination of several pathways, including nucleotide excision repair (NER), homologous recombination (HR) and non-homologous end-joining (NHEJ). High-dose melphalan (HDM) followed by autologous stem-cell transplantation (ASCT) has been the gold-standard of treatment for eligible patients with Multiple Myeloma (MM), while the combination with prednisone has been the reference regime for first line treatment of elderly patients for several decades. MM is the second most prevalent hematological malignancy. Despite recent advances in MM treatment, it remains an incurable disease. Hence, there is an urgent need for optimizing melphalan-based MM regimen by improving the patient classification, and identifying the patients who could most benefit.

Aims: In the present study, we focused on the potential involvement of HR and NHEJ pathways in the repair of melphalan-induced DNA damage in MM and on the link between DNA repair efficiency and clinical response to melphalan.

Methods: We examined two human MM cell lines (HMCLs; melphalan-sensitive RPMI-8226 and resistant LR5) and bone marrow plasma cells (BMPCs) from 30 newly diagnosed MM patients (16M/14F; median age 58 years) who consequently received HDM and supported by ASCT. All patients provided written informed consent. Patient response status was assessed according to the IMW Criteria. Primary cells and HMCLs were *ex vivo* treated with melphalan either alone or in combination with inhibitors of HR (RI-1) or NHEJ (SCR7) and the levels of the N-ras-specific monoadducts and ICLs were evaluated by Southern blot analysis, as well as DSBs (intermediates of ICL repair) by immunofluorescence γH2AX staining. The induction of apoptosis by melphalan, using a photometric enzyme-immunoassay, was also studied.

Results: Monoadducts, ICLs and DSBs repair efficiencies in BMPCs were correlated with clinical response of MM patients and categorized them into 2 groups, responders (≥PR, n=18) and non-responders (<PR, n=12) to melphalan therapy, with non-responders exhibiting significantly higher DNA repair efficiencies (all P<0.001). Also, melphalan-induced apoptosis in BMPCs inversely correlated with the repair efficiencies of all lesions examined, with the toxicity being significantly higher in responders than in non-responders (all P<0.01). To note, LR5 cells showed higher repair efficiencies of monoadducts, ICLs and DSBs and lower toxicity than RPMI-8226 cells. Furthermore, we found that the combined treatment of melphalan with the HR or NHEJ inhibitors had no effect on the repair efficiency of both monoadducts and ICLs, while suppressed the removal of DSBs. The γ-H2AX foci formation followed the timing of ICL formation and reached maximal levels within 8h. Thereafter, γ-H2AX foci levels declined rapidly, suggesting the resolution of the intermediate DSBs by downstream pathways (HR, NHEJ). Interestingly, the repair efficiency of DSBs in BMPCs was significantly higher in non-responders ($t_{1/2}$ of damage removal, 9h) than in responders ($t_{1/2}$ 12h) (P<0.02). Finally, the combined treatment of cells with melphalan and DNA repair inhibitors strongly enhanced the cytotoxic activity of melphalan (all P<0.01).

Summary and Conclusions: Our findings provide a mechanistic correlation between the efficiency of HR and NHEJ pathways and response to melphalan therapy. Specific inhibition of these DNA repair pathways might be an effective strategy to enhance sensitivity of cancer cells.

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***EX VIVO* HIGH THROUGHPUT DRUG SENSITIVITY AND RESISTANCE TESTING IDENTIFIES ONCOGENIC PATHWAY ADDICTION AND NOVEL TREATMENT OPTIONS FOR MULTIPLE MYELOMA**

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Background: Multiple myeloma (MM) is an incurable malignancy characterized by clonal expansion of malignant plasma cells. The introduction of novel therapies including proteasome inhibitors and immunomodulatory drugs has significantly improved overall survival in recent years. However, relapsed disease and drug resistance remain a major challenge.. *Ex vivo* drug testing offers a powerful tool to predict drug sensitivity and guide treatment decisions. This in turn also helps to understand dependency of oncogenic signaling networks based on the response against specific targets.

Aims: In the current study we aimed to identify drug sensitivity patterns, novel therapeutic options and elucidate critical signaling networks in newly diagnosed and relapsed MM patients using a chemical systems biology approach.

Methods: Plasma cells (CD138+) were enriched from fresh bone marrow aspirates derived from 8 diagnosed and 17 relapsed myeloma patients. The cells were screened against 306 oncology drugs (both approved and investigational) representing diverse mechanisms of actions. In order to detect any cytotoxic effects on other bone marrow cell populations, CD138 negative cells from the same sample were screened in parallel for 5 patients. A drug sensitivity score (DSS) and selective drug sensitivity score (sDSS) were calculated based on previously described methods (Pemovska *et al.*, 2013). These were used to compare drug responses across the sample cohort.

Results: Proteasome inhibitors, melphalan, CDK inhibitors and survivin inhibitors were cytotoxic to all cell populations tested in the *ex vivo* assay. Auranofin, an anti-rheumatoid drug targeting IKK showed select cytotoxicity towards

MM cells. In addition, CD138+ cells were selectively sensitive to epigenetic modulators such as the demethylase inhibitor GSK-J4 and the p53 activator PRIMA-1MET. Based on the *ex vivo* drug responses, three major subgroups of patients were identified. One subgroup showed extreme sensitivity to IGF1R, PI3K and ATP competitive mTOR inhibitors. Two subgroups showed varied sensitivity to MEK inhibitors. However, these drugs had no effect on the CD138 negative population, suggesting the CD138+ cell sensitivities were disease specific responses possibly due to the drugs targeting specific underlying molecular aberrations. We also identified a subgroup that was extremely refractory to most targeted drugs tested in our assay. However, this subgroup showed sensitivity to BCL2 inhibitors. In addition, approximately 33% of the MM samples tested responded to glucocorticoids. No clear difference in drug response was observed between MM samples obtained from diagnosed or relapsed patients. Currently we are gathering genomic, transcriptomic and epigenetic data to understand detailed molecular events that help to explain drug responses in each patient subgroup

Summary and Conclusions: Based on an *ex vivo* high throughout chemical biology assay, we identified three subgroups of myeloma patients. The subgroups were defined based on select sensitivity to specific classes of targeted drugs. From the drug sensitivity profiles, PI3K/mTOR, MAPK, IGF1R mediated pathways appeared to be active in malignant plasma cells and likely play important roles in MM pathogenesis. These results suggest that targeting those pathways may be an extremely effective strategy to selectively eradicate MM cells, and argues in favor of individualized treatment design based on *ex vivo* drug response and disease molecular profiles.

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TARGETING ANGIOGENESIS IN MULTIPLE MYELOMA: A POSSIBLE ROLE FOR EPHA3 AND A SPECIFIC MONOCLONAL ANTIBODY

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Background: Multiple myeloma (MM) remains an incurable malignancy despite important recent advances in treatments. Neo-vascularization entails a crucial aspect of interactions between neoplastic plasma cells (PCs) and their microenvironment. Without it, MM would be unable to grow and progress, and would probably regress to a low-mass steady-state comparable to monoclonal gammopathy of undetermined significance (MGUS). To overcome drug resistance and improve clinical response to novel therapeutic approaches halting both PC growth and the increased bone marrow (BM) microvascular density are needed. In this setting, monoclonal antibodies against MM-specific cell surface antigens represent a promising therapeutic approach, which is, however, hampered by a lack of appropriate membrane target structures expressed across all MM cells. The Eph receptors, a large family of receptor tyrosine kinases, have been implicated in many processes involved in malignancy, including alteration of the tumour microenvironment, and in angiogenesis, in both of which EphA3 likely plays an active role. Interestingly, the over-expression of EphA3 is sufficient to confer tumorigenic potential, although probably further mechanisms can occur to abnormally activate the receptor. A first-in-class engineered IgG1 antibody targeting the EphA3 was developed and it is now under phase I clinical trials in USA and Australia for the treatment of EphA3 over-expressing hematological myeloid malignancies refractory to conventional treatment.

Aims: We investigated the role of EphA3 in MM angiogenesis and the effect of a specific monoclonal antibody to define EphA3 as a new possible therapeutic target in MM.

Methods: EphA3 mRNA and protein were evaluated in endothelial cell (ECs) of 40 patients with active MM (MMECs), 10 with monoclonal gammapathies of undetermined significance (MGECs), and 8 with benign anemia (ECs controls) by absolute real time PCR and by western blot coupled to immunofluorescence and FACS analysis respectively. Immunoistochemistry was also performed on MM BM biopsies. The biological effects of EphA3 targeting were studied *in vitro* silencing (siRNA) the EphA3 mRNA in MMECs and using the anti EphA3 antibody testing them in series of *in vitro* functional assays including viability, apoptosis, adhesion, migration, wound healing and angiogenesis tests.The expession of EphA3 pro-angiogenic targets in silincing MMECs was studied by gene expression profiling. We further examined the inhibitory capacity of anti-EphA3 antibody (Ab) on tumor growth in SCID mice bearing MM tumor cell xenografts. Finally, we assessed morphology, vessel density, and apoptosis of excised xenotransplanted tumors.

Results: Briefly, our data showed that EphA3 mRNA and protein levels were progressively increased from ECs to MGECs, reaching the highest values in MMECs. EphA3 stained intensely and diffusely MM microvessels and PC in MM BM biopsies. The EphA3 targeting by either siRNA or anti EphA3 Ab impaired the MMECs angiogenesis related functions both *in vitro* (Figure 1) and *in vivo*. In particular, tumour masses developed in xenograft mice treated with anti-EphA3 Ab were smaller in size and showed foci of ischemic-hemorrhagic necrosis, in association with a significant ($P < 0.05$) reduction in the number of intact tumor microvessels compared to the mice treated with control isotype antibody. Finally, the transcriptional profiles of EphA3-siRNA MMECs showed a downregulation of the adhesion, migration and angiogenesis molecules such as RYK, JAM2, VEGFA, FLNA, CD148.

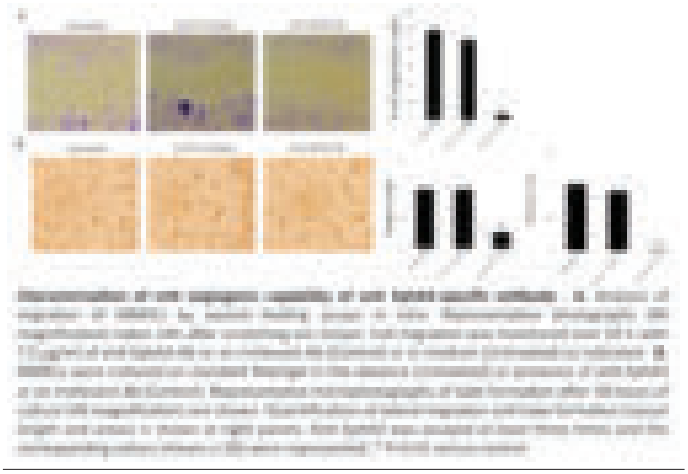

Figure 1.

Summary and Conclusions: EphA3 plays a critical role in MM angiogenesis; the anti EphA3 Ab inhibits this process *in vitro* and *in vivo*. EphA3 targeting could represent a new possible strategy for the treatment of MM patients.

Myeloma and other monoclonal gammopathies - Clinical 1

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INITIAL RESULTS FROM A PHASE 2 STUDY OF CARFILZOMIB, LENALIDOMIDE, AND LOW-DOSE DEXAMETHASONE (KRD) PLUS AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background: In a previous phase 1/2 study, KRd provided a high stringent complete response (sCR) rate of 55% and durable disease control with a 3-year progression-free survival (PFS) of 79% and overall survival (OS) of 96% after extended treatment, with the majority of transplant-eligible patients (pts) deferring ASCT per study design (Jakubowiak *et al.* Blood 2012; Jasielec *et al.* ASH 2013).
Aims: We conducted a phase 2 study (NCT01816971) to assess whether incorporating ASCT into an extended KRd treatment plan can further improve outcomes in transplant-eligible pts with NDMM.
Methods: Pts with symptomatic NDMM per IMWG criteria and transplant candidates were eligible for enrollment. All pts provided written informed consent. For induction (cycles 1–4), pts received 28-day cycles: carfilzomib (CFZ) IV on days 1, 2, 8, 9, 15, and 16 at 20 mg/m^2 days 1 and 2 of cycle 1 and 36 mg/m^2 IV thereafter; daily lenalidomide (LEN) 25 mg PO (d1–21); and weekly dexamethasone (dex) 40 mg IV/PO. After cycle 4, pts proceeded to stem cell collection with G-CSF+plerixafor followed by high-dose melphalan 200 mg/m^2 with ASCT. Post-ASCT (70–90 days), KRd was resumed as consolidation (cycles 5–8) with CFZ at the pre-transplant dose, dex reduced to 20 mg, and LEN reduced to 15 mg for the first consolidation cycle with the option to increase to pre-transplant tolerated doses for the remaining cycles (7+). For extended treatment (Cycles 9–18), KRd was given with a modified CFZ schedule (days 1, 2, 15, 16) followed by single-agent LEN maintenance until progression. The primary endpoint is rate of sCR at the end of cycle 8. Based on a sCR rate of 30% after 8 cycles in the prior study, we estimate that a sCR of 50% with KRd plus ASCT after 8 cycles will support further evaluation.
Results: As of February 24, 2014, 26 pts have been enrolled. Median age was 62y (range 44–76) and 58% of pts were ISS stage I/II. All 26 pts were evaluable for response and toxicity and continue protocol treatment: 13 pts completed induction, 11 pts completed ASCT, 9 started consolidation, and 8 completed consolidation. At a median of 4 KRd cycles (range 1–10), all 26 pts achieved ≥partial response (PR), 62% ≥very good PR (VGPR), and 23% sCR. After ASCT, ≥VGPR was 91% and sCR 27%.The rate of sCR at the end of 8 cycles (primary end-point) was 50%. In patients who completed ASCT and ≥1 cycle of KRd consolidation (range 5–10 total KRd cycles), the sCR rate was 75% and the rate of negative minimal residual disease by 9-color flow cytometry was 66%. After a median follow-up of 7 months (range 1–13), all pts were alive and progression free. There were no new or unexpected toxicities in the induction phase and no unexpected toxicities in the consolidation phase compared with those previously reported. Enrollment is continuing, with a target of 53 pts. Updated results will be reported during the meeting.
Summary and Conclusions: These preliminary data are very encouraging and suggest that pairing KRd with ASCT in NDMM can further increase the depth of response, possibly translating into improved survival outcomes.

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THE USE OF SINGLE AGENT ZOLEDRONIC ACID PREVENTS THE DEVELOPMENT OF BONE DISEASE COMPLICATIONS IN MYELOMA PATIENTS WITH ASYMPTOMATIC RELAPSES WITH NO DEMONSTRATED EFFECT ON SYMPTOMS PROGRESSION

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Background: Zoledronic Acid (ZOL) is presumed to have anti-myeloma effect, although it is small and the real clinical effect remains to be elucidated, especially without chemotherapy. Current therapies induce response in most patients, but virtually all of them relapse, usually with an initial M-component re-growth (biochemical relapse) with no clinical symptoms. In such a case, the usual decision is not to treat the patient at this point, a perfect situation to explore the antitumor benefit of ZOL in the absence of any other cytotoxic therapy.
Aims: To evaluate the potential anti-tumor effect of ZOL treatment in MM patients under asymptomatic biochemical relapse after prior standard therapy. Primary end-point was Symptomatic Progression Free Survival (sPFS). Secondary evaluation variables were response rate, skeletal related events and time to next chemotherapy.
Methods: Patients and methods: Since JUN10 to JUL12, 100 patients were recruited in a randomized, prospective, open label phase IV trial in which a group of patients received ZOL (4 mg iv./4 wk, 12 doses) or no ZOL. All patients were monitorized every 4 wk until AUG13 in which database was closed.
Results: Results: 51 pts were randomized to receive ZOL while 49 received no ZOL. All patients were in Asymptomatic Biochemical Relapse, median age 68yr (40-87) and male/female 53/47. M-component was IgG (72%), IgA (25%) and only light chain (3%). Relapse presented after 1, 2 or ³3 lines of therapy in 67%, 22% and 11% of cases, respectively. Prior treatment included transplant (66%), bortezomib (53%) or IMiDs (36%). 1-2 skeletal related events (SRE) had presented in 32% of cases. Bone marrow plasma cell infiltration by flow cytometry was low 1,65% (0,01%>96%), 44% abnormal (2-100%). FISH/cytogenetics was abnormal in 52% of cases: t(11;14) 19%, Rb deletion (alone) 17%, del(p53) 8%, t(4;14) 4% and t(14;x) 4%. ZOL and no ZOL groups were well balanced for prognostic features, prior response, and time from diagnosis the inclusion in the trial. 40 patients completed the 12 visits programmed in the trial and four terminated before completion due to patient refusal (n=2) and development of other diseases (n=2). The remaining 56 patients stopped the trial due to progression before 12 months (n=55) and osteonecrosis of the jaw development (n=1). Median sPFS since inclusion was of 309 days (10.1 months), being slightly longer for the ZOL group (13 months) *vs.* no ZOL group (9 months), although differences were not statistically significant. The 30-month projected sPFS was 38% *vs.* 19% for ZOL *vs.* no ZOL pts. (p>0,1). The pattern of symptomatic progression varied according to the group of therapy. Thus, in the group of patients not treated with ZOL, 33 progressions were noted: 17 progressed with more advanced bone disease (12 cases of new bone lesions or regrowth of prior lesions, 2 spinal cord compressions, and 3 cases of hypercalcemia), 14 with anemia and 2 with renal dysfunction. By contrast, 28 progressions were seen in the group of patients treated with ZOL: 6 with new or enlarged bone lesions, 19 with anemia, 1 with renal dysfunction, 1 with extramedullary growth and one hyperviscosity (P=0.031). There were 8 SRE that presented only in the group of patients treated without ZOL (p=0.003) (Figure 1).

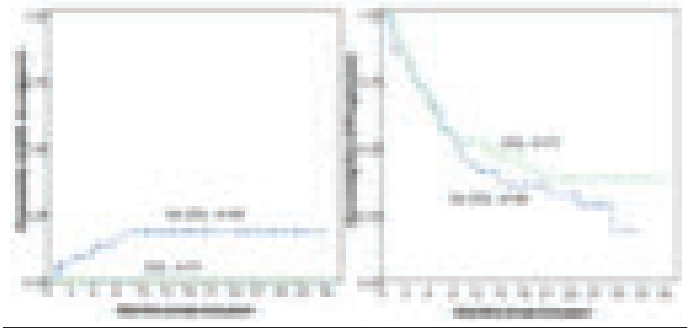

Figure 1.

Summary and Conclusions: Zoledronic Acid single therapy reduces the risk of progression with symptomatic bone disease and SREs in MM with asymptomatic relapse. The possible antitumor effect of Zoledronic Acid alone in biochemical relapses could not be completely demonstrated. These results support the use of Zoledronic acid alone in patients with biochemical relapses or progressions in whom the physician decides not to initiate cytotoxic therapy based on the absence of symptomatic disease.

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LENALIDOMIDE-DEXAMETHSONE (RD) *VS* MELPHALAN-LENALIDOMIDE-PREDNISONE (MPR) *VS* CYCLOPHOSPHAMIDE-PREDNISONE-LENALIDOMIDE (CPR) IN ELDERLY COMMUNITY-BASED NEWLY DIAGNOSED MYELOMA PATIENTS

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Background: Rd and MPR showed to be effective and safe in newly diag-

nosed multiple myeloma (MM) patients. Cyclophosphamide is a less toxic alkylating alternative agent. A formal comparison between 2-drug and 3-drug lenalidomide-containing combinations has not yet been performed.
Aims: To compare a not-alkylating regimen (Rd) *vs* two different alkylating-based regimens (MPR or CPR) in a community-based setting of MM pts ≥65 years old or not eligible for autologous stem cell transplantation. The primary endpoint was progression-free survival (PFS).
Methods: 662 patients with newly diagnosed MM were randomized to receive nine 28-day cycles of Rd, MPR or CPR. Upfront dose reductions of dexamethasone, melphalan and cyclophosphamide were performed according to patients age (Rd: lenalidomide 25 mg/day for 21 days; dexamethasone 40 mg on days 1,8,15 and 22 in pts 65-75 years old and 20 mg in those >75 years; MPR: lenalidomide 10 mg/day for 21 days; melphalan orally 0.18 mg/Kg for 4 days in pts 65-75 years old and 0.13 mg/Kg in >75 years pts; prednisone 1.5 mg/Kg for 4 days; CPR: lenalidomide 25 mg/day for 21 days; cyclophosphamide orally 50 mg/day for 21 days in pts 65-75 years old and 50 mg every other day in >75 years pts; prednisone 25 mg every other day). After induction, patients were randomized to receive maintenance with lenalidomide alone (R) or with prednisone (RP), until disease progression.
Results: Patients characteristics were well balanced in all groups. 212 patients in Rd, 210 in MPR and 220 in CPR arm were evaluable for response. After induction, at least partial response (PR) rate was 78%, 73% and 73% in Rd, MPR and CPR arms, respectively (Rd *vs* MPR p=0.138; Rd *vs* CPR p=0.205). After a median follow-up of 26 months, median PFS was 22 months in Rd, 27 months in MPR and 23 months in CPR patients (Rd *vs* MPR p=0.231; Rd *vs* CPR p=0.862). Grade ≥3 hematologic adverse events were 29% in Rd, 67% in MPR and 32% in CPR arms (Rd *vs* MPR p<0.0001; CPR *vs* MPR p<0.0001). No difference was noticed in grade ≥3 non-hematologic adverse events between the treatment groups: 29% with Rd, 31% with MPR, 30% with CPR. 203 patients in the R group and 198 in the RP group started maintenance treatment. After a median follow-up of 18 months, 2-year PFS from the beginning of maintenance treatment was 52% in R and 58% in RP patients (p=0.570). A sub-group analysis according to patient's frailty, as established by ADL, IADL, Charlson scores and age (Larocca A, *et al.* Blood ASH meeting 2013; 122: abstract 687) was conducted. No significant PFS difference was reported among the three arms in fit and frail patients. Grade ≥3 hematologic toxicity was sensibly higher in the MPR arm in both fit and frail patients.
Summary and Conclusions: In a community-based population, triplet alkylating combinations do not offer a significant PFS advantage over doublet therapy without alkylating agents. In a subgroup analysis according patient's frailty, PFS was similar in fit, unfit and frail patients regardless of treatment, yet grade ≥3 hematologic toxicity was higher with MPR. Updated data will be presented at the meeting.

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SAFETY AND EFFICACY OF DARATUMUMAB WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED OR RELAPSED, REFRACTORY MULTIPLE MYELOMA.

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Background: Daratumumab (DARA) (HuMax™-CD38), a human IgG1κ monoclonal antibody effectively mediates destruction of CD38-expressing malignant plasma cells. In the first-in-human dose-escalation study, 42% of heavily pretreated patients with relapsed or relapsed, refractory (RR) multiple myeloma (MM) treated with DARA alone (≥4mg/kg) achieved partial response (PR) and 25% had minimal response (MR) (modified IMWG guidelines). In preclinical studies, DARA+lenalidomide (LEN) enhanced killing of MM cells *in vitro.*
Aims: We evaluated safety, pharmacokinetics (PK) and efficacy of DARA+LEN+dexamethasone (DEX) in patients with relapsed or RR MM.
Methods: In this ongoing phase I/II open-label multicenter dose-escalation (part 1) study, patients (≥18 years old) with life expectancy ≥3 months and ECOG status 0, 1 or 2 received DARA+LEN+DEX: (DARA [2-16 mg/kg] per week [8 wks], twice a month [16 wks], then, once monthly until disease progression, unmanageable toxicity or 24 months in total; LEN [25 mg]; DEX [40 mg] once weekly). Cohort expansion (part 2) study explores testing of maximum DARA dose determined in part 1.
Results: Data from 12 patients (10 men, 2 women), median age 62 years (48-76) are evaluable to date. Median prior therapies: 4 (2-4); median ECOG status: 0.5 (0-1); median DARA infusions: 14.5 (1-23); median infusion time: 6.6 (5.9-7.3) hours. One patient (2 mg/kg dose) withdrew from study due to recurrent grade 1 QT prolongation and hypokalemia. Most frequent (>40% patients) adverse events were neutropenia and diarrhea; 17 were ≥grade 3 with 70% hematological (neutropenia, thrombocytopenia, anemia). MTD was not reached. DARA+LEN+DEX PK-profile was similar to DARA alone suggesting LEN and DEX do not affect the DARA PK-profile. Available preliminary efficacy data from 11 patients demonstrated marked decrease in M-protein in all patients; 8/11 patients achieved PR or better, 5/11 with VGPR, 2/11 with MR. Median time to response was 4.1 weeks (2.1-4.3).
Summary and Conclusions: DARA+LEN+DEX has favorable safety profile with manageable toxicities in relapsed and RR MM. Encouraging early activity is seen with marked reduction in M-protein and 8/11 patients (72%) achieving PR or better. Longer follow up is necessary to estimate overall depth of response.

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POMALIDOMIDE, BORTEZOMIB, AND LOW-DOSE DEXAMETHASONE (PVD) IN LENALIDOMIDE-REFRACTORY AND PROTEASOME INHIBITOR-EXPOSED MYELOMA: THE MM-005 TRIAL

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Background: The combination of an immunomodulatory drug (lenalidomide [LEN] or pomalidomide [POM]) with the proteasome inhibitor (PI) bortezomib (BORT) and low-dose dexamethasone (LoDEX) has demonstrated activity in multiple myeloma patients (pts; Richardson PG. *Blood.* 2014; Nooka AK. *Leukemia*. 2013). POM has been approved in Europe in combination with LoDEX for the treatment (Tx) of relapsed and refractory multiple myeloma (RRMM) pts who have received ≥2 prior Tx, including LEN and BORT, and have demonstrated progressive disease (PD) on the last therapy.
Aims: MM-005 is a phase 1 study designed to identify the optimal dose of POM, BORT, and LoDEX combination therapy (PVD) for a phase 3 trial (MM-007) comparing PVD *vs.* BORT+LoDEX in RRMM pts.
Methods: Eligible pts received 1-4 prior lines of Tx, including ≥2 consecutive cycles of LEN and a PI. Pts must have been LEN refractory and PI exposed but not BORT refractory. The maximum tolerated dose (MTD) was determined using a 3+3 design. Each cohort received 21-day cycles of POM (1-4 mg/day on days 1-14), intravenous (IV) BORT (1-1.3 mg/m^2 on days 1, 4, 8, and 11), and LoDEX (20 mg/day on days 1, 2, 4, 5, 8, 9, 11, and 12; pts aged >75 years [yrs] received 10 mg/day on days 1, 2, 4, 5, 8, 9, 11, and 12). A 6-pt cohort was added to receive subcutaneous (SC) BORT at the MTD. Tx continued until PD or unacceptable adverse event (AE). MTD was the primary endpoint; additional endpoints included safety, overall response rate (ORR; ≥partial response [PR]), time to response (TTR), duration of response (DoR), pharmacokinetics, and the safety and efficacy of SC BORT.
Results: The trial is fully enrolled (N=28): 22 pts in IV BORT and 6 pts in SC BORT cohorts. Median prior Tx was 2 (range, 1-4). No dose-limiting toxicities (DLTs) were observed. Recommended PVD dose (21-day cycles) was found to be POM 4 mg on days 1-14; BORT 1.3 mg/m^2 on days 1, 4, 8, and 11 for cycles 1-8 and days 1 and 8 for cycle 9+; and LoDEX 20 mg (10 mg for pts >75 yrs) on days 1, 2, 4, 5, 8, 9, 11, and 12 for cycles 1-8 and days 1, 2, 8, and 9 for cycle 9+. The most common grade 3-4 AEs that occurred in the IV cohort included: neutropenia (36%) and thrombocytopenia (27%); for the SC cohort, a total of 7 grade 3 AEs were recorded (1 pt for each); no grade 4 AEs were observed. Peripheral neuropathy (PN) in IV cohorts was 0% grade 3-4, 14% grade 2, and 32% grade 1; 1 pt reported grade 2 PN in the SC cohort. No pt discontinued due to Tx-related AEs. ORR (IV cohorts) was 71% (38% ≥very good PR [VGPR]), median TTR was 1 cycle, and median DoR was 11 cycles. Preliminary ORR (median 5 cycles received) for SC pts was 67% (1 PR, 2 VGPRs, 1 complete response [CR]). Four of 6 pts receiving SC BORT still remain on Tx, 1 of whom has not yet responded. Within these small cohorts, BORT administration route (IV *vs.* SC) did not appear to impact POM exposure, which was within the range previously observed. Additional long-term follow-up data will be presented.
Summary and Conclusions: PVD was well tolerated in LEN-refractory/BORT-exposed pts with no DLTs or discontinuations due to Tx-related AEs. PVD was effective in all cohorts, with an encouraging ORR of approximately 70%, including a high rate of VGPRs and a CR. These results provide further evidence that POM is tolerable and effective as part of combination therapies and support its use as a backbone in Tx strategies for RRMM. The optimal PVD dose identified is under further evaluation in the ongoing MM-007 phase 3 trial (N=782).

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THE REVII TRIAL: LENALIDOMIDE AND DEXAMETHASONE AS SECOND LINE TREATMENT IN MYELOMA FOLLOWED BY EXTENDED LENALIDOMID *VS* LEN/DEX

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Background: The current indication for the use of lenalidomid in Europe is relapsed or refractory multiple myeloma patients and is based upon a subset analysis from two phase III studies (Weber 2007, Dimopoulos 2007), where the benefit of initiating lenalidomide plus betamethasone at first relapse was analyzed (Stadtmauer 2009). The analysis found that patients with only one prior therapy had significantly longer median TTP compared with those treated in later lines (17.1 months *vs.* 10.6 months).
Aims: Here we report outcomes from the REVII trial in 2nd line treatment of MM, a two part study with first an observational part with standard treatment of lenalidomide combined with corticosteroids and then, a randomized part, where patients that had responded in the first part, were randomized to lenalidomide combined with dexamethasone or lenalidomide as a single drug.
Methods: 132 patients with multiple myeloma in first relapse were included in the first, observational part of the study, and treated with up to 9 cycles of lenalidomide combined with betamethasone or dexamethasone according to local routines. Patients that had achieved at least partial response (PR) and had consolidation with at least two additional cycles were offered to take part in the second part of the study. Next where 63 patients were randomized to be treated with either lenalidomide combined with dexamethasone or lenalidomide as a single drug for a maximum of 24 cycles (96 weeks). In the first part the primary endpoint was time to response and the secondary endpoints were quality of life, safety and cytogenetic markers. In the second part primary endpoint was time to progression and secondary was safety. Survival data were calculated from first entry in the observational study. Data cutoff was 31st January 2014, with a median follow-up of 12 months.
Results: A very high proportion of patients achieved PR or better (≥PR 88%). [SP1] Of the patients 5% were primary refractory and progressed without any prior response and 7% got a minor response or stable disease. Very good partial response or better (≥VGPR) was achieved in 44% of the patients. First response was achieved after a median of 7.5 weeks and best response after a median of 10 weeks. The PFS for all patients at 12 and 18 months was 68% and 62%, respectively and the OS was 88% and 81% respectively. After randomizations no differences in responses between the Len and the Len/dex group were found. The PFS at 12 and 18 months was 84%/72% for the Len group and 86%/75% for the Len/Dex group (p=0.64). OS was 100%/86% for the Len group and 100%/92% for the Len/Dex group (p=0.94). Further results will be presented at the conference.
Summary and Conclusions: Len/Dex was associated with a very high response rate at 2nd line treatment of MM. On achieving at least PR, continuing with dexamethasone does not add any benefit for the patients regarding responses, PFS or OS.

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PRELIMINARY PRE-RANDOMIZATION RESULTS FROM A PHASE 3 STUDY OF FRONTLINE SUBCUTANEOUS (SC) BORTEZOMIB-BASED INDUCTION AND CONSOLIDATION IN TRANSPLANTATION-ELIGIBLE MULTIPLE MYELOMA (MM) PATIENTS (PTS)

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Background: Induction chemotherapy followed by high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) remains standard frontline treatment for eligible MM pts. Post-ASCT consolidation/maintenance may produce deeper/more prolonged responses leading to improved outcomes. Several studies have shown pre- and post-ASCT bortezomib (Btz)-based consolidation/maintenance to be well tolerated, and lead to increased CR and VGPR rates. SC Btz has demonstrated equivalent efficacy and improved tolerability compared to IV Btz in relapsed MM (Moreau *et al.*, Lancet Oncol 2011); however, data on frontline SC Btz are limited.
Aims: The overall study aim is to evaluate if SC Btz-thalidomide-prednisolone (VTP) consolidation after SC Btz-cyclophosphamide-dexamethasone (SC VCD) induction and HDT-ASCT produces an improved CR+VGPR rate compared with thalidomide-prednisolone (TP) consolidation (NCT01539083). This analysis was performed to evaluate the safety/tolerability of SC VCD induction and obtain preliminary response data prior to pt randomization to consolidation.
Methods: Consenting adults with previously untreated MM, ECOG PS 0–2, and who were HDT-ASCT-eligible were included. Pts received 3×21-d cycles of SC VCD induction (Btz 1.3 mg/m² SC d 1, 4, 8, 11; cyclophosphamide 300 mg/m² PO d 1, 8, 15; dexamethasone 20 mg PO d 1, 2, 4, 5, 8, 9, 11, 12), followed by peripheral blood stem cell (PBSC) mobilization, HDT (melphalan 200 mg/m², IV), and ASCT. Pts without evidence of disease progression post-ASCT were then randomized 1:1 to VTP or TP consolidation. Adverse events (AEs) were graded by NCI-CTCAE v4.0. Responses were investigator-assessed by IMWG criteria.
Results: At data cut-off (Nov 30, 2013), 187 pts had been enrolled at centers in Australia (88%), China (3%), and Korea (9%). Median age was 59 yrs (range 32–71), 55% were male, 32%/51%/17% had ISS stage I/II/III MM, and adverse cytogenetics included 7% t(4:14), 6% del 17p, 17% amp 1q, and 3% t(14:16). Pts received a median of 3 cycles of SC VCD (range 1–3); mean Btz dose intensity was 5.12 mg/m²/cycle (98% of planned). 152/162 (94%) pts had proceeded to the PBSC phase and 135/153 (88%) to ASCT. Overall, 31 pts had discontinued treatment (10, 8, and 13 during induction, PBSC phase, and ASCT, respectively), 8 due to AEs. 48 pts were ongoing (25, 9, and 14 in the induction, PBSC, and ASCT phases, respectively). At clinical cut-off for this analysis, 108/139 (78%) pts had proceeded to randomization to consolidation. Most common any-grade (G) treatment-emergent AEs during induction were constipation (35%), nausea (33%), and fatigue (25%). 34% of pts had G ≥3 AEs, including 6% neutropenia. 29% of pts had peripheral neuropathy (1 pt G 3), and 21% had injection site reactions (no G ≥3). Best confirmed response rates (≥PR) were 79% (25% CR+VGPR; 8% CR) post-induction, 83% (32% CR+VGPR; 10% CR) post-PBSC phase, and 87% (35% CR+VGPR; 13% CR) post-HDT-ASCT.
Summary and Conclusions: These data suggest that VCD incorporating SC Btz is a tolerable and active induction therapy in previously untreated MM pts. Rates of neuropathy and discontinuations due to AEs were low, and a substantial proportion of pts proceeded to ASCT. Preliminary response rates appear similar to previous large studies investigating VCD induction (Einsele *et al.*, ASH 2009, Goldschmidt *et al.*, ASH 2013). Randomization to consolidation is ongoing.

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GERIATRIC ASSESSMENT PREDICTS SURVIVAL AND RISK OF SERIOUS ADVERSE EVENTS FOR ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS.

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Background: Multiple myeloma is a neoplastic disease typical of elderly patients and aging is associated with worse survival. Elderly patients are highly heterogeneous. Aging and frailty have a negative impact on treatment tolerance and outcome of cancer patients. A geriatric assessment can better define patient status and help physicians tailor treatment.
Aims: The primary endpoint was to define a frailty score and assess its impact on outcome and toxicity. The secondary endpoint was to assess its value in subgroup of patients defined at high risk by International Staging System (ISS) and Fluorescence *In Situ* Hybridization (FISH).
Methods: Newly diagnosed multiple myeloma (NDMM) patients enrolled in 3 prospective trials including lenalidomide, bortezomib or carfilzomib were retrospectively analyzed. At diagnosis, a geriatric assessment was performed to assess co-morbidities (Charlson index), cognitive and functional conditions [Activity of Daily Living (ADL) and Instrumental Activity of Daily living (IADL) scores].
Results: We assessed 869 NDMM elderly patients. The prognostic role of age (‹75, 75-80, ›80 years), Charlson (≤1, ≥2), ADL (›4,≤4) and IADL (›5, ≤5) indices on overall survival (OS) was investigated using the Cox proportional hazard

model. An additive frailty score was calculated (range 0-5) and three groups of patients were identified: fit (score=0, 39%); unfit (score=1, 31%), and frail (score ≥2, 30%). Progression-free survival (HR=1.68, CI 1.31-2.15, p<0.001) and OS (HR=3.57, CI 2.37-5.39, p<0.001) were significantly shorter for frail patients compared with fit patients. In a Cox's model including ISS, FISH risk group and type of therapy (lenalidomide *versus* no lenalidomide), frail patients showed a higher risk of disease progression (HR 1.48, CI 1.15-1.92, p=0.003) and death (HR 2.88, CI 1.88-4.40, p<0.001). The risks of grade 3 or higher non-hematologic adverse events (HR 1.57, CI 1.12-2.19, p=0.008) and drug discontinuation (HR 2.21, CI 1.57-3.09, p<0.001) were higher for frail patients. The cumulative incidence of non-hematologic grade 3 or higher adverse events was higher for frail patients, and 4-month rate was 7.4% in fit and 19.2% in frail patients. The combination of the frailty score with ISS staging significantly improved risk assessment (Figure 1).

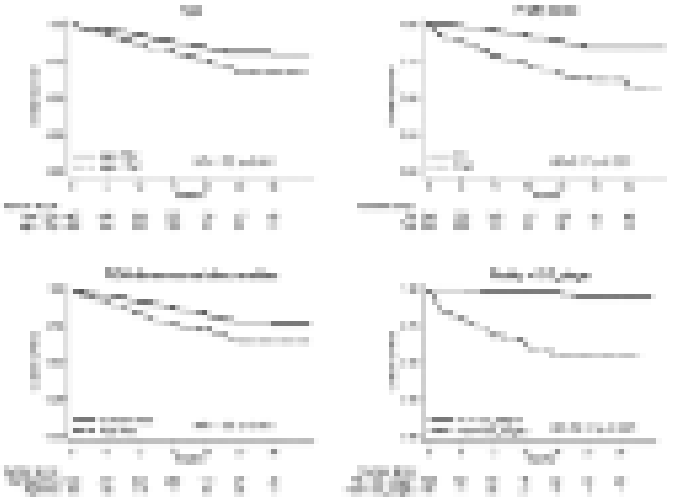

Figure 1.

Summary and Conclusions: This simple score system based on age and geriatric assessment is able to identify frail patients at higher risk of toxicities and with a poor outcome. This approach should be used in the clinical practice to improve patient outcome and quality of life, reducing adverse events and health-care related costs.

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ORAL INVESTIGATIONAL PROTEASOME INHIBITOR IXAZOMIB CITRATE (MLN9708) PLUS LENALIDOMIDE-DEXAMETHASONE IN ELDERLY PATIENTS (PTS) WITH PREVIOUSLY UNTREATED MULTIPLE MYELOMA

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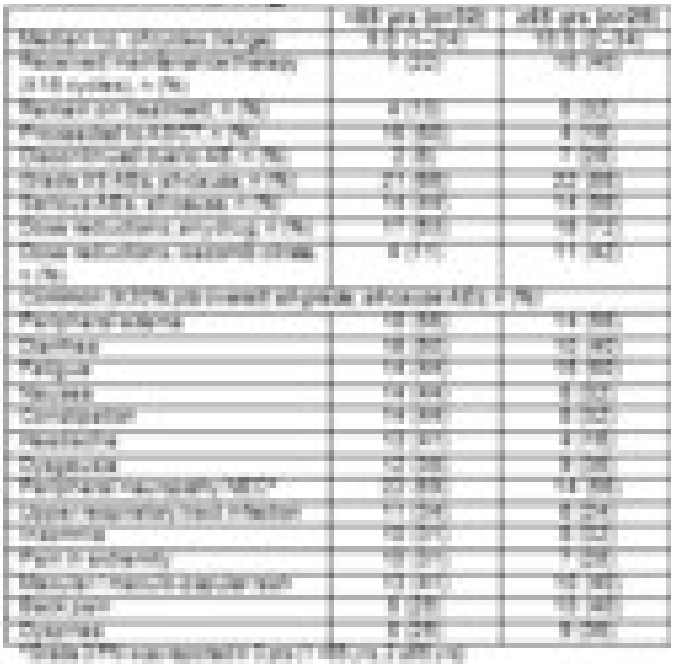
Background: Safe and effective novel long-term combination regimens are required for elderly multiple myeloma (MM) pts ineligible for autologous stem cell transplant (ASCT). Ixazomib citrate is the first oral proteasome inhibitor with reported clinical activity in MM. This phase 1/2 study investigated twice-weekly oral ixazomib citrate plus lenalidomide-dexamethasone in ASCT-eligible and -ineligible pts with previously untreated MM (NCT01383928). This subanalysis evaluates efficacy and safety in elderly *vs* younger pts.

Aims: Phase 1 objectives included evaluation of safety, tolerability, maximum tolerated dose (MTD), and recommended phase 2 dose (RP2D). Phase 2 objectives included assessment of CR+VGPR rate and overall response rate (ORR). This subanalysis evaluated efficacy and safety in elderly (≥65 yrs) *vs* younger (<65 yrs) pts, and includes data up to Jan 9, 2014 (median treatment duration, 6.9 months).

Methods: Pts received oral ixazomib citrate on days 1, 4, 8, and 11, lenalidomide 25 mg on days 1-14, and dexamethasone 20 mg on days 1, 2, 4, 5, 8, 9, 11, and 12 (reduced to 10 mg in cycles 9–16) in 21-day cycles. After 16 cycles, pts received only ixazomib citrate, on the same dose and twice-weekly schedule (maintenance therapy) until progression or unacceptable toxicity. Transplant-eligible pts could undergo stem cell collection after 3 cycles and ASCT after 6 cycles. In the phase 1 study, two fixed doses of ixazomib citrate were investigated: 3.0 and 3.7 mg. No AEs met the DLT criteria in cycle 1 at either dose of ixazomib citrate. The RP2D was selected as 3.0 mg.

Results: 64 pts (14 phase 1, 50 phase 2) were analyzed, including 38 aged <65 yrs and 26 aged ≥65 yrs (of whom six were aged ≥75 yrs); 32 and 25 pts, respectively, were treated at the RP2D. Disease characteristics were similar between the two age groups, though there was a trend for a higher ISS stage in older patients (39% and 58% stage II/III MM in pts aged <65 and ≥65 yrs, respectively). The CR+VGPR rate in pts aged <65 and ≥65 yrs was 67% and 65%, respectively (including 33% and 23% CR, and 33% and 42% VGPR), and ORR was 92% and 96%, respectively. At the time of data cut-off, 8 patients, all <65 yrs, had disease progression. Data on treatment exposure, disposition, and common AEs at the RP2D are shown in the Table 1. The types of treatment-emergent AEs were similar between the age groups, although the frequency could be different; for example, the incidences of diarrhea, nausea, and PN were higher in the <65 yrs group, whereas the incidence of fatigue was higher in the ≥65 yrs group, and the incidence of rash was similar in the two groups. Patients aged ≥65 yrs experienced more Grade ≥3 and serious AEs and required more dose reductions, but also stayed on the study drug longer. There was one on-study death at cycle 4, due to cardiorespiratory arrest (likely PE) in a pt aged 82 yrs (considered related to lenalidomide by the investigator). There was no evidence of cumulative toxicities.

Table 1. Treatment exposure, disposition, and common AEs at the RP2 ofixazomib citrate (3.0 mg).



Summary and Conclusions: These data suggest that the oral triplet combination of ixazomib citrate plus lenalidomide-dexamethasone is associated with encouraging antitumor activity across the age groups analyzed, with CR+VGPR rates of 65-67% and CR rates of 23-33%. Reversible and manageable toxicities, with limited grade 3 PN, were seen regardless of age. The data support further studies using weekly dosing, including ongoing phase 3 trials.

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DOSE-DEPENDENT EFFICACY OF DARATUMUMAB (DARA) AS MONOTHERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RR MM)

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Background: Pts with RR MM received DARA for 9 wks in doses of 0.005-24mg/kg in the GEN501 dose-escalation part[1].

Aims: The purpose of the GEN501 expansion part was to evaluate safety and efficacy of 2 doses of DARA for up to 24 mths using alternate dose schedules.
Methods: Pts ≥18 yrs, RR to at least 2 prior lines of therapy, incl. IMiDs and proteasome inhibitors, and ineligible for salvage ASCT were enrolled at 2 dose levels: A) 8mg/kg +/- pre-dose (10mg) wkly for the first 8 infusions; B) 16mg/kg without pre-dose with a 3-wk washout period between the first 2 doses followed by 7 wkly doses. All pts were then dosed every 2nd wk for 16 wks followed by dosing every 4th wk until disease progression, toxicity or for max 24 mths.
Results: Data from 30 pts in the 8mg/kg cohort and 15 pts in the 16mg/kg cohort recruited into the GEN501 expansion part are presented. Median age was 58.2 (35.1-76.9) and 64.1 (50.5-75.0) years, prior treatment lines were 5 (3-11), and 4 (2-8) and time since diagnosis was 5.5 (2.1-15.2) and 7.1 (0.4-13.3) years, respectively. Median number of DARA infusions was 10.5 *vs* 7.0, reflecting the more recent initiation of the 16mg/kg cohort. Mean infusion times were 3.5 *vs* 3.4 hours in the 8 and 16mg/kg groups, respectively (Table 1).

Table 1. Response.

	PD	SD	MR	PR	VGPR	ORR[A]
8 mg/kg n=30	9	14	5	2	0	7%
16 mg/kg n=13[B]	3	3	1	3	3	46%

[A]PR or better.
[B]2 pts had 1st dose at data cut-off.

Safety. No dose-related increase in adverse events (AEs) was observed. Most common AEs reported (in ≥20% of all pts) were pyrexia, allergic rhinitis, fatigue, upper respiratory tract infection, diarrhea, dyspnea and cough. Only mild (Gr 1 & 2) infusion-related reactions (IRRs) were reported with 20% in the 8mg/kg group *vs.* 27% in the 16mg/kg group. Six patients had thrombocytopenia; one was considered related to DARA. One SAE of lymphocytopenia observed was considered related to DARA. One pt was withdrawn after first full dose due to decreased platelet count Gr 4. This patient received the first full infusion when the platelets were Gr 3. Omission of the pre-dose increased neither the incidence nor the severity of IRRs.
Summary and Conclusions: DARA monotherapy in RR MM pts resulted in high single agent activity when administered at 16 mg/kg (46% ORR). Toxicities proved manageable, with an overall favorable safety profile. Full response data will be presented at the meeting including bone marrow assessments.

Reference
1. Lokhorst: EHA 2013 abstract S576.

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EFFICACY AND SAFETY OF THREE SUBCUTANEOUS BORTEZOMIB COMBINATIONS IN ELDERLY, NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma is primarily a disease of the elderly. Frail elderly patients are more susceptible to adverse events (AEs) and drug discontinuation, thus suggesting the need for an adapted-reduced dose strategy. Weekly and subcutaneous (sc) bortezomib administration was associated with a significant reduction in AEs, particularly peripheral neuropathy (PN), without affecting efficacy. (Moreau P, *et al. Lancet Oncol*. 2011;12(5):431-40)
Aims: To assess efficacy and safety of 3 reduced-dose intensity sc bortezomib-based treatments in elderly, newly diagnosed multiple myeloma (NDMM) patients.
Methods: Patients with NDMM ≥75 years, or younger with comorbidities, were enrolled. Induction phase consisted of nine 28-day cycles with sc bortezomib 1.3 mg/m^2 on days 1, 8, 15, 22, plus oral prednisone 50 mg every other day (VP) or VP plus oral cyclophosphamide 50 mg every other day (VCP) or oral melphalan 2 mg every other day (VMP), followed by maintenance with sc bortezomib every 2 weeks until progression. A geriatric assessment was performed at baseline and included Charlson comorbidity index, Activity of Daily Living score (ADL) and Instrumental Activity of Daily Living score (IADL).
Results: Overall, 152 patients were enrolled: 51 patients in the VP, 51 in the VCP and 50 in the VMP groups. Median age was 78 years and 30% of patients were older than 80 years. By combining the geriatric assessment with age, patients were classified as fit (18%), unfit (28%) or frail (54%). International Staging System III and High-Risk Interphase Fluorescence *In Situ* Hybridization profile [presence of del17 and/or t(4;14) and/or t(14,16)] were well balanced in the three groups. The overall response rate (ORR) was 67% in the VP, 67% in the VCP, and 80% in the VMP groups. After a median follow-up of 21 months, the median PFS was 15, 15 and 22 months (p=not significant) and 2-year OS estimates were 62%, 76% and 81% (p=not significant) in the VP, VCP and VMP group, respectively. Grade ≥3 hematological AEs occurred in less than 10% in all the three groups during induction. Grade ≥3 non-hematologic AEs were mainly infections (12%) and cardiovascular events (8%), and they were particularly higher in patients receiving melphalan (22% of infections and 14% of cardiac events). Grade ≥3 peripheral neuropathy (PN) was 6% in all groups. From start of maintenance, the median follow-up was 9 months and the median PFS was 6 months, no significant differences between the cohorts were noticed. Maintenance was well tolerated and grade ≥3 AEs were less than 4%. The median PFS 14 months in patients <75 years, 15 months in patients 75-80 years and 16 months in patients >80 years (p=not significant) and the respective 2-year OS was 67%, 76%, 74%, respectively; p=not significant). A slight increase in discontinuation rate for toxicities or death associated with age was detected (16%, 20%, 25%, respectively). The 2-year OS was 88% in fit, 79% in unfit and 64% in frail patients (p=0.019 for frail *vs* fit), the respective grade ≥3 AEs were 30%, 39% and 44%. Discontinuation rate due to toxicity or death was 11% in fit, 21% in unfit and 26% in frail patients.
Summary and Conclusions: The combinations VP, VCP and VMP with subcutaneous bortezomib showed similar efficacy in elderly NDMM patients, whereas a worse safety profile was observed in patients receiving 3 drugs, particularly when melphalan was used. Geriatric assessment is essential to identify at baseline fit, unfit and frail patients and should be routinely performed.

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RICOLINOSTAT (ACY-1215), THE FIRST SELECTIVE HISTONE DEACETYLASE 6 INHIBITOR, IS ACTIVE AND WELL TOLDERATED IN COMBINATION WITH LENALIDOMIDE OR BORTEZOMIB IN PATIENTS WITH REFRACTORY MYELOMA

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Background: Ricolinostat, an oral selective HDAC6 inhibitor, is well tolerated in the clinic as monotherapy and demonstrates potent synergistic activity with bortezomib (BORT) (Raje Blood, V20(210):4061) and with lenalidomide (LEN) in preclinical models. Ricolinostat is being developed as a combination agent in relapsed or refractory (R/R) multiple myeloma (MM).
Methods: Two parallel open label single arm phase I/II trials study ricolinostat with BORT (ACY-100), and LEN (ACE-MM-101) with dose escalation in a 3+3 design in R/R MM patients (pts). In ACY-100, R/R pts who received at least two lines of therapy (tx) including a proteasome inhibitor and an immunomodulatory agent, ineligible for or refused autologous stem cell transplant (ASCT), had adequate marrow reserve and hepatic function with creatinine clearance (CrCl)>30mg/mL, were eligible for the phase 1b study. BORT 1.0-1.3 mg/m^2 on days 1, 4, 8 and 11 and Dex 80 mg/week were combined with escalating doses of ricolinostat up to 160 mg bid days 1-5, 8-12 on a 21 day schedule. ACE-MM-101 included R/R pts who received at least one prior tx and have CrCl>50 mg/mL and other entry criteria as above. LEN 25 mg is given daily on 21 days of a 28 day schedule, with ricolinostat on a schedule of up to 160 mg bid days 1-5, 8-12 and 15-19 or continuous 21 day dosing if tolerated. Peripheral blood samples were obtained for PK and PD assessment of acetylated tubulin and acetylated histones in both trials.
Results: As of Jan 31, 2014 25 heavily pretreated pts enrolled in ACY-100. 72% were refractory to the most recent tx. Treatment emergent AEs were mostly grade 1-2 and not attributed to study drug. AEs ≥grade 3 were hematologic abnormalities (8), elevated amylase (2), other asymptomatic laboratory abnormalities (3), and one pt with stomach cramps, diarrhea, and fatigue. One pt with cardiac history had a fatal pulmonary embolism after 3 cycles of tx. 17/25 pts were evaluable for response; the overall response rate was 47% in evaluable pts, or 33% of the ITT population. Eleven pts refractory to BORT were evaluable for response. One pt had CR, one had PR and 9 had SD with median time on study 4 (2-18) cycles. 21 pts have been enrolled in the ACE-MM-101 trial and completed a median of 6 (1-17) cycles. 9 pts received ≥3 prior txs, and 8 pts were refractory to the most recent tx. Treatment emergent AEs were mostly low grade and not considered related to ricolinostat and included diarrhea, fatigue, headache, muscle spasms, neutropenia, rash and upper respiratory infection. Two grade 3 neutropenia events were considered possibly related to ricolinostat. All pts had ≥SD as the best response. 66.6% had ≥PR, including 1 CR, 4 VGPR, 9 PR, 3 MR, seventeen pts had prior LEN tx. Seven patients were refractory to either full dose or maintenance LEN. One pt had VGPR, one PR, one MR and four SD. PK and PD: There were no drug-drug interactions with ricolinostat and LEN or BORT. PK was dose proportional up to 160 mg with

C_{max} 1-2 μM. PD showed increased acetylated tubulin, a marker of HDAC6 inhibition, in all pts ≥160 mg and showed increased acetylated histone, a Class 1 HDAC marker, at 240 mg.
Summary and Conclusions: Ricolinostat (qd and bid) can be safely combined with full doses of BORT or LEN. Durable responses have been observed in pts previously refractory to the combination partner and phase 2 studies are planned.

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A PHASE 2 STUDY OF CIRCULARLY PERMUTED TRAIL (CPT) PLUS THALIDOMIDE AND DEXAMETHASONE *VERSUS* THALIDOMIDE AND DEXAMETHASON IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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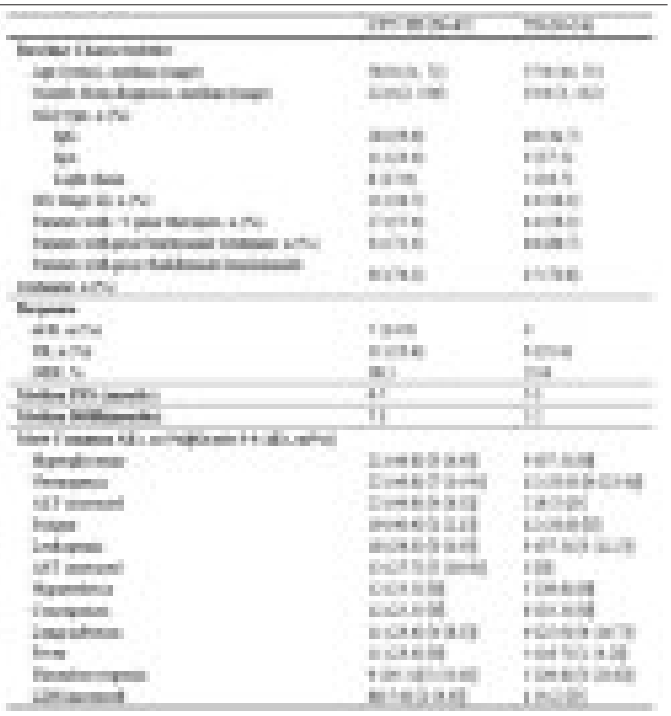
Background: CPT is a recombinant mutant of human Apo2L/TRAIL developed by Beijing Sunbio Biotech Co. Ltd. as a targeted therapy for multiple myeloma (MM) and other hematologic malignancies. CPT is a dual pro-apoptotic receptor agonist that directly activates both pro-apoptotic receptors TRAIL-R1 (DR4) and TRAIL-R2 (DR5). CPT selectively induces apoptosis in a variety of cancer cells, while sparing most normal cells in preclinical models. CPT as a mono-therapy has shown definitive activities for patients with relapsed or refractory multiple myeloma (RRMM) in phase 1 and 2 studies.
Aims: This multi-center, open-label phase 2 study was designed to compare the efficacy and safety of CPT plus thalidomide and dexamethason (CPT+TD) *versus* thalidomide and dexamethason (TD) in RRMM patients.
Methods: Eligible RRMM patients were randomized (2:1) to receive CPT+TD or TD. Each cycle was 28-days: CPT IV 10mg/kg on days 1-5, thalidomide PO 150mg daily, and dexamethason PO 40mg on days 1-4. Treatment was continued until having finished 4 cycles or disease progression or unacceptable toxicity. Treatment beyond 4 cycles was permitted at physician's discretion, yet with the treatment cycle adjusted to 6 weeks. The primary endpoint was overall response rate (ORR, defined as ≥partial response [PR]) based on EBMT criteria. Secondary endpoints included progression free survival (PFS), duration of response (DOR) and safety. Safety was assessed according to NCI-CTCAE 3.0. All patients provided written informed consent.
Results: A total of 71 RRMM patients were enrolled, and 47 patients were randomized to CPT+TD group and 24 patients to TD group. Baseline demographics and disease characteristics were well balanced between CPT+TD and TD (Table 1), including the median age (58.0 years *vs.* 57.0 years), the proportion of patients with ≥3 prior anti-myeloma therapies (57.4% *vs.* 58.3%), with prior bortezomib treatment (72.3% *vs.* 66.7%) or with prior thalidomide or lenalidomide (74.5% *vs.* 70.8%), and ISS (Stage III: 44.7% *vs.* 58.3%), etc. With CPT+TD and TD treatments□no complete response (CR) was observed, near CR was 14.9% *vs.* 0, PR was 23.4% *vs.* 25.0%, and ORR was 38.3% *vs.* 25.0% (Table 1). The ORR difference, however, was not significant owing to the small sample size (P =0.08). The median PFS was 6.7 months in CPT+TD *vs.* 3.1 months in TD (P=0.16), and the median DOR in response patients was 7.1 months *vs.* 3.2 months (P=0.49). The addition of CPT to TD treatment significantly improved the ORR and the median PFS in patients with refractory disease (P=0.02, P=0.03, respectively), the median PFS in patients received ≥3 prior therapies (P=0.01), and the median PFS in those with IgG type MM (P=0.01). In both groups, the ORR, the median PFS and the median DOR seemed superior in patients never treated with bortezomib or immunomodulatory drugs (thalidomide or lenalidomide), but no statistical significance obtained possibly due to the small sample of the trial.
The majority of most common adverse events (AEs) were similar for both treatments except that the occurrences of ALT and AST elevation were more frequent in CPT+TD (P=0.001, P=0.003, respectively) (Table 1). Common grade 3-4 AEs for CPT+TD and TD included neutropenia (14.9% *vs.* 25.0%), thrombocytopenia (10.6% *vs.* 20.8%), increased AST (10.6% *vs.* 0), etc. Treatment-related dose reductions and/or interruptions in CPT+TD and TD group were 38.3% and 29.2%, respectively. In CPT+TD group, dose reductions and/or interruptions due to CPT-related AEs occurred in 8.5% (4/47) of patients.

Table 1. Baseline characteristics, efficacy and safety profile of patients treated with CPT+TD or TD.



Summary and Conclusions: This study showed that the combination of CPT with TD regimen was effective and well tolerated for patients with RRMM. The addition of CPT to TD improved the clinical responses and the PFS. Although a CPT-related liver toxicity was observed, most AEs were mild to moderate and controllable. Further confirmatory study is indicated.

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PRELIMINARY SAFETY AND EFFICACY OF TH-302, AN INVESTIGATIONAL HYPOXIA-TARGETED DRUG, AND DEXAMETHASONE (DEX) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RR MM)

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Background: While alkylators, IMiDs and proteasome inhibitors are current standard treatment for patients (pts) with multiple myeloma (MM), the presence of hypoxia in the diseased bone marrow (Colla *et al.*, *Leukemia* 2010) presents a new therapeutic target for MM. TH-302 is a novel 2-nitroimidazole prodrug of the DNA alkylator bromo-isophosphoramide mustard that is selectively activated under hypoxic conditions. Synergistic induction of apoptosis in MM cells by TH-302 and bortezomib was shown in MM models *in vivo* and *in vitro* (Hu *et al.*, *Mol Cancer Ther* 2013). This current Phase 1/2 study investigates TH-302 with dex in pts with relapsed/refractory multiple myeloma (RR MM). In the dose-escalation stage of the study, the maximum tolerated dose (MTD) of biweekly TH-302 was established at 340 mg/m^2 and preliminary activity has been reported based on modified IMWG criteria (Ghobrial *et al.*, ASH 2013). The 340 mg/m^2 plus dex expansion arm is ongoing.
Aims: Preliminary assessment of safety and efficacy of TH-302 at the MTD (340 mg/m^2) plus dex in pts with RR MM.
Methods: This Phase 1/2 open-label multicenter study is evaluating IV TH-302 (240-480 mg/m^2) plus PO dex (40 mg) on Days 1, 4, 8 and 11 of a 21-day cycle

in heavily pretreated RR MM pts. At the MTD, a Simon two-stage mini-max design was implemented to pursue a regimen with ≥25% response rate or discontinue if ≤5% (90% power, 10% alpha).
Results: Sixteen pts (11 male, 5 female) were enrolled through completion of the initial stage of the Simon design, including 9 at the MTD. Median number of lines of prior therapy were 6 (3-11) and median age was 60 years (53 – 86). All pts had previously received both bortezomib and lenalidomide/thalidomide containing regimens and an alkylating agent. The most common ≥Gr 3 AEs included thrombocytopenia (44%) and leukopenia (38%). Dose limiting Gr 3 stomatitis was reported in the 480 mg/m^2 cohort but not seen at lower doses. Seven pts had SAEs, 6 of which were related to TH-302, including 3 pts with pneumonia. Pre-specified target for response for the initial 9-pt Simon stage at the MTD was achieved with 1 PR, 2 MRs, 4 SDs, 1 PD and 1 NE (ORR 33%). To date, 15 of 24 pts have been enrolled to evaluate safety and efficacy of TH-302 at the MTD (340 mg/m^2) plus dex.
Summary and Conclusions: TH-302 can be administered at 340 mg/m^2, biweekly with dex. Encouraging preliminary clinical activity has been noted in pts with heavily pre-treated RR MM. Data from pts in the Simon two-stage treated at the MTD will be updated and presented at the meeting.

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POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM) WITH DELETION DEL(17P) AND/OR TRANSLOCATION T(4;14).

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Background: Multiple myeloma with del(17p) and/or t(4;14) is characterized with short survival related to early relapse rates (median TTP <4 months) and rapid development of resistance mechanisms to multiple agents. IMiDs® imunomodulatory agents and proteasome inhibitors (bortezomib) also display a shortened median survival of approximately 9 months in RRMM. We and others have shown that pomalidomide plus low-dose dexamethasone has prolonged time to progression (TTP) in RRMM who have progressed after multiple treatment options. However, the median TTP appeared shorter <4 months for patients with del17p and/or t(4;14).
Aims: We have designed a phase 2 multicenter, open-label study aimed to determine the efficacy and safety profile of pomalidomide in RRMM patients with del(17p) and/or t(4;14).
Methods: This study enrolled patients with progressive RRMM that were relapsing to lenalidomide (minimum two cycles). Del(17p) and/or t(4;14) was identified centrally using FISH on bone marrow plasma cells. The response was evaluated centrally according to IMWG criteria. The primary objective was to evaluate TTP. Pomalidomide was given orally at 4 mg daily on days 1–21 of each 28-days and dexamethasone orally at 40 mg daily on days 1, 8, 15 and 22 of each cycle. Venous thrombotic event (VTE) prophylaxis was mandatory. The primary analysis was conducted on the ITT population. An interim analysis is reported.
Results: 50 patients (gender ratio 1.5) were enrolled, the median age was 59 yrs (range, 30-80). The median time from diagnosis to enrolment was 3 years (IQ 2-4), 40% had ISS 3, and 60% high β2m. All patients had loss of 17p (46%) and/or t(4;14) (64%). The median number of prior lines of therapy was 3 (1-10). 84% were refractory to lenalidomide, 54% to proteasome inhibitor, 36% were refractory to an alkylating agent, and 76% were refractory to the last line of therapy prior to study entry. The overall response rate (ORR) was 20%, 27% in del(17p) and 16% in t(4;14));54% had stable disease. The median duration of response was not reached. With a median follow-up of 5 months (IQ 3-11), 66% have stopped treatment including 76% due to progression of MM, and 38% had died. The median TTP (range) for the cohort as a whole is approaching 3 months (2-5), with a clear difference in favour for del(17p), 8 months (3;nr) *versus* 3 months (2;4) for t(4;14). The median OS for the cohort as a whole is 12 months (CI95% 5;nr), with a 8-months event-free survival rate of 59%. Interestingly, del(17p) patients benefited more from pomalidomide plus low-dose dexamethasone as median OS was not reached, with 63% survival at 8-month, while for t(4;14) patients median OS was 9 months (4.5;16). However, it appears that t(4;14) patients were able to be rescued in some extent by investigators following pomalidomide therapy. Toxicity was manageable in these fragile RRMM patients with 40% of serious adverse events (SAEs) reported related to the studied treatment.
Summary and Conclusions: Pomalidomide plus low dose dexamethasone is active and well tolerated in this RRMM population characterized with high and rapid development of a refractoriness state. This study provides further evidence that pomalidomide is active in patients with adverse FISH cytogenetic and that ongoing triplet-based combination should demonstrate improved response rates and survival in future studies.

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PERSISTENT PFS BENEFIT AND SUPERIOR PFS2 WITH VTD *VS* TD FOR NEWLY DIAGNOSED, TRANSPLANT ELIGIBLE, MULTIPLE MYELOMA (MM) PATIENTS: UPDATED ANALYSIS OF GIMEMA MMY-3006 STUDY

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Background: The phase 3 GIMEMA-MMY-3006 study comparing bortezomib-thalidomide-dexamethasone (VTD) *vs.* thalidomide-dexamethasone (TD) as induction therapy before, and consolidation after, double autologous stem-cell transplantation (ASCT) provided demonstration of superior CR/nCR rates after induction (the primary study endpoint) and across all subsequent treatment phases, a gain which translated into significantly longer PFS for patients randomized to the VTD arm (Cavo, Lancet 2010; Blood 2012).
Aims: We herein report an updated analysis of the study with a focus on long term outcomes, including PFS2, time to second anti-myeloma therapy, treatment-free interval and post-relapse OS.
Methods: Overall, 474 patients were included in the trial. After a median follow up of 65 months, 251 patients (53%) have progressed and of these 221 (88%) had available data on salvage therapy after relapse.
Results: On an intention-to-treat basis, median PFS was 57 months for patients randomized to the VTD arm as compared to 42 months for those assigned in the TD arm (HR 0.67; p=0.001). Overall, randomization to VTD was associated with a 34% reduction in the risk of relapse or progression (median TTP, 61 *vs* 45 months for patients in the TD arm; p=0.001). No statistically significant difference in 5-years estimates of OS was observed between VTD and TD groups (80% *vs* 73%). PFS2, defined as the time from initial randomization to second objective disease progression or death from any cause, was significantly longer for patients randomised to VTD than for those in the TD group (76% *vs.* 63% at 5 years, respectively; HR 0.64, p=0.009). Globally, 73% and 83% of patients in the VTD and TD arms required the start of salvage therapy due to symptomatic relapse. Median time to subsequent anti-myeloma therapy (defined as the interval between start of induction treatment and the first dose of second-line therapy) was significantly longer for patients assigned to VTD than for those who experienced relapse in the TD cohort (40 *vs* 31 months, p=0.014). Similarly, median treatment-free interval (defined as the time between last administration of front-line therapy and start of salvage treatment) was 26 months in VTD group *vs* 16 months in TD arm (p=0.016). Most of the patients received a novel agent-containing salvage therapy, while 18% was treated with conventional chemotherapy. As expected, a greater percentage of patients in the TD arm were treated with second-line bortezomib-based combinations in comparison with those relapsing in the VTD arm (72% *vs* 46%, respectively, p=0.001). Within the VTD group, no difference in PFS2 was seen regarding the use of bortezomib or an IMiD as (part of) second-line therapy (64 *vs* 62 months, respectively). Clinical benefit from primary randomization to VTD *vs* TD was also observed in terms of second PFS (defined as the interval between first and second progression) (HR 0.61, p=0.032). The median OS after relapse was 36 months for both TD and VTD groups. No differences in post relapse OS were observed for patients primarily assigned to VTD or TD who received a subsequent bortezomib-based salvage therapy.
Summary and Conclusions: With an extended follow-up of approximately 5 years, VTD was superior to TD in terms of extended PFS, TTP, time to subsequent anti-MM therapy and treatment-free interval. PFS2, which has been recently proposed as a surrogate for OS, was significantly longer for patients randomized to VTD, with no difference regardless of the use of bortezomib or an IMiD as part of second-line therapy. This finding, along with similar post-relapse OS values across the two groups, suggest that short-term exposure to VTD did not select the emergence of more resistant, or bortezomib- resistant, clones at the time of relapse.

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HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN PATIENTS (PTS) WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): THE FIRST TRIAL

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Background: In the pivotal phase 3 FIRST trial, transplant-ineligible NDMM pts treated continuously with lenalidomide and low-dose dexamethasone (Rd) showed improved progression-free survival (PFS) and overall survival (OS) compared with either Rd treatment (Tx) given for a fixed duration (Rd18) or melphalan-prednisone-thalidomide (MPT) (Dimopoulos, Blood 2013). The assessment of QOL is of particular importance in elderly pts.
Aims: Evaluate changes in HRQOL during Tx with Rd, Rd18, and MPT in NDMM pts from the FIRST trial.
Methods: Transplant-ineligible NDMM pts were randomized to continuous Rd until disease progression (n=535), Rd for 18 cycles (Rd18; 72 weeks [wks]; n=541), or MPT (12 cycles; 72 wks; n=547). HRQOL was assessed using three validated questionnaires, the EORTC QLQ-C30, QLQ-MY20, and EQ-5D which were completed at: baseline (beginning Cycle 1); at the end of Cycle 1; after 3, 6, 12, and 18 months (mos) of Tx; and at study discontinuation. Cross-sectional and longitudinal analyses were performed. Changes from baseline at each time point were estimated with a focus on 7 pre-selected clinically-relevant domains: 1) Global QOL, 2) Physical Functioning, 3) Pain, 4) Fatigue, 5) Disease Symptoms, 6) Side Effects of Treatment, and 7) Health Utility (EQ-5D). A sub-analysis based on age ≤75 *versus* (*vs.*) >75 of QOL was performed.
Results: Across Tx arms, questionnaire compliance was high (≥84%) at the end of Cycle 1 and after 3 and 6 mos. Compliance rates were lower in the MPT arm than both the Rd arms at 12 mos (81% *vs.* 91%; $P \leq 0.002$) and 18 mos (67% *vs.* 89%; $P \leq 0.002$). In all Tx arms, scores for Global QOL, Physical Functioning, Pain, Fatigue, Disease Symptoms, and Health Utility improved significantly ($P \leq 0.05$) from baseline at most time points. For the Side Effects of Treatment domain, Tx with MPT was associated with a consistent and significant worsening from baseline at all time points. Side Effects of Treatment scores in the MPT arm showed significantly greater deterioration *vs.* the continuous Rd arm at Cycle 1 and after 3 and 12 mos of Tx ($P \leq 0.05$). Disease progression was associated with a worsening in HRQOL scores for all domains, across all 3 Tx groups ($P < 0.001$). The analysis performed, stratifying groups by age, showed that in pts >75, treatment does not impair HRQOL with regards to the domains of interest. In particular this subset analysis demonstrated a trend in pain reduction from baseline for pts >75.
Summary and Conclusions: The improvement in PFS and OS observed with continuous Rd Tx in the FIRST trial were accompanied with benefit observed in clinically relevant HRQOL measurements. Disease progression was associated with worsening HRQOL scores across all Tx groups. Side Effects of Treatment scores were significantly worse in the MPT arm than the continuous Rd arm.

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MM-003, A PHASE 3 STUDY OF POMALIDOMIDE+LOW-DOSE DEXAMETHASONE *VS.* HIGH-DOSE DEXAMETHASONE IN REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: OUTCOMES BY PRIOR THERAPY AND RESPONSE DEPTH

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Background: Once patients (pts) become refractory or intolerant to treatment (Tx) with novel agents, survival is significantly shortened (median overall survival [OS] of only 9 mos) (Kumar SK, *Leukemia.* 2012). Depth of response to Tx has been shown to be a predictor of favorable outcomes (Harousseau J-L. *Haematologica.* 2010). Pomalidomide (POM) has been approved for pts with relapsed/refractory multiple myeloma (RRMM) who have received ≥2 prior Tx, including lenalidomide (LEN) and bortezomib (BORT), and demonstrated disease progression on their last line of Tx. MM-003, a randomized pivotal phase 3 trial, compared the efficacy and safety of POM+low-dose dexamethasone (LoDEX) *vs.* high-dose dexamethasone (HiDEX) in RRMM pts. Trial results demonstrated significantly longer progression-free survival (PFS) and OS for POM+LoDEX, with acceptable tolerability (San Miguel J. *Lancet Oncol.* 2013).
Aims: To analyze the impact of the previous Tx and depth of response on response rate and/or survival from the MM-003 trial.
Methods: Pts must have been refractory to the last prior Tx (progressive disease [PD] during Tx or within 60 days) and must have exhausted LEN and BORT after ≥2 consecutive cycles of each agent (alone or in combination). Pts were randomized 2:1 to receive 28-day (D) cycles of POM 4 mg D1-21/28 days+LoDEX 40 mg (20 mg for pts >75 yrs) weekly or HiDEX 40 mg (20 mg for pts >75 yrs) D1-4, D9-12, and D17-20. Tx continued until PD or unacceptable toxicity. PFS was the primary endpoint; secondary endpoints included OS, overall response rate (ORR; ≥partial response [PR]), time to progression (TTP), and safety. This analysis examined pt outcomes by prior Tx history and depth of response, with a median follow-up of 15.4 mos.
Results: 455 pts were randomized: 302 pts received POM+LoDEX and 153 pts received HiDEX. Pt characteristics were well balanced between arms. Median number of prior Tx was 5 (range, 2-17). Most pts (75%) were double refractory to LEN and BORT. POM+LoDEX significantly prolonged PFS *vs.* HiDEX regardless of number or type of prior Tx (Figure 1A), with favorable OS outcomes as well (Figure 1B). Significant PFS and OS benefits were observed even in pts who were refractory to LEN as the last prior Tx. The ORR was significantly higher for pts receiving POM+LoDEX *vs.* HiDEX (32% *vs.* 11%; $P < .001$), with similar response rates regardless of prior Tx. An exploratory analysis found that TTP for pts treated with POM+LoDEX in this trial was similar to that observed with their last prior line of Tx (4.6 mos *vs.* 4.4 mos; $P = .32$), whereas it was significantly shorter in pts who received HiDEX *vs.* the last prior line of Tx (2.1 mos *vs.* 4.3 mos; $P < .001$). When examining outcomes by depth of response, median PFS was longer for pts with M-protein reductions ≥50% (8.4 mos) or ≥25% (7.4 mos) *vs.* 0% to 25% (2.3 mos). Additionally, median OS was longer for pts with M-protein reductions ≥50% (19.9 mos) or ≥25% (17.2 mos) *vs.* 0% to 25% (7.5 mos).

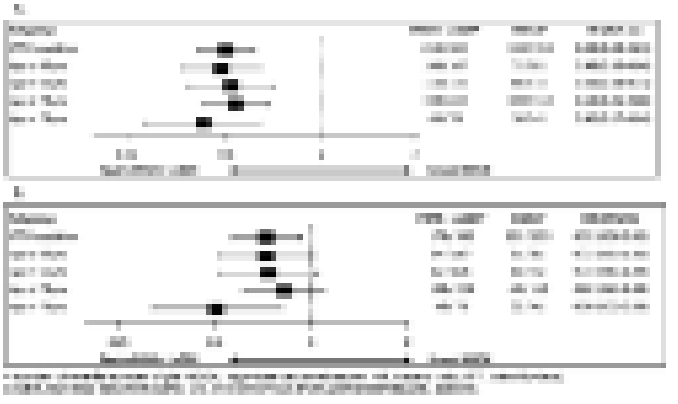

Figure 1.

Summary and Conclusions: In heavily pretreated RRMM pts, POM+LoDEX provided consistent efficacy regardless of number or type of prior Tx. Importantly, LEN as the last prior Tx did not impact response, PFS, or OS *vs.* the overall intent-to-treat population. Pts achieving responses ≥minimal response attained a significant survival benefit, which was higher in pts achieving PR. Taken together, POM+LoDEX should be considered a standard Tx option in RRMM pts, regardless of last prior Tx.

Myeloma and other monoclonal gammopathies - Clinical 2

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ACTIVITY OF MV-NIS IN A PHASE I TRIAL FOR PATIENTS WITH RELAPSED, REFRACTORY MULTIPLE MYELOMA (MM)

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Background: MV-NIS is an Edmonston-lineage measles virus that expresses the human sodium-iodide symporter (hNIS). The virus is oncolytic to primary myeloma cells and cell lines, and its activity can be monitored by noninvasive imaging of radioiodine uptake by hNIS. CD46, the receptor for MV, is overexpressed in myeloma cells.
Aims: To conducted a Phase I study to determine the safety and efficacy of MV-NIS in patients with relapsed, refractory MM.
Methods: The trial is a standard 3+3 dose escalation design but with two patient cohorts. Cohort 1 included single agent intravenous MV-NIS administered at TCID50 titers of: 10^6, 10^7, 10^8, 10^9, 10^{10}, and 10^{11}. Cohort 2 patients started treatment with cyclophosphamide 10 mg/kg 2 days prior to MV-NIS at MTD/100. Antibody response to measles was tested pre and post therapy. Bone marrow myeloma (CD138+) and non-myeloma cells (CD138-) were tested for CD46 expression and MV infectivity. PK and biodistribution were followed, respectively, by MV-N by Q-RT-PCR in blood, urine, and gargle samples and by serial nuclear imaging. Eligible patients had adequate bone marrow and organ reserve and had explored all other myeloma treatment options.
Results: Thirty-one patients have been treated. Two patients were replaced for lack of protocol completion. Cohort I initially enrolled 13 patients receiving one dose 10^6, 10^7 10^8 or 10^9 TCID50 of MV-NIS. Since no DLT was observed, per protocol Cohort 2 began accrual, recruiting 8 patients. At trial conception, manufacturing allowed doses only up to 10^9, but technology improvements subsequently allowed for two higher dose levels (10^{10} and 10^{11}). Therefore, Cohort 2 accrual was suspended, and Cohort 1 accrual was resumed to test these 2 higher levels (11 additional patients). Grade 3-4 AEs deemed at least possibly related to protocol therapy in both cohorts at all dose levels were: neutropenia (n=8); thrombocytopenia (n=4); anemia (n=3); and lymphopenia (n=1). One patient treated in cohort 2, dose level 3 (*e.g.* CTX and TCID50 $9x10^7$) had a grade 3 left ventricular failure possibly related to therapy. In Cohort 1, MTD has not been reached, and TCID50 10^{11}, will be the dose used in an upcoming Phase II trial of single agent MV-NIS. Grade 1-2 AEs seen in at least 5 patients were: nausea (n=11); chills (n=9); fever (n=6); and rash, neutropenia, thrombocytopenia, and vomiting (each n=5). Most patients had low baseline anti-IgG MV titers which boosted by six weeks post MV-NIS treatment. MV-N RNA sequences were amplified from gargle specimens, blood and urine. The most dramatically positive ^{123}I scan was seen in 1 patient at the 10^{11} demonstrating proof of principle of NIS. One patient treated at TCID50 10^{11}, achieved a CR that persists for more than 8 months. Transient drops in serum FLCs were seen in other patients.
Summary and Conclusions: Intravenous administration of MV-NIS is feasible and produced a CR in one patient at top dose level. The virus is capable of replicating before being cleared by the immune system. The Phase II trial will open in a month using the 10^{11}, dose. Oncolytic viruses offer a promising new modality for the targeted infection and destruction of disseminated cancer.
This work was supported by funds from the NIH/NCI (R01CA125614, R01CA168719), Al and Mary Agnes McQuinn, the Richard M Schulze Family Foundation. The NCI (RAID Program) supported cGMP virus manufacture and toxicology/pharmacology studies.

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OUTCOMES OF PRIMARY SYSTEMIC LIGHT CHAIN (AL) AMYLOIDOSIS IN PATIENTS TREATED UPFRONT WITH BORTEZOMIB OR LENALIDOMIDE AND THE IMPORTANCE OF RISK ADAPTED STRATEGIES

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Background: Bortezomib and lenalidomide are increasingly used in patients with AL amyloidosis, but long-term data of their use as first line therapy in AL amyloidosis are lacking. Furthermore, despite the use of these effective therapies early death remains a significant complication of AL and new startegies are neede in order to reduce early mortality
Aims: We report our experience after long follow up of the outcomes of patients who received bortezomib or lenalidomide as a primary therapy for AL amyloidosis. Furthermore, in order to reduce early mortality and retain the rapid activity of bortezomib, three years ago we implemented a risk-adapted approach for bortezomib dose and schedule, based on cardiobiomarkers, age and systolic blood pressure.
Methods: We analyzed the outcomes of 85 consecutive patients, who received primary therapy with bortezomib (N=49) or lenalidomide (N=36)(maximum 12 cycles – no maintenance). Standard bortezomib/dexamethasone was given in 26 patients and risk-adapted bortezomib in 23 patients (of which N=11 received full dose/schedule bortezomib/dexamethasone and N=12 weekly (attenuated) bortezomib/ dexamethasone), based on cardiac biomarkers, systolic blood pressure, presence of neuropathy and age.
Results: On intent to treat, 67% of patients achieved a hematologic response (24% hemCRs and 8% VGPRs) and 34% an organ response; both were more frequent with bortezomib *vs* lenalidomide. Onset of hematologic remission was significantly more rapid with bortezomib. Median survival is 47 months. An early death occurred in 20%: in 36% of patients treated with standard bortezomib, in 22% of lenalidomide-treated patients (p=0.940); but only in 4.5% of patients treated with the risk-adapted bortezomib approach. Activity, in terms of hemPR/hemCR and NTproBNP response, was similar for bortezomib regimens. In patients with a minimal follow up of 3 years, 19% of patients with Mayo stage-3 disease remain alive, the survival of patients with stage-2 disease is 28 months and has not been reached for patients with Mayo stage-1 disease (the 3-year survival is 88%) but is similar for patients treated with bortezomib or lenalidomide.
Summary and Conclusions: long term follow up indicates that remissions obtained with lenalidomide or bortezomib may be durable. There was no difference in the survival of patients treated with bortezomib *vs* lenalidomide, although bortezomib acts faster and induces deeper responses. A risk-adapted treatment strategy based on bortezomib may reduce early mortality and preserve activity but longer follow up is needed in order to evaluate whether this strategy improves other outcomes.

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BORTEZOMIB COMBINATION THERAPY, AND SUBSEQUENT BORTEZOMIB RE-TREATMENT, IS A USEFUL TREATMENT STRATEGY FOR MULTIPLE MYELOMA PATIENTS WITH RAS MUTATIONS

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Background: Mutations of the RAS gene family are prevalent in MM. A synthetic lethality screen in RAS mutant cells identified genes encoding for proteasome subunits, suggesting that MM tumours bearing RAS mutations may be particularly sensitive to bortezomib.
Aims: To investigate if MM disease response to bortezomib is linked to NRAS/KRAS mutational status.
Methods: 70 patients treated at a single centre with bortezomib for relapsed disease were analysed for RAS mutation status to correlate with clinical outcomes.
Results: Patients were mainly relapsed/refractory (92.8%), had received a median of 4 prior lines of therapy and 97% were bortezomib naïve. Pre-treatment bone marrow samples were obtained with informed consent and RAS mutation analysis performed on RNA extracted from CD138 selected cells. RAS mutations were detected in 31 patients (44.3%). 22 patients (31.4%) had mutations in NRAS and 10 patients (14.3%) in KRAS (1 patient had mutations in both). NRAS mutations were found in codons 61 (n=12), 13 (n=5), 12 (n=4) and 64 (n=1). KRAS mutations were detected in codons 61 (n=6), 12 (n=3) and 117 (n=1). There was no association between RAS mutation and high risk FISH.[KY1] All patients were treated with bortezomib regimens: single agent bortezomib (4 patients), bortezomib/dexamethasone (23), bortezomib/dexamethasone/cyclophosphamide (31), bortezomib/dexamethasone/doxorubicin (9) and bortezomib/dexamethasone/thalidomide (3), with an even split between patient groups. 86.7% of patients with RAS mutations responded to treatment compared to 72.2% with WT RAS. No difference in PFS was observed between the two groups (8.0 months WT *vs* 8.0 RAS). OS was shorter in patients with RAS mutations (27.0 months *vs* 40.7, NS). Analysis of NRAS and KRAS mutations separately showed response rates (≥PR) were similar (85.7% NRAS *vs* 88.9% KRAS). Patients with KRAS mutations had a trend to shorter OS (24.8 KRAS *vs* 28.2 NRAS *vs* 40.7 WT). At relapse post initial exposure to bortezomib a similar percentage of patients in each group received further therapy (6/8 KRAS, 16/19 NRAS, 31/33 WT). Median OS from date of relapse was shorter in patients with RAS mutations (11.7 months KRAS *vs* 22.1 NRAS *VS* 33.8 WT, p=0.04 for KRAS *vs* WT). Patients with RAS mutations had similar PFS and OS whether treated with repeat bortezomib or lenalidomide (PFS 8.5 months bortezomib *vs* 7.0 lenalidomide, OS 28.67 *vs* 33.67). In contrast in patients with WT RAS PFS was significantly shorter with bortezomib re-treatment compared to lenalidomide (5.67 months *vs* 10.5, p=0.015), as was OS (25.6 months with bortezomib *vs* 46.6 with lenalidomide, NS).
Summary and Conclusions: Although RAS mutation status had no impact on the outcome of initial bortezomib therapy, at disease progression patients with KRAS mutations fared worse. This may reflect the association of KRAS mutations with advanced disease, but also suggests tumours with mutant KRAS are

relatively more sensitive to bortezomib combination therapy than other subsequent therapy. In contrast to a recent report on the resistance of NRAS mutant tumours to single agent bortezomib, we found no impact of NRAS mutations on sensitivity to combination bortezomib regimens. Patients with WT RAS fared worse when retreated with bortezomib than if they received lenalidomide but those with RAS mutations had similar outcomes with either therapy, suggesting RAS mutation status may confer added bortezomib sensitivity. Although our findings await confirmation in a larger patient cohort they suggest that bortezomib combination therapy, and bortezomib re-treatment, is a useful strategy for MM patients with RAS mutations.

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EUROPEAN POST-APPROVAL SAFETY STUDY (PASS) OF RELAPSE/REFRACTORY MULTIPLE MYELOMA (RRMM): PATTERNS OF ANTITHROMBOTIC PROPHYLAXIS IN A LARGE COHORT OF MM PATIENTS TREATED WITH LENALIDOMIDE

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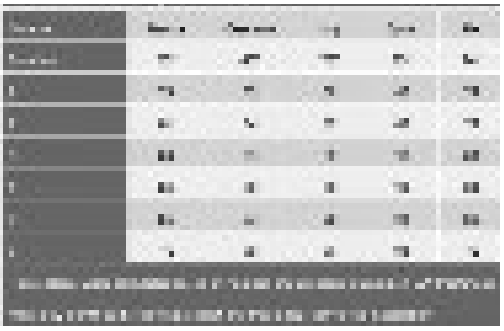
Background: EU PASS is an observational, non-interventional post-authorization study designed to investigate the safety of lenalidomide (LEN) and other agents in the treatment of RRMM patients (pts) in a real-world setting. Venous thromboembolic events (VTE) are common among cancer pts (7%) and recognized as a possible adverse event (AE) associated with the administration of LEN+dexamethasone (DEX) therapy (Tx). Among RRMM pts treated with LEN and high-dose DEX, grade (Gr) 3–4 VTE was experienced by 11-14% of pts (Dimopoulos, NEJM 2007; Weber, NEJM 2007). Recommendations for thromboprophylactic Tx based on the number of VTE risk factors have been published. Larocca (Blood 2012) reported that the incidence of VTE was similar in NDMM pts treated with LEN+low dose DEX, who were randomized to receive either acetylsalicylic acid (ASA) 100 mg/day (2%) or low-molecular-weight-heparin (LMWH) enoxaparin 40 mg/day (1%).
Aims: To investigate the frequency and patterns of thromboprophylactic medication used in LEN-treated pts, and describe the proportion of pts who experienced a VTE while receiving RRMM Tx.
Methods: RRMM pts who had received ≥1 prior Tx were enrolled at the investigator's discretion into a LEN- or background cohort (other regimens). This analysis will focus only on pts who were treated within the LEN cohort. Thromboprophylactic medication was administered as per local standard practice. AEs were graded according to NCI-CTCAE (v3). Patterns of thromboprophylactic usage were examined using descriptive statistics.
Results: As of November 2013, a total of 3,520 pts across 269 institutions in 17 European countries were enrolled; of those, 62% received LEN (n=2,166). Pts in the LEN cohort had a median age of 68 years (range 25–95) and 54% were male. Median number of prior RRMM Tx was 2 (range 0–6); 18% had 1 prior, 57% had 2 prior, and 24% had ≥3 prior Tx; 12% of pts had a history of prior VTE. A total of 71% (n=1527) of pts were receiving thromboprophylactic Tx prior to starting LEN; 81% (n=1558) received Tx on commencing LEN and this number increased to 84% (n=871) for those remaining on LEN by month 6. On commencing LEN, thromboprophylaxis included 42% (n=800) ASA, 31% (n=589) LMWH, 5% (n=90) warfarin, and 8% (n=149) other agents. The proportion of pts receiving prophylaxis at 6 months was 91-93% in Italy and Spain, 75–76% in UK and France, and 60% in Germany (Table 1). A total of 5% (n=117) out of the 2,166 pts in the LEN cohort developed VTE while on Tx; among these 59% (n=69) occurred within the first 6 months. The occurrence of VTE Gr 3-4 was 3% (n=56).

Table 1. Percent of patients receiving any form of thromboprophylaxis in the first 6 months of LEN treatment by country and by month.



Summary and Conclusions: Patterns of thromboprophylactic medication in the big 5 EU countries were comparable and highest in Italy and Spain, intermediate in the UK and France and lowest in Germany. ASA was the most frequently prescribed Tx. In RRMM pts treated with LEN, there was sustained compliance to thromboprophylaxis during the first 6 months. The incidence of VTE during LEN Tx was 5%. Further analyses will examine the incidence by type of prophylaxis used as well as other risk factors.

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RANDOMIZED PHASE 2 STUDY OF BORTEZOMIB, THALIDOMIDE, AND DEXAMETHASONE WITH OR WITHOUT CYCLOPHOSPHAMIDE AS INDUCTION THERAPY IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA (MM): LONG-TERM FOLLOW-UP RESULTS

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Background: Bortezomib in combination with thalidomide and dexamethasone (VTD) is an effective regimen in patients (pts) with previously untreated MM. We conducted an open-label phase 2 study to evaluate the efficacy and safety of VTD and VTD plus cyclophosphamide (VTDC) as induction therapy prior to high-dose therapy plus stem cell transplant (HDT/SCT) (NCT00531453). Primary results showed that both VTD and VTDC are active induction regimens resulting in bone marrow confirmed complete response (CR) rates of 29% and 31% post-induction, and 57% and 61% post-HDT/SCT, respectively (Ludwig *et al. JCO* 2012). There were no significant differences in efficacy between the 2 regimens, indicating no benefit from the addition of cyclophosphamide.
Aims: To assess any differences in long-term outcomes between the 2 regimens, this long-term extension follow-up phase of the study evaluated final time-to-event data: updated post-treatment results are reported.
Methods: Adult pts were randomized (1:1) to receive i.v. bortezomib (1.3 mg/m^2), thalidomide (100 mg), and dexamethasone (40 mg), with or without cyclophosphamide 400 mg/m^2 for 4x21-day cycles, followed by HDT/SCT. This long-term extension study assessed time-to-event endpoints, including time-to-next therapy (TTNT), progression-free survival (PFS) and overall survival (OS).
Results: 98 pts (49 VTD, 49 VTDC) were included in the intent-to-treat and safety populations. At the final analysis, median duration of follow-up was 64.8 months (65.3 months VTD, 64.7 months VTDC). Median TTNT was 51.8 months (95% CI 31.9, NE) and 47.9 months (95% CI 28.7, NE) in the VTD and VTDC treatment groups, respectively. The type of subsequent therapy was similar in both treatment groups; dexamethasone (41.8%), lenalidomide (33.7%), bortezomib (31.6%) and thalidomide (17.3%) were the most commonly used agents. In a sensitivity analysis (10 pts starting subsequent therapy after 'relapse from CR' or 'clinical relapse' but without IMWG criteria-based progression were considered to have progressed), PFS and TTP were similar in the VTD and VTDC groups: median PFS was 34.1 months (95% CI 23.5, NE) and 34.2 months (95% CI 23.5, 48.2), and median TTP was 34.7 months (95% CI 23.9, NE) and 34.5 months (95% CI 23.5, 50.6), respectively. The 3-year and 5-year OS rates were similar for both treatment groups: 79.6% (95% CI 65.4, 88.5) *vs* 83.7% (95% CI 70.0, 91.5) at 3 years, and 69.1% (95% CI 54.1, 80.1) *vs* 65.3% (95% CI 50.1, 76.8) at 5 years for the VTD and VTDC treatment groups, respectively (Figure 1A). Of pts with bone-marrow-confirmed CR available for MRD analysis, 34 were MRD-negative and 8 MRD-positive by multiparameter flow cytometry. When analyzed by MRD status, OS was longer in MRD-negative *vs* MRD-positive pts (hazard ratio [HR] 3.66, p=0.0318) (Figure 1B). Although not significant, a similar trend was seen for PFS (HR 2.29, p=0.0849). During this follow-up period, no new adverse events and no second primary malignancies were reported.
Summary and Conclusions: The VTD induction regimen followed by HDT/SCT provides long-term disease control (median TTNT 51.8 months, 5-year OS 69.1%). Consistent with the primary analysis results, there was no significant difference in long-term outcomes between the VTD and VDTC groups.

Analysis of OS and PFS by MRD status confirms the importance of achieving an immunophenotypic CR after treatment.

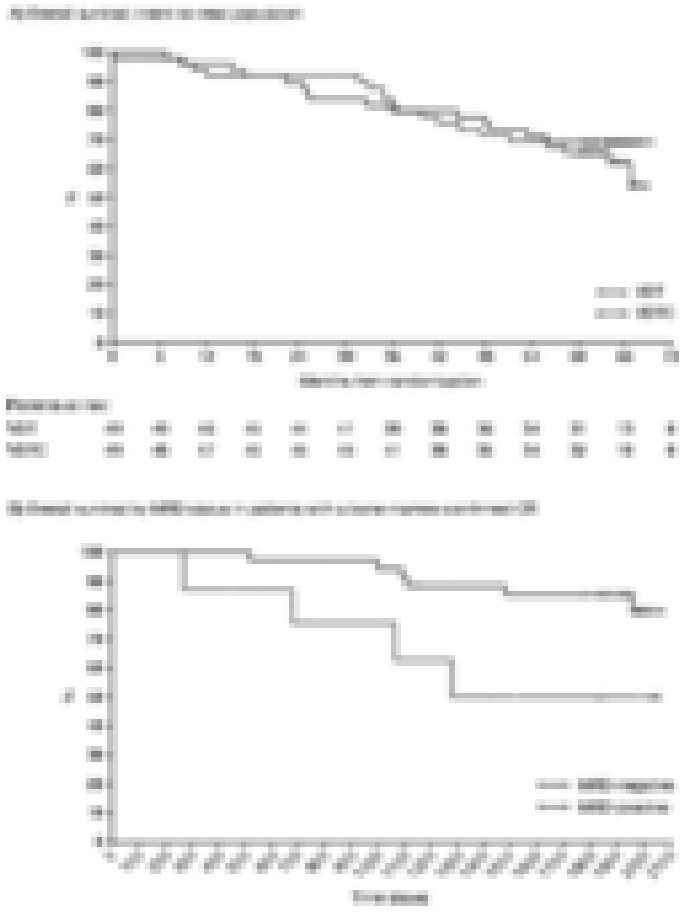

Figure 1.

P370

A PHASE 1 STUDY OF FILANESIB (ARRY-520) WITH BORTEZOMIB (BTZ) AND DEXAMETHASONE (DEX) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Filanesib is a novel KSP inhibitor with encouraging activity in patients (pts) with RRMM. In preclinical models, the activity of filanesib is synergistic with BTZ, providing a rationale to combine these drugs in the clinic.
Aims: ARRAY-520-111 is a Phase 1 study to identify the maximum tolerated dose of filanesib, BTZ and dex.
Methods: Eligible pts have RRMM with ≥2 prior lines of therapy, including a proteasome inhibitor (PI) and an IMiD. Filanesib is administered intravenously (IV) on days (D) 1, 2, 15, 16 (Schedule 1) or on D1, 15 (Schedule 2); BTZ is administered IV or subcutaneously weekly on D1, 8, 15; and 40 mg oral dex, if given, is taken on D 1, 8, 15 in a 28D cycle. G-CSF is given for 5-7D starting D3 or 4 and again D17 or 18. The safety of filanesib+BTZ in the early portion of dose escalation has been reported previously. This abstract will focus on the safety and activity of higher doses of filanesib in this dose escalation study (≥1.25 mg/m^2/D filanesib+1.3 mg/m^2/D BTZ).
Results: In Schedule 1, 19 pts have been treated at filanesib doses of 1.25 and 1.5 mg/m^2/D with 1.3 mg/m^2/D BTZ, without and with dex. Pts received a median of 5 prior therapies and 42% were BTZ refractory. The maximum planned dose in this schedule has been established as 1.5 mg/m^2/D filanesib and 1.3 mg/m^2/D BTZ+40 mg dex. In Schedule 2, 10 pts have been treated at doses of 2.25 and 3.0 mg/m^2/D filanesib with 1.3 mg/m^2/D BTZ+dex. Pts had received a median of 4 prior therapies. The recommended Phase 2 dose (RP2D) in this schedule has been established as 3.0 mg/m^2/D filanesib and 1.3 mg/m^2/D BTZ+40 mg dex. Both schedules have shown acceptable tolerability, with the most common adverse events (AEs) being transient, non-cumulative myelosuppression: based on laboratory data, Grade (Gr) 3-4 neutropenia was observed in 38% of pts and Gr3-4 thrombocytopenia in 27% of pts. A low overall rate of non-hematological toxicity was observed: no Gr3-4 non-hematologic AEs were observed in more than 9% of patients. Among all 29 pts treated at doses of ≥1.25 mg/m^2/D filanesib+1.3 mg/m^2/D BTZ, treatment-emergent neuropathy (Gr2) was observed in only 1 pt. In the 19 pts in Schedule 1 who received ≥1.25 mg/m^2/D filanesib+1.3 mg/m^2/D BTZ, the overall response rate (ORR, ≥PR) was 42% (8/19) and the clinical benefit rate (CBR, ≥MR) was 58%. In the subset of pts with BTZ-sensitive disease, an ORR of 56% (5/9) and a CBR of 89% was observed. In pts with PI-refractory disease, an ORR of 30% (3/10) was observed. The median time on treatment in Schedule 1 has been 12 months (range 1, 14.3+). The observed duration of response to-date ranged from 3.1+ to 11.4+ months. The 10 pts in Schedule 2 had received a median of 1 cycle of treatment at the time of data cutoff and 8 pts remained on study with 2 MR observed to date. High pre-dose levels of alpha 1-acid glycoprotein (AAG) are a potential marker of lack of response to filanesib. To date in PI-refractory pts, responses occurred only in pts with low AAG levels.
Summary and Conclusions: Filanesib combined with weekly BTZ, dex, and prophylactic G-CSF appears well tolerated with manageable AEs in heavily pretreated pts. This combination has shown promising activity both in BTZ-sensitive (ORR=56%; CBR=89%) and in PI-refractory MM (ORR=30%). In addition, a more convenient schedule of filanesib on D1, 15+weekly BTZ, is feasible. Expansion cohorts of 21 pts of both schedules are ongoing in pts with BTZ-sensitive disease who received 1-3 prior therapies. These data will be updated at time of presentation and will include pts in the expansion cohorts.

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A COMPARISON BETWEEN NEXT-GENERATION SEQUENCING AND ASO-QPCR FOR MINIMAL RESIDUAL DISEASE DETECTION IN MULTIPLE MYELOMA: THE CLINICAL VALUE IN ASCT SETTING

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Background: Although molecular complete remission (mCR) in multiple myeloma (MM) can be assessed by allele-specific oligonucleotide (ASO)-PCR, this technique requires preparation of clonotype-specific primers for each individual which is laborious and time-consuming. We utilized a sequencing method, termed the LymphoSIGHT™ platform, which employs consensus primers and high-throughput sequencing to amplify and sequence all rearranged immunoglobulin gene segments present in a myeloma clone.
Aims: We compared the LymphoSIGHT™ method with ASO-qPCR for MRD detection in autografts in the autologous peripheral blood stem cell transplantation (ASCT) setting.
Methods: Forty-four Japanese patients with newly diagnosed MM who received various induction regimens prior to ASCT were retrospectively analyzed. All patients had achieved a partial response (PR) or complete response (CR) after ASCT. BM slides from 29 MM patients and fresh BM cells from 15 MM patients at diagnosis as well as autografts were obtained for DNA extraction. IGH-based ASO-qPCR was performed as described previously (van der Velden *et al.*, Methods Mol Biol 2009). We performed next-generation sequencing (NGS) using the LymphoSIGHT method (Faham *et al.*, Blood 2012). Using universal primer sets, we amplified IGH variable (V), diversity (D), and joining (J) gene segments, IGH-DJ, and IGK from genomic DNA. Amplified products were subjected to deep sequencing using NGS. Reads were analyzed using standardized algorithms for clonotype determination. Myeloma-specific clonotypes were identified for each patient based on their high frequency in BM samples. The presence of the myeloma clonotype was then assessed in follow-up samples.
Results: MRD in autografts could be assessed in 44 of 44 patients (100%) by NGS and 36 of 44 patients (82%) by ASO-qPCR. MRD in autografts was detected in 35 of 44 (80%) by NGS and 16 of 36 (44%) by ASO-qPCR. Two cases where MRD was not detected by NGS (MRD^{NGS}(-)) and 19 MRD^{NGS}(+) cases received post-ASCT therapy using novel agents such as bortezomib/lenalidomide/thalidomide while 7 MRD^{NGS}(-) cases and 16 MRD^{NGS}(+) cases were followed without post-ASCT therapy. The MRD^{NGS}(-) cases without post-ASCT therapy showed a significantly better PFS than those MRD NGS(+) cases without post-ASCT therapy (P=0.025) although overall survival rates were comparable among the three groups (Figure 1A). To investigate the value of sensitive detection by NGS, we compared PFS in 7 MRD^{NGS}(-) cases (Group 1) with the 6 MRD^{NGS}(+) cases where MRD was not detected by ASO-qPCR (MRD^{ASO}(-)) (Group 2). The patients in both groups did not receive any post-ASCT therapy. Group 1 tended to show a better PFS than Group 2 (P=0.091) (Figure 1B). Additional patients and samples are being analyzed and results will be presented.
Summary and Conclusions: Detection of MRD in the post-ASCT setting is a significant predictor of PFS, and MRD-negativity in autografts revealed by NGS

may be more closely associated with durable remission of MM than that revealed by ASO-qPCR.

Figure 1. Comparison between NGS and ASO-qPCR for MRD detection in MM. PFS according to MRD in autograft by NGS (A), and PFS comparison between NGS and ASO-qPCR in cases without post-ASCT Tx.

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VORINOSTAT (V) IN COMBINATION WITH BORTEZOMIB (B), DOXORUBICIN (D) AND DEXAMETHASONE (D) (VBDD) IN PATIENTS WITH REFRACTORY OR RELAPSED MULTIPLE MYELOMA (RRMM): AN INTERIM PHASE I/II ANALYSIS

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Background: The combination of bortezomib, doxorubicin and dexamethasone (BDD) is well tolerated and induces a high overall response rate (ORR). Preclinical studies have demonstrated that vorinostat, a histone deacetylase inhibitor, is synergistic with bortezomib and doxorubicin.
Aims: The aim of this phase I/II study was to determine the tolerability and activity of the combination of BDD with vorinostat (VBDD) in RRMM.
Methods: Patients received escalated vorinostat-doses (provided by MSD) at 100mg (dose level [DL] 0), 200mg (DL +1) and 300mg (DL +2) on days 1-4, 8-11, 15-18, combined with bortezomib 1.3mg/m^2 day 1,8,15 (provided by Janssen-Cilag GmbH), dexamethasone 40mg day 1,8,15,22 and doxorubicin on 9mg/m^2 day 1+8 (Figure 1A). The primary objective was the maximum tolerated dose (MTD; 3+3 dose escalation design). Secondary objectives were safety, response assessed by EBMT and IMWG criteria, progression-free survival and overall survival. ORR (>PR) was assessed every 4 weeks and at discontinuation or end of treatment (EOT). Correlative endpoints include prognostic MM-parameters, organ function, QoL-, comorbidity-assessments and translational studies (*e.g.* HDAC-activity decrease in PB MNCs, global acetylation and gene expression analysis). Dose limiting toxicities (DLTs) were defined as any possibly drug related adverse events (AEs) ≥grade 3 (CTCAE) within the 1st cycle. After completion of 6 cycles, patients could continue with bortezomib maintenance or proceed to (most often 2nd) ASCT.
Results: To date, 26/30 patients have been enrolled (median age: 63 years [range 54-78], 58% males). Median prior therapy lines were substantial with 3 (range 1-8): bortezomib, thalidomide or lenalidomide had been given in 81%, 23% and 31%, respectively; 84% of patients had undergone prior SCTs. Cytogenetic abnormalities included del(17p) (n=4), t(4;14) (n=2), gain(1q) (n=4), t(11;14) (n=4) and hyperdiploidy (n=7). The median Karnofsky Performance Status was 90% (range 70-100%). No DLTs have been observed to date; with 3 patients each being included in DL 0 and DL +1, the following patients safely proceeded to DL +2 (Figure 1B). Nine SAEs occurred in 7/26 patients (27%): bacteraemia (n=1) and herpes zoster reactivation (n=1) were suspected to be related to all VBDD-drugs. No causal relationship to study drugs was suspected for pneumonia (2), syncope (1), pathological fracture (1), respiratory infection (1) and 2 death due to PD with persisting plasma cell leukemia (PCL) or refractory disease. Best response and response at EOT defined by ORR was 60% and 45%, and the clinical benefit rate (CR, PR, SD) 92% and 90%, respectively (Figure 1C). At a median follow-up of 12 months (range 1-28), there have been only 2 patients with PD (1 with RRMM and 1 with PCL). The analysis of HDAC activity (before cycle 1, d1 and under VBDD treatment, cycle 2, d8) after VBDD initiation demonstrated HDAC-downregulation in 7/10 (70%) patients (Figure 1D). Further analyses will determine whether HDAC activity and treatment response may correlate and whether this HDAC downregulation may precede and/or indicate depth of response.
Summary and Conclusions: VBDD is a well tolerated and effective regimen in heavily pretreated RRMM patients. There have been no observed DLT and the MTD of vorinostat was set at 300mg, with all reported SAEs being in line with the known safety profile of the investigated drugs. Our alternative vorinostat-schedule (dosing of 4 days on and 4 days off) induced excellent tolerability and seems to enhance the antimyeloma response, warranting completion of this study.

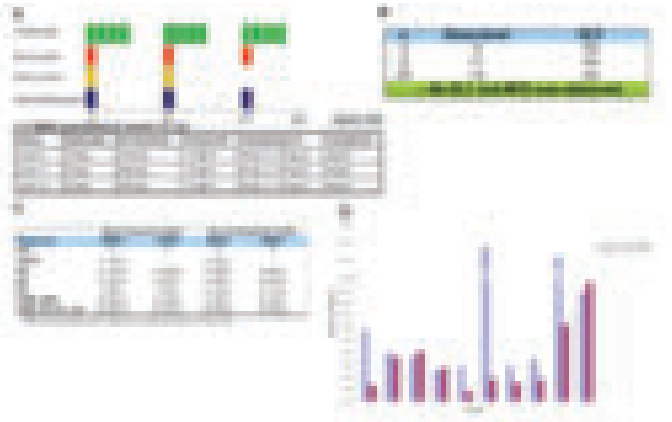

Figure 1. A)VBDD-treatment schedule; B) DLT in dose levels 0,+1 and +2; C) best response (n=25) and EOT (n=20) according to IMWG and EBMT criteria and D) HDAC activity (before cycle 1, d1 and under VBDD treatment, cycle 2, d8) after VBDD initiation demonstrated HDAC-downregulation in 7/10 (70%) patients.

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A PHASE IB DOSE ESCALATION TRIAL OF SAR650984 (ANTI-CD-38 MAB) IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: SAR650984 (SAR) is a humanized IgG1 monoclonal antibody that binds selectively to a unique epitope on human CD38 receptor. SAR kills tumor cells via antibody-dependent cellular-mediated cytotoxicity, complement-dependent cytotoxicity, direct apoptosis induction without secondary crosslinking and allosteric inhibition on CD38 enzymatic activity. We present data on the dose escalation phase of the study (NCT01749969)
Aims: To determine the safety profile of SAR650984 in combination with Lenolidomide and dexamethasone in heavily pretreated patients with refractory and relapsing multiple myeloma
Methods: Three dose levels (DL) of SAR 3, 5 and 10 mg/kg were evaluated in combination with lenalidomide (LEN) and dexamethasone (Dex). LEN 25 mg was given on days (d) 1 – 21 and D 40 mg on d 1, 8, 15 and 22 every 28 d's. SAR was given IV on d 1 and 15 and escalated using the classic 3+3 design. All patients signed an IRB approved informed consent.
Results: 13 patients (pts) with RRMM were treated; median age 61 yrs (48 - 73); median prior treatment regimens 6 (2 - 12), 100% had received prior LEN (23% prior pomalidomide) and 92.3% previously received bortezomib (38.5% prior carfilzomib). The median time from diagnosis to first SAR dosing was 4.5 yrs (3 - 11). The maximum tolerated dose was not reached. The most frequent adverse events included nausea, cough (n=6 each); fatigue, muscle spasm, infection (n=5 each); vomiting, diarrhea, dehydration and insomnia (n=4 each). Grade (G) ≥3 hematologic abnormalities were neutropenia (n=4) and thrombocytopenia (n=3). One pt discontinued therapy (cycle 1, d 1) due to an infusion reaction (bronchospasm G 3). The ORR (≥PR), according to IMWG criteria, among 12 evaluable pts was 58%. Responses occurred at each DL of 3mg/kg (1PR), 5mg/kg (1PR, 1 VGPR) and 10 mg/kg (1PR, 3 VGPR). Clinical benefit response (≥MR) was 67% with 1 MR at 3 mg/kg. Median time on treatment was 20.6 weeks (0 - 35) and 7 pts remain on therapy. PK showed non linearity at select dose levels, SAR plasma trough levels were above target for tumor eradication from preclinical data.
Summary and Conclusions: The combination of SAR with LEN/Dex was tolerated and no DLT's were reported at any DL. SAR+LEN/Dex demonstrated encouraging efficacy in pts with heavily pretreated RRMM. An expansion cohort of 18 pts recently enrolled on the trial and the results will be reported at the meeting.

P374

IDENTIFICATION OF REVERSIBLE RENAL DAMAGE AND EARLY MARKERS OF RESPONSE TO CHEMOTHERAPY IN AL AMYLOIDOSIS: A STUDY ON 732 PATIENTS

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Background: The kidney is involved in 70% of patients with AL amyloidosis, but little is known on factors affecting progression and potential reversibility of renal damage, and the criteria for renal response have never been validated.
Aims: To identify prognostic factors, response and progression criteria for renal involvement in AL amyloidosis.
Methods: We systematically searched the databases of the Pavia and Heidelberg amyloidosis canters for previously untreated patients with renal AL amyloidosis diagnosed between 2004 and 2012. Italian patients (n=461) were used as testing cohort and German patients (n=271) as validation cohort. All the patients gave written informed consent. The study endpoint was time from diagnosis to dialysis initiation (renal survival). Patients who died off-dialysis were censored.
Results: Seventy-one (15%) patients required dialysis in the Italian group and 84 (31%) in the German series after a median time of 85 and 69 months, respectively. Baseline proteinuria >5 g/24 hours and estimated glomerular filtration rate (eGFR) <50 mL/min per 1.73 m^2 were associated with poorer renal survival. Patients with proteinuria below and eGFR above the thresholds were at low-risk of dialysis (0 and 4% at three years in the testing and validation cohorts, respectively), whereas subjects with both high proteinuria and low eGFR were at high-risk (60% at three years in the Italian and 85% in the German series). Patients with either proteinuria above the cutoff or eGFR below the threshold were at intermediate-risk of progression (18% and 31% at three years in the testing and validation cohorts, respectively). The difference in renal survival between the three stages was significant (P<0.001 in both groups). Response and progression were assessed at three and six months. A decrease in eGFR by ≥25% was associated with poor renal survival in both cohorts and was adopted as the criterion for renal progression. A decrease in proteinuria by ≥30% or below 0.5 g/24 hours in the absence of renal progression was the criterion for renal response, being associated with longer renal survival in both series. Hematologic very good partial or complete remission improved renal outcome.
Summary and Conclusions: We identified and validated a staging system for renal involvement and criteria for early assessment of renal response and progression in AL amyloidosis which should be used in routine clinical practice and clinical trial design.

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MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) IS ASSOCIATED WITH A 30% INCREASED RISK OF DYING AT 8 YEARS OF FOLLOW-UP: RESULTS FROM A SCREENED CROSS-SECTIONAL POPULATION-BASED STUDY

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Background: Multiple myeloma (MM) is a hematological malignancy where abnormal plasma cells proliferate and accumulate in the bone marrow. All cases of MM are preceded by the asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS), where an M-protein is present in the blood but there are no other signs of hematologic malignancy. The prevalence of MGUS increases with age, and has been estimated to be 3-4% in a population aged 65 years or above. However, since MGUS by its nature is asymptomatic and likely to remain undiscovered, there is there is limited information on prevalence and survival in MGUS.
Aims: This study aims to determine the prevalence of MGUS in a screened, elderly population, and to evaluate the effect of MGUS on survival.
Methods: The cohort under study was the longitudinal AGES-Reykjavik Study. The participants were men and women born 1907-1934 in Iceland, who were examined up to six times by the Icelandic Heart Association. Blood samples from all participants, collected in 2002-2006, were screened for M-protein using serum protein electrophoresis (SPEP) as well as free light chain (FLC) analysis. Information on outcome was supplemented by matching hospital, nursing home, and mortality records to the cohort. The survival of individuals with MGUS *versus* those without was compared by calculating hazard ratios (HRs) and confidence intervals (CIs), based on a Cox proportional hazard model adjusting for age and gender, and by using Kaplan Meier analysis.
Results: Out of 5,784 participants, 313 participants (5.4%) had 1, 2 or 3 M-protein bands on SPEP. A total of 464 participants (8.0%) had a pathological FLC ratio (reference interval 0.26-1.65), but normal SPEP. Due to missing information, 36 observations were excluded from the analyses, and 2 were excluded because of conflicting results on SPEP and FLC. The median age at inclusion in the cohort was 77 years (range 66-98). Individuals with MGUS and with FLC-MGUS were older (78.5 years) than individuals without MGUS (76.8 years). Among individuals with MGUS, 53.7% were male, compared to 50.2% of individuals with FLC-MGUS and 40.9% of individuals with no MGUS. Of visible M-protein bands on SPEP, IgG was the most common isotype (56.5%), followed by IgM (30.7%), IgA (10.5%), free kappa/lambda only (1.9%) and IgD (0.3%). After a median follow-up time of 8 years, we found that individuals with MGUS had a significantly higher risk of death (HR=1.3, 95% CI 1.1-1.5) than individuals with no MGUS, whereas individuals with FLC-MGUS did not (HR=1.1, 95% CI 0.9-1.2) (Figure 1).

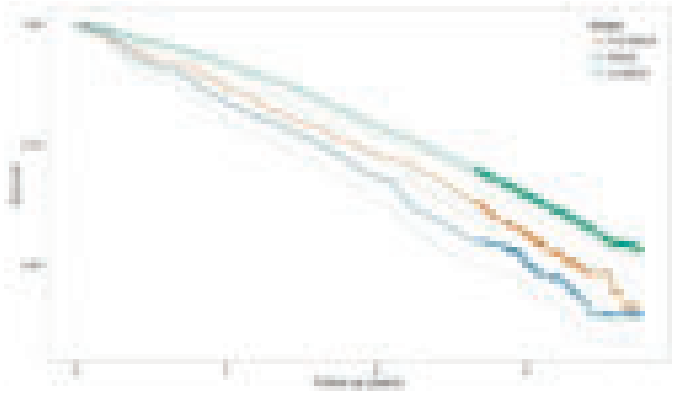

Figure 1.

Summary and Conclusions: In this large, population-based cohort screening study, we found that compared to individuals without MGUS, individuals with MGUS have a 30% increased risk of dying at a median of 8 years of follow-up. This is in contrast to previous, hospital-based studies. Our findings also suggest that MGUS is perhaps even more common than previously assumed. Attention should be directed towards characterizing causes of death as well as finding the underlying reasons for inferior survival in MGUS.

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INTERIM RESULTS FROM A PHASE IIA STUDY OF THE ANTI-CXCL12 SPIEGELMER OLAPTESED PEGOL (NOX-A12) IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Olaptesed pegol (NOX-A12) is a novel L-stereoisomer RNA aptamer (Spiegelmer®) that binds and neutralizes the chemokine CXCL12 with high affinity. CXCL12, through interaction with the chemokine receptors CXCR4 and CXCR7, is responsible for homing and trafficking of healthy and malignant blood cells to the bone marrow (BM) stroma. Preclinical studies have shown synergistic activity of CXCL12-targeting and anti-myeloma agents, specifically bortezomib, suggesting that targeting of the myeloma niche may enhance treatment efficacy.
Aims: Here we aim to assess the activity and safety of olaptesed in combination with bortezomib and dexamethasone in patients with relapsed/refractory multiple myeloma (MM).
Methods: Twenty-nine patients with relapsed or refractory MM were enrolled. At this time, data from 20 patients (10 males and 10 females) are evaluable. Median (range) age was 65 years (48-79). Patients had received 2 (1-6) prior treatment lines. Pre-treatment consisted of dexamethasone in 19, lenalidomide in 15, bortezomib in 10 and carfilzomib in 1 patient. Seven patients underwent stem cell transplantation. Four patients presented with ISS stage I, 8 with stage 2 and 8 with stage III. Twelve patients had IgG, 2 presented with IgA and 6 with light chain myeloma. Thirteen patients had "high-risk" cytogenetic aberrations, defined by the presence of t(4;14) or del17p. Six patients were refractory to their last prior treatment, 10 were progressing during or shortly after maintenance therapy, mostly lenalidomide-dexamethasone. Patients were treated using a dose titration design with intravenous olaptesed at doses increasing from 1

mg/kg to 2 mg/kg and 4 mg/kg at cycles 1, 2 and 3, respectively, at 1 hour prior to bortezomib administration. During cycles 4 to 8, olaptesed was dosed at the highest individually titrated dose. Bortezomib (1.3 mg/m^2) was given on days 1, 4, 8 and 11 as intravenous injection. Oral dexamethasone (20 mg) was added on the day of and the day after bortezomib administration. Response was evaluated based on the uniform response criteria of the IMWG (Rajkumar SV et. al. Blood 2011; 117: 4691-5). To study PK/PD, a pilot dose of 1 to 4 mg/kg olaptesed alone was administered to the initial 10 patients before the start of the regular treatment regimen.

Results: The median number of completed cycles was 7.5 (0-8). The overall response rate was 70% with 6 patients achieving VGPR and 8 PR. Minimal response was noted in 1 patient, and 3 patients had progressive disease. Levels of circulating CD138$^+$/CD38$^+$, plasma cells were studied for 72 hours in the pilot phase and for 24 hours at cycles 1 and 4. A 2-fold increase of plasma cells compared to baseline was noted already after one hour and an increased mobilization persisted throughout the observation period (Figure 1). Treatment with olaptesed in combination with VD was safe and well tolerated without a significant increase in adverse events compared to VD-treated MM patients. The dose of olaptesed was titrated up to 4 mg/kg in all 15 patients treated beyond cycle 3.

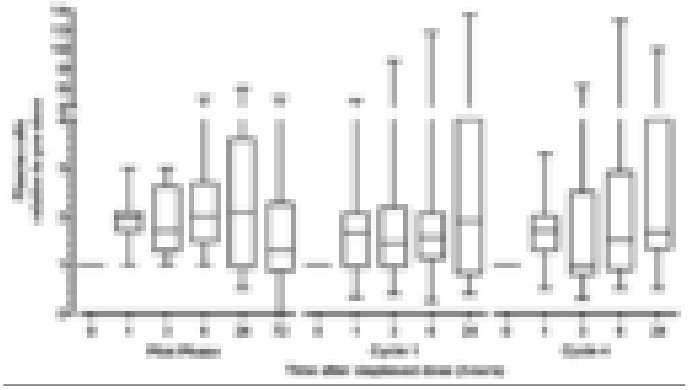

Figure 1.

Summary and Conclusions: The analysis of 20 evaluable patients documented an ORR of 70%, which is comparable to published data of the VD regimen. Considering that the study population included patients retreated with bortezomib and a marked proportion of patients with unfavorable cytogenetics, these results support the further development of olaptesed pegol in MM.

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IDENTIFICATION OF MYELOMA-SPECIFIC CLONOTYPES IN DIAGNOSTIC SAMPLES FROM PATIENTS WITH MULTIPLE MYELOMA USING NEXT-GENERATION SEQUENCING METHOD

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Background: Recent reports support the prognostic importance of minimal residual disease (MRD) levels in multiple myeloma (MM) patients and suggest that novel methods for MRD assessment can play a role in the evolving MM treatment paradigm. The application of next-generation sequencing-based MRD assessment has been previously demonstrated in multiple lymphoid malignancies (Faham *et al.* Blood 2012, Ladetto *et al.* Leukemia 2013). This quantitative method, termed the LymphoSIGHT™ platform, relies on amplification and sequencing of immunoglobulin gene segments using consensus primers, with a demonstrated applicability higher than 90% in MM patients (Vij *et al.*, Clin Lymphoma Myeloma Leukemia 2013).

Aims: This technical study is aimed at assessing optimal sample type and preparation techniques. Previous experience suggested that myeloma cells may be diminished in a PBMC preparation. In this study, we evaluated this hypothesis by comparing the myeloma level in PBMC preparations and samples following RBC lysis. In addition, we evaluated the effectiveness of identifying myeloma clonotypes using DNA obtained from Bone Marrow Aspirate (BMA) slides.

Methods: Baseline samples were collected from 23 patients with MM. The following samples were provided at baseline: BMA slides, BMA cell preparations using a Ficoll protocol, and BMA cell preparations using an RBC lysis protocol. The Ficoll BMA cell preparations were divided into the mononuclear cell fraction and the lower Ficoll fraction which is typically comprised of granulocytes and erythrocytes. Identification of myeloma cells was performed using Sequenta's LymphoSIGHT™ platform. Briefly, using universal primer sets, we amplified immunoglobulin heavy chain (IGH) and light chain (IGK) variable, diversity, and joining gene segments from genomic DNA. Amplified products were sequenced and analyzed using standardized algorithms for clonotype determination. Tumor-specific clonotypes were identified for each patient based on their high-frequency (>5%) within the B-cell repertoire. Following identification of the myeloma-specific clonotypes, we assessed each of the cell preparations for the presence of the myeloma clonotype(s).

Results: The sequencing assay detected the presence of a myeloma-specific clonal gene rearrangement in 22 of 23 (96%) of patients with MM. Applicability rates were 17/18 (94%) in BMA slides and 18/19 (95%) in RBC lysis cell preparations. These applicability rates are consistent with previous reports of sequencing applicability in MM patients. In thirteen patients, we investigated the potential loss of myeloma-specific clonotypes due to Ficoll cell preparation. The variation in myeloma cell loss was typically low but ranged from essentially no loss to the loss of more than 90% of the myeloma cells in the PBMC of one patient compared to the RBC lysis preparation. The myeloma cells were detected in the typically discarded lower layer of the Ficoll preparation which explained the loss.

Summary and Conclusions: These results suggest that sequencing based MRD analysis is applicable in >95% of patients with MM. Further evaluation and optimization of sample processing methods is ongoing to enable application of the sequencing method for clinical MRD assessment in MM patients.

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MULTIPLE MYELOMA PATIENTS THAT DEVELOP SECOND PRIMARY MALIGNANCY HAVE A WORSE PROGNOSIS THAN MULTIPLE MYELOMA PATIENTS TREATED BEFORE THE INTRODUCTION OF NOVEL AGENTS: A POPULATION BASED-STUDY

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Background: The survival of patients with multiple myeloma (MM) has significantly improved over the last decades due to increasingly effective therapies. With this improvement the incidence of second primary malignancies (SPM), including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), has become a concern. In a recent meta-analysis, exposure to lenalidomide plus melphalan significantly increased hematological SPM. However, the effect of SPM on survival in patients with MM has not been established.

Aims: The aim of this population-based study was to assess the effect of SPM on survival in patients with MM.

Methods: All MM patients diagnosed in Sweden 1958-2011 were identified from the Swedish Cancer Registry. We identified information on all subsequent SPM diagnoses among patients in the MM cohort. For each MM patient with SPM, two controls without SPM from the MM cohort were selected and matched by year of birth, sex, and date of MM diagnosis. Survival was estimated from SPM diagnosis (and corresponding date for controls) until death, with follow-up to 2012. Cox regression was used to calculate hazard ratios (HR) and 95% confidence intervals (CIs), adjusting for gender, age at MM diagnosis, and calendar period.

Results: Among 26,627 patients diagnosed with MM in Sweden 1958-2011, a total of 1,812 developed SPM and 5,132 MM patients were matched controls. Median age at MM diagnosis was 70 years and 74 years at SPM diagnosis. Of the 1,812 patients that developed SPM, 25% were gastrointestinal, 12% hematological, 13% non-melanoma skin cancer, 3% malignant melanoma, 5% respiratory, 3% nervous system tumors, 15% male reproductive system, 6% breast, 3% female reproductive system, 8% kidney and urinary tract, and 6% unspecified. Overall patients with SPM had a statistically significant 1.8-fold (95% CI 1.7-1.9) increased risk of death in comparison to control MM group. Median survival was 0.9 years after SPM diagnosis and 2.5 years after corresponding date for controls. MM patients with SPM diagnosed 2001-2011 had a significant 1.2-fold (1.01-1.42) increased risk of death in comparison to MM patients without SPM diagnosed 1958-2000 (Figure). MM patients with SPM 1958-2000 had a significant 1.6-fold (1.4-1.9) increased risk of death in comparison to MM patients with SPM 2001-2011. There was a statistically significant increased risk of death in MM patients with the following cancer groups (compared to without): gastrointestinal (HR=2.3; 2.1-2.5), hematological (2.9; 2.5-3.3), non-melanoma skin cancer (1.2; 1.0-1.3), respiratory (3.7; 3.0-4.5), nervous system tumors (3.9; 3.0-5.1), male reproductive system (1.3; 1.2-1.6), female reproductive system (2.0; 1.5-2.7), kidney and urinary tract (1.9; 1.6-2.3), and unspecified tumors (2.5; 2.1-3.1). There was no significant effect on survival in patients with malignant melanoma (1.1; 0.8-1.5) and breast cancer (1.3; 1.0-1.6) (Figure 1).

Summary and Conclusions: In this large population-based study including more than 25,000 MM patients, we found that despite the improvement in MM

survival in recent years, patients diagnosed with SPM in the last decade have an increased risk of death compared to MM patients without SPM before the introduction of the novel agents. Although MM patients in general benefit from the new therapies, the prognosis in patients with SPM is a concern. Clearly, there is need to clarify the underlying biological mechanisms and risk factors for SPM in the clinical setting to be able to provide effective MM treatment without increasing SPM.

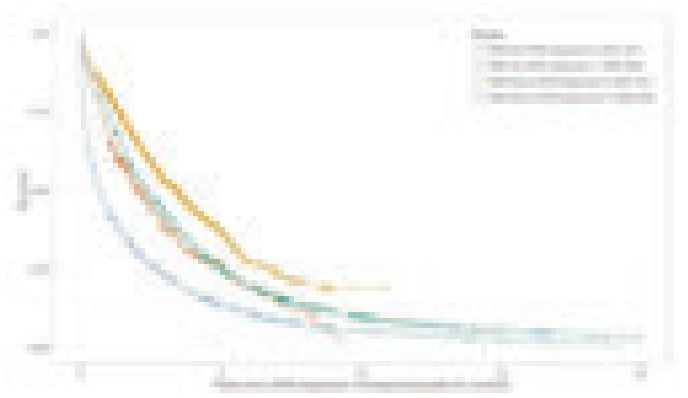

Figure 1.

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BORTEZOMIB OR THALIDOMIDE INCORPORATED INTO AUTOLOGOUS STEM CELL TRANSPLANTATION *VERSUS* NOVEL AGENT-BASED TREATMENTS FOR ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: A CASE-MATCH COMPARISON

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Background: In the novel agent era, the role of autologous stem cell transplantation (ASCT) for newly diagnosed multiple myeloma (MM) patients (pts) over 65 years of age still remains an open issue.
Aims: We aimed at exploring the role of novel agents incorporated into ASCT in comparison with novel agents alone in elderly MM pts.
Methods: We retrospectively analyzed 63 pts with a median age of 68 years (range 66-72) who were considered eligible to receive up-front novel agents plus ASCT, and we performed a case-match comparison with a series of pts who were enrolled in two GIMEMA trials for elderly pts and were treated with melphalan-prednisone plus thalidomide (MPT) (78 pts) or bortezomib (MPV) (111 pts).
Results: At diagnosis, 50% of ASCT-eligible pts had ISS stage 2-3 and 24% had renal impairment. Cytogenetic abnormalities were evaluable in half of the pts: t(4;14) was detected in 19% of them and 17p deletion in 15%. Induction therapies were bortezomib-based (37 pts) or thalidomide-based (26 pts) (median number of cycles received: 4) and affected at least a VGPR in 57% and 36% of the pts, respectively. A median of $7.2x10^6$ CD34+ cells/kg (IQR 5.8-10.9) was collected following cyclophosphamide plus G-CSF (58 pts) or G-CSF alone (5 pts). Melphalan dose prior to ASCT was 200 mg/m^2, in 39/63 pts, and 140 mg/m^2 in the remaining pts due to impaired renal function; 16 pts received a tandem ASCT. Hematologic recovery occurred at day 12 both for platelets and neutrophils (range 7-16 and 10-15). No transplant-related mortality was observed at 100 days. Two pts experienced grade 4 mucositis, and 13 pts (20%) presented grade 3 toxicities, mainly mucositis and infections. Overall, 98% of the pts achieved at least PR, including 79% at least VGPR and 51% CR. With a median follow up of 48 months, 4-years OS was 77%, median TTP and PFS were 42 and 43.4 months, respectively. Case matching was performed with a 1:3 ratio, with respect to age (+/-2 years), ISS stage and year of treatment; ASCT-eligible pts who had received bortezomib-based induction therapy were matched with pts treated with MPV, whereas pts who had received thalidomide-based induction were matched with pts treated with MPT. Baseline characteristics were similar between pts who received or not ASCT. The median number of MPT or MPV cycles actually received was 6. Novel agent-based ASCT significantly increased the probability to achieve CR as a best response (51% *vs* 29%, P=0.002), and extended TTP (43 *vs* 25 months, P=0.0013), PFS (43 *vs* 24 months, P=0.0007) and time to next treatment (33 *vs* 23 months, P=0.0077), in comparison to the control group. OS was similar between the two groups (66% at 48 months), as well post relapse OS (41% at 48 months in both groups). We performed a multivariate Cox regression analysis, that included different therapies, along with baseline and treatment-related features as prognostic factors. Variables significantly related to extended TTP and PFS included novel agent-based ASCT (P=0.006 and P=0.003), ISS 1 *vs* 2 and 3 (P=0.03 and P=0.01) and receiving a bortezomib-based therapy followed or not by ASCT (P=0.02 and P=0.03). Prolonged OS was significantly related to attainment of CR as a best response and ISS 1 (P=0.005 for both).
Summary and Conclusions: This analysis suggests that ASCT is feasible and well tolerated in selected MM pts aged >65. With the limitations of a retrospective case-match analysis and different treatments, novel agents incorporated into ASCT seem to offer better outcomes in comparison with novel agent-based treatments alone and should be considered a valid option for fit elderly pts.

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CAN 24-HOUR URINE COLLECTION BE REPLACED BY AN EARLY MORNING SAMPLE FOR BENCE JONES PROTEIN DETECTION AND QUANTIFICATION?

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Background: Detection and quantification of Bence Jones protein (BJP) is necessary in multiple myeloma, AL amyloidosis, and other monoclonal gammopathies. For BJP quantification a 24h urine collection is required; however, this can be inconvenient and subject to errors, particularly in the outpatient setting. Total protein excretion can be measured in an early morning void (EMV) and expressed as protein-to-creatinine ratio.
Aims: In the present study we tested if an EMV sample could be used to detect and quantify BJP in 337 outpatients from two hematology Clinics.
Methods: Two-hundred four had AL amyloidosis, 56 MGUS, 51 MM, 17 SMM, 9 WM. All the patients gave written informed consent. Patients were asked to provide the 24h urine collection and the early morning void (EMV) obtained at the end of the collection. The BJP was detected by agarose gel immunofixation. The BJP quantification was performed by densitometric scanning of the electrophoretic monoclonal peak.
Results: In 132 patients BJP was detected in both samples, in 13 only in the EMV, and in 1 only in the 24h sample (agreement 96%, Kappa-statistic 0.91 in the overall population). When the BJP was visible at electrophoresis (68 pairs of samples) it was quantified by densitometric scanning of the peak and expressed in mg/24h or mg/g-creatinine. There was very good agreement between BJP expressed as mg/24h and mg/g-creatinine in the 24h samples (Lin's correlation coefficient [cc] 0.95), mg/g-creatinine in the 24h and EMV samples (cc 0.95), and mg/24h in the 24h sample and mg/g-creatinine in the EMV (cc 0.91). In 25 patients with ≥2 measurements, agreement between changes in BJP expressed in mg/24h in the 24h sample and in mg/g-creatinine in the EMV was suboptimal (cc 0.72). However, agreement between BJP changes expressed in mg/g-creatinine in the 24h sample and in the EMV was better (cc 0.85).
Summary and Conclusions: An EMV can be used for BJP detection and measurement. The better agreement in BJP changes between the 24h and EMV samples when BJP is expressed in mg/g-creatinine probably reflects patients' errors in timed urine collection.

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PHASE II TRIAL OF LENALIDOMIDE, DEXAMETHASONE AND CYCLOPHOSPHAMIDE (LENDEXAL) FOR PREVIOUSLY UNTREATED PATIENTS WITH LIGHT-CHAIN AMYLOIDOSIS

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Background: Oral melphalan and dexamethasone is the standard treatment for newly diagnosed patients with immunoglobulin light chain (AL) amyloidosis not eligible for high-dose melphalan and stem cell transplantation (HDM/SCT). However, new treatment options are still needed for this patient population.
Aims: Based on the activity of IMiDs® (immunolodulatory drugs) in relapsed/refractory AL amyloidosis, we designed a prospective and multicenter phase II trial with lenalidomide, dexamethasone and cyclophosphamide for newly diagnosed patients not eligible for autologous transplant (clinicaltrials.gov identifier: NCT01194791).
Methods: The main inclusion criteria were newly diagnosed AL amyloidosis confirmed by biopsy, significant organ involvement, cardiac ejection fraction

over 50%, serum creatinine below 3 mg/dL, and not being eligible for autologous transplant. Treatment schedule consisted of 6 cycles of lenalidomide at 15 mg orally (po) on days 1 to 21, dexamethasone at 20 mg po on days 1 to 4 and 9 to 12, and cyclophosphamide at 300 mg/m^2 intravenously (iv) on days 1 and 8 every 28 days, followed by 6 more cycles of lenalidomide at the same dose, dexamethasone at 20 mg po on days 1 to 4 and cyclophosphamide 300 mg/m^2 iv on day 1. After these 12 cycles, maintenance with lenalidomide (10 mg po on days 1 to 21) and dexamethasone (20 mg po on days 1 to 4) was planned until discontinuation due to intolerance or disease progression. All patients received prophylaxis of thromboembolic events with oral aspirin (100 mg) or subcutaneous low-molecular-weight heparin. The primary end-point was hematologic response.
Results: From September 2010 to December 2012, 28 patients were included in the study. Twenty-three patients had cardiac involvement, with cardiac stage 3 in 13 of them. The overall hematologic response rate was 46%, including 14% complete responses. The organ response rate was 32%. In 5 patients lenalidomide was reduced or discontinued due to toxicity. A therapy-related serious adverse event was reported in 7 patients. So far, 11 patients remain on the study and 17 have been discontinuated mostly due to death secondary to AL-related cardiac or renal events (8 patients), progression or lack of response (4), and toxicity (3).
Summary and Conclusions: In agreement with previous reports, our results support the efficacy and tolerability of the combination lenalidomide/dexamethasone/cyclophosphamide in this poor prognostic population of patients with AL amyloidosis.

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THE ADDITION OF BORTEZOMIB TO STANDARD MELPHALAN/DEXAMETHASONE IMPROVES THE QUALITY OF RESPONSE BUT DOES NOT REDUCE THE RATE OF EARLY DEATHS IN AL AMYLOIDOSIS: A MATCHED CASE CONTROL COMPARISON

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Background: Oral melphalan and dexamethasone (MDex) is standard treatment in patients with AL amyloidosis who are not eligible for stem cell transplantation. Following encouraging reports on the activity of bortezomib combined with alkylators and dexamethasone, these combinations are being moved to frontline therapy.
Aims: We compared the outcome of 87 patients treated with bortezomib plus MDex (BMDex) with that of 87 controls treated with MDex.
Methods: Patients and controls were matched for all known prognostic factors, including age, cardiac and renal function and clonal burden. All the patients gave written informed consent.
Results: There was no significant difference in the rate of severe adverse events (17% in the MDex cohort and 22% in the BMDex group, P=0.444). Most common were fluid retention and cytopenia (9% and 7% in both cohorts, respectively). Peripheral neuropathy was observed only in BMDex patients (5%). A higher rate of complete responses was observed with BMDex (42% *vs.* 19%, P=0.002), but this did not result in a survival improvement. However, when patients with class III or IV heart failure and N-terminal pro-natriuretic peptide type-B >8500 ng/mL where excluded, a significant survival advantage for BMDex appeared (median survival 61 months *vs.* not reached, P=0.014). Patients treated with full-dose dexamethasone (40 mg) had similar response rates and survival whether they received bortezomib or not.
Summary and Conclusions: The addition of bortezomib to MDex does not overcome the poor prognosis of advanced cardiac amyloidosis. Intermediate-risk patients, who are not deemed eligible for high-dose dexamethasone, are likely to take the greatest advantage from combinations of bortezomib, alkylators and dexamethasone.

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THE PERCENTAGE OF CLONAL PLASMA CELLS IN BONE MARROW BY CIG-FISH: COMPARISON AMONG LIGHT CHAIN CARDIAC AMYLOIDOSIS, MONOCLONAL GAMMOPATHY OF UNDERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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Background: Amyloid light chain (AL) amyloidosis is a disease of clonal plasma cells synthesizing structurally abnormal light chain. Since infiltration of plasma cells in in bone marrow (BM) is minimal in amyloidosis and mature plasma cells have low proliferative activity, classic cytogenetic analysis is hampered. Thereon, we performed immunofluorescence detection of cytoplasmic light-chain (clg FISH) in AL amyloidosis, monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM).
Aims: The aim of this stud is to investigate the characteristics of cytogenetic abnormaliteis found in AL amyloidosis as compared with other plasma cell neoplasms, and to determine the significance of proportions of abnormal clones measured by the clg FISH method.
Methods: A total of 230 patients was enrolled; 25 cardiac AL, 25 MUGS, and 180 MM patients. Plasma cell Sorting was performed by clg-FISH targeting IGH, RB1, TP53, 1p25/1q32, CEP 9/p16.
Results: Of 25 amyloidosis patients, 16 (61.5%) presented at least one cytogenetic abnormalities. IGH rearrangement was most frequent in 13 (50%; 13/16, 81.3%) patients, followed by RB1 deletions in 3. Eleven AL amyloidosis patients with ≤30% BM plasma cells showed only IGH rearrangements without other cytogenetic abnormalities and% of clonal cells with IGH among among total plasma cells in BM ranged from 16% to 100%. In 5 patients with >30% BM plasma cells showed multiple abnormalities. In these patients, clg-FISH revealed abnormalities in >90% of plasma cells. The 6-month mortality rate of AL amyloidosis patients with single IGH rearrangements were 27.3% (3/11), which higher than those of amyloidosis patients with multiple abnormalities (0/5). Among 25 MGUS patients, 8 (32%) patients showed cytogenetic abnormalities. Of note, the most frequent abnormality was trisomy 9, which was found in 7 (28%; 7/8, 87.5%) patients. Five MGUS patients presented trisomy 9 as single abnormality and the% clonal plasma cells ranged from 55% to 90%. Among 180 MM patients, 150 (83.3%) presented ≥2 cytogenetic abnormalities, 110 (61.1%) patients presented IGH rearrangements, 91 (50.6%) had trisomies or tetrasomies of chromosome 9, and 37 (20.6%) patients presented both abnormalities. The% clonal plasma cells ranged from 12-100%. The difference in% clonal plasma cells in BM was not significantly between AL amyloidosis and MGUS (mean, 63.8% *vs* 71.9%, P=0.971), and higher in MM (mean, 91%; P <0.001).
Summary and Conclusions: AL amyloidosis patients presented IGH rearrangement as a most frequent single cytogenetic abnormality. The percentage of clonal plasma cells in BM by Ig-FISH was not significantly different among AL amyloidosis and MGUS. The clinical presentation of cardiac amyloidosis was not dependent of the plasma cell burden or the clone size harboring cytogenetic abnormalities.

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PLATELET MICROPARTICLE PROCOAGULANT AND PROANGIOGENIC FEATURES IN ESSENTIAL THROMBOCYTHEMIA (ET)

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Background: Circulating microparticles (MP) are altered in number and composition in various diseases associated to a high thrombotic risk, including ET. In addition to their role in the process of thrombus formation, MP are also important in tumor progression, particularly in the neoangiogenesis process. Platelets, which circulate in an activated status in ET, represent the main source of MP in these patients.
Aims: To characterize the procoagulant and proangiogenic properties of MP released *in vitro* by platelets isolated from patients with ET.
Methods: A total of 23 ET (11M/12F) and 16 healthy (CTR: 9M/7F) subjects were included into the study after informed consent. Platelets were isolated from venous blood samples collected in sodium citrate (3.2%) according to a standardized procedure, and re-suspended in Hepes Tyrode buffer at the concentration of $1x10^9$ platelets/ml and left unstirred for 20 min at 37 C° for the releasing of MP. At the end of incubation, platelet MP (PMP) were isolated by two serial high speed centrifugations (4,000 rpm for 20 min and 14,000 rpm for 60 min) and re-suspended in a fixed volume of the appropriate buffers for the different analyses. PMP were quantified by flow cytometry (Accuri C6, Becton Dickinson) as CD41 positive events. The calibrated automated thrombography (CAT assay by Stago) was used to measure the PMP thrombin generation (TG) potential, while the P-PPL/1 assay (Stago) to evaluate phospholipid-dependent procoagulant activity (PCA). The capillary-like tube formation assay in Matrigel was employed to characterize the PMP proangiogenic potential. Finally, VEGF levels in PMP lysates were measured by ELISA.
Results: Significantly higher PMP levels were found in plasma of ET patients compared to controls (3,359±345 *vs* 1,562± 134/uL; p<0.05). After correction for the respective *in vivo* platelet counts, we observed that ET patients displayed significantly lower rate of circulating PMP per platelets compared to controls (6.5 *vs* 11.4 PMP/plt, p<0.05). The *in vitro* study, showed that platelets from ET patients released **s**ignificantly (p<0.05) lower quantity of PMP compared to controls (1.85±1.0 *vs* 6.04±1.34 $x10^6$/ml). Concerning the TG assay, PMP obtained by platelets from ET patients showed a lower TG activity compared to controls (p<0.05), as demonstrated by longer lag-time (23±0.9 *vs* 21.8±1.9 min) and lower peak of thrombin (91.6±11.8 *vs* 183.5±17.9 nM FIIa). Likewise, PMP from ET patients induced significantly lower phospholipid-dependent PCA compared to controls (54.8±5.53 *vs* 34.9±2.56 sec; p<0.05). After normalization of the PMP count, the procoagulant potential of PMP from ET patients remained lower than that of controls. Finally, in the capillary like tube formation assay, the PMP from ET patients were less proangiogenic compared to control PMP (At $5x10^6$, MP/ml: 560±36 *vs* 713±48 mm/cm²; p<0.05), although VEGF levels were higher in PMP from ET than controls (68±43 *vs* 33±22 pg/ml; p=n.s).
Summary and Conclusions: Our data show that platelets isolated from ET patients generate *in vitro* less PMP than controls, when resuspended at the same concentration. This finding is in agreement with the observation that *in vivo* the rate of PMP per platelets is significantly lower in ET than in controls. Moreover, PMP released *in vitro* from ET patients show a lower procoagulant and proangiogenic potential. Therefore, the demonstrated higher PCA of MP *in vivo* might be mainly due to the presence of elevated number of MP in plasma but not to an intrinsic increased procoagulant potential.

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ACTIVATING MUTATIONS IN TRANSCRIPTION FACTOR NF-E2 ALTER PROTEIN-PROTEIN INTERACTIONS

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Background: We have recently described indel mutations in the gene encoding the transcription factor NF-E2 in patients with myeloproliferative neoplasms (MPN). These alterations lead to the formation of truncated proteins, which, despite having lost the ability to bind DNA, nonetheless increase the activity of wild type NF-E2. To date, the mechanism of action by which truncated NF-E2 augments the transactivating potential of its wild type counterpart remains unclear.
Aims: We hypothesized that truncation of NF-E2 alters the range of protein-protein interactions exerted and that these aberrant interactions confer the activating phenotype.
Methods: Using co-transfection and co-immunoprecipitation assays, we investigated protein-protein interactions of wild type NF-E2 and its truncation mutants.
Results: Here we demonstrate for the first time that NF-E2, which is known to form heterodimers with small Maf proteins, can alternatively form homodimers. The truncated mutants, in contrast, have lost both the ability to interact with Maf proteins as well as the ability to homodimerize.
The E3-ubiquitin ligase ITCH has previously been reported to bind wild type NF-E2, thereby sequestering and stabilizing the protein in the cytoplasm. We have recently demonstrated mislocalization of NF-E2 in patients with PMF (primary myelofibrosis). Compared to either healthy controls or patients with ET (essential thrombocythemia), PMF patients display nuclear NF-E2 in a significantly higher number of early erythroblasts, an observation that can be exploited diagnostically to identify early, pre-fibrotic PMF patients.
We hypothesized that altered NF-E2 / ITCH interactions may underlie the observed protein mislocalization in PMF patients. However, even the shortest truncation mutant, which is comprised of only 79 amino acids of the NF-E2 N-terminus, retains the ability to interact with ITCH. Co-expression of ITCH increases the amount of truncated NF-E2 protein detectable, suggesting that the interaction stabilizes the mutant protein, just as it does wild type NF-E2.
Summary and Conclusions: Consequently, we propose that mutant NF-E2 occupies the available ITCH protein, thereby preventing it from sequestering the wild type transcription factor in the cytoplasm. This leads to the observed increase in nuclear NF-E2 in PMF patients with the concomitant increase in NF-E2 transcriptional activity observed in the presence of mutant NF-E2. While only a subset of PMF patients carries NF-E2 mutations, the vast majority of MPN patients express significantly elevated NF-E2 levels. An excess of wild type NF-E2 may likewise prevent ITCH from effectively sequestering the transcription factor. The mechanism causing increased nuclear NF-E2 activity by decreasing cytoplasmic sequestration, elucidated here, may thus be active in the majority of MPN patients and constitute a paradigm for explaining the elevated NF-E2 activity observed in MPN.

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MORGANA IS A NEW ONCOSUPPRESSOR IN CHRONIC MYELOID LEUKEMIA

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Background: We recently described morgana as an essential protein able to regulate centrosome duplication and genomic stability, by inhibiting ROCK. We have previously generated a morgana null murine model which is characterized by an embryonic lethal phenotype. From 12 months of age onwards, *morgana* +/- mice showed clear pathological signs, such as rapid weight loss, hunched posture and severe hypokinesia and died as a result.
Aims: First, we aimed to identify the cause of death of morgana +/- mice and then we assessed whether morgana could play a role in a corresponding human pathology.
Methods: Peripheral and bone marrow samples from Morgana +/- mice have been studied using conventional methods, including flow cytometry, colony formation assay, cytogenetics and bone marrow transplantation assays. Human samples have been collected from informed patients upon ethical committee approval. Bone marrow samples have been used to assess the expression levels of morgana using both western immunoblot, immunofluorescence, quantitative PCR and immunohystochemistry. Furthermore primary cells have been treated with different ROCK inhibitors to assess apoptosis induction.
Results: Here we show that morgana +/- mice spontaneously develop a lethal myeloproliferative disease resembling human atypical chronic myeloid leukemia (aCML), preceded by ROCK hyperactivation centrosome amplification and cytogenetic abnormalities in the bone marrow (BM). Moreover, we found that morgana is often underexpressed in the BM of patients affected by aCML, a rare disorder characterized by non-recurrent cytogenetic abnormalities, whose molecular basis is poorly understood. Morgana is also underexpressed in the BM of a portion of patients affected by Philadelphia positive CML (Ph+ CML) caused by the BCR-ABL oncogene. Notably, we found that morgana underexpression predicts a worse response to Imatinib, the Ph+ CML standard treatment.
Summary and Conclusions: Morgana can act as an oncosuppressor in CML with different modalities: morgana underexpression induces centrosome amplification and cytogenetic abnormalities and in Ph+ CML, it synergizes with BCR-ABL signaling, therefore reducing the efficacy of Imatinib treatment. Importantly, ROCK inhibition in the BM of patients underexpressing morgana restored the efficacy of Imatinib to induce apoptosis, suggesting that ROCK inhibitors, in combination with Imatinib treatment, can overcome suboptimal responses in patients in which morgana is underexpressed.

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CD4+ T CELL FUNCTIONS ARE POTENTLY SUPPRESSED BY THE JANUS KINASE 1/2 (JAK1/JAK2) INHIBITOR RUXOLITINIB

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Background: Recent discoveries of activating JAK mutations in patients with myeloproliferative diseases (MPNs) coupled with the so far known biology of JAKs in cytokine signalling provided the rationale for targeting these kinases in MPNs. Ruxolitinib (INCB018424) is the first JAK1/JAK2 inhibitor approved for treatment of patients with myelofibrosis (MF). Although ruxolitinib shows limited anti-clonal activity, a profound improvement of quality of life and splenomegaly in MF patients is observed and linked to a substantial reduction of MF-associated circulating pro-inflammatory and pro-angiogenic factors. JAK/STAT-signalling is known to be involved in the regulation of various immune cells including $CD4^+$ T cells, which critically orchestrate inflammatory responses. To better understand how ruxolitinib is modulating $CD4^+$ T cell response, we here provide an in depth analysis of $CD4^+$ T cell function upon ruxolitinib exposure.
Aims: We aim to characterise in detail the JAK1/2 inhibition on CD4+ T cells by phenotypic and functional assays.
Methods: Highly purified $CD4^+$, T cells isolated from healthy human PBMCs were stimulated with i) plate bound anti-CD3, ii) plate bound anti-CD3 and soluble anti-CD28 antibodies, iii) IL-2 in the presence of increasing concentrations of ruxolitinib (0.1μM–10μM) or the respective vehicle control (DMSO). Cytokine production was quantified by flow-based bead assays (Human Th1/Th2 FlowCytomix Multiplex). Proliferation was detected by CFSE dilution analysis. Differentiation was analyzed by using $CD4^+CD62L^+$ T cells isolated from C57BL/6 mice exposed to TH1, TH17 or iTreg differentiation cocktails in combination with anti-CD3 and anti-CD28 stimulation. For analysis of apoptosis/necrosis induction, annexin/propidium iodide staining was applied. Signalling events were analyzed by using phospho-flow technology to evaluate ruxolitinib-mediated changes of TCR- and/or cytokine-induced signalling cascades (using pS6, pSTAT5, pERK, pAKT, pP38, pcFos, pcJun and pCD3Z antibodies). Additionally we analysed patients treated with ruxolitinib (n=9) for changes in their T-cell compartment. Therefore, cells were collected prior to and during ruxolitinib therapy in weekly intervals. T-cells were analyzed according to their expression of CD3 and CD4 and further characterized as activated (HLA-DR+), Treg (CD25hi/CD127low) or TH1 (IFNg+) and Th17 (IL17+) cells.
Results: $CD4^+$ T cell proliferation is dose-dependently suppressed by ruxolitinib when T cells were activated by each of the three conditions tested. In line with previous studies, production of pro-inflammatory cytokines such as IL-1β, IL-5, IL-6 and TNF-α was dose-dependently inhibited in ruxolitinib, whereas expression of the pro-inflammatory IL-8 was increased in a dose-dependent manner. Interestingly, despite the complete proliferation block, we also observed an increase in IL-2 particularly at the lower ruxolitinib concentrations (0.1μM) followed by a dose dependent reduction at higher dose-levels (10μM). After short-term activation of ruxolitinib-exposed $CD4^+$ T cells by anti-CD3 and anti-CD28, proximal TCR signaling events were not affected, whereas a clear down-regulation of IL-2 induced STAT5 phosphorylation could be detected. After wash-out, ruxolitinib-induced inhibitory effects on $CD4^+$ T cell function were fully reversible, as shown by re-induction of the T cell activation markers CD25 and CD69 upon stimulation. Finally, we could provide clear evidence that the differentiation capacity of naïve $CD4^+$ T cells into TH1, TH17 and iTreg was markedly reduced. Finally, we could prove tha *in vivo* significance of our *in vitro* findings, as patients treated with ruxolitinib showed reduced numbers of activated T cells as well as reduced numbers in pro-inflammatory TH1 and TH17 cells. Similarly and in line with our *in vitro* results, Treg were clearly reduced in patients during ruxolitinib therapy.
Summary and Conclusions: We could show that ruxolitinib potently affects $CD4^+$ T cell biology. These data provide a rationale for testing JAK inhibitors in diseases triggered by hyperactive $CD4^+$, T cells, such as autoimmune diseases. In addition, they also provide an explanation for the increased infection rates (*i.e.* viral reactivation and urinary tract infection) seen in ruxolitinib-treated patients.

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INTRACELLULAR CALCIUM DEPOSITS AND STORE OPERATED CALCIUM ENTRY IN CD34+ CELLS FROM PATIENTS WITH MYELOFIBROSIS CARRYING A CALR MUTATION

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Background: Mutations at exon 9 of the calreticulin gene (*CALR*) have been recently identified in about 70% of patients with primary myelofibrosis (PMF) or essential thrombocythemia (ET), who were wild type for *JAK-2* and/or *MPL* mutations. *CALR* gene product is an endoplasmic reticulum (ER) Ca^{2+} binding chaperone, which is involved also in the regulation of glycoprotein folding. In patients with PMF or ET, *CALR* mutations, which occur in a multipotent hematopoietic progenitor cell, are mainly represented by either a 52-bp deletion or a 5 bp insertion, resulting in a novel C-terminal peptide sequence. This is the domain responsible for the Ca^{2+}-binding activity of the protein and this mutation is predicted to lower Ca^{2+} storage capacity in the ER. ER-Ca^{2+} content is tightly coupled to a well known mechanism of Ca^{2+} influx in hematopoietic cells, namely, store-operated Ca^{2+} entry (SOCE), which is activated upon a fall in ER Ca^{2+} content to refill ER and activates specific Ca^{2+}-dependent signaling pathways. The interaction between ER-dependent Ca^{2+} release and SOCE is involved in the regulation of gene expression, apoptosis, cell migration, and cell proliferation/differentiation. The mechanisms by which *CALR* mutations produce a myeloproliferative phenotype are unknown.
Aims: Based on the role that *CALR* plays in regulating Ca^{2+} homeostasis, we have studied intracellular Ca^{2+} deposit and SOCE in CD34+ cells obtained from the peripheral blood of 3 patients with PMF carrying the 52-bp deletion of CALR gene, 3 patients with PMF carrying a JAK2V617F mutation, and 1 healthy subject.
Methods: CD34+ cells were loaded with 4 mcM fura-2 acetoxymethyl ester for 1 hour at room temperature. Depletion of intracellular Ca^{2+} stores was obtained by the addition of 10 mcM cyclopiazonic acid (CPA) to a 0 Ca^{2+} bathing medium. Subsequent replenishment of Ca^{2+} (1.5 mM) to the extracellular solution elicited a rise in $[Ca^{2+}]_i$ due to Ca^{2+} influx through open store-operated Ca^{2+} channels. The amplitude of the peak Ca^{2+} response to CPA was measured as the difference between the ratio at the peak and the mean ratio of 1 min baseline before the peak. SOCE amplitude was measured as the difference between the peak and the mean ratio of 1 min baseline recorded before Ca^{2+} readdition. Data are expressed as mean +/- SE.
Results: Resting Ca^{2+} levels in CD34+ cells from *CALR*-mutated patients were slightly lower as compared to V617F*JAK2* mutated patients (68.7±24.0 nM *vs* 82.2±13.2 nM), but considerably higher than the healthy donor (33.5 nM). CD34+ cells from CALR-mutated patients release more Ca^{2+} from ER stores as compared to V617F*JAK2* mutated patients (52.8 nM±28.6 *vs* 37.7 nM±19.1, respectively) and compared to the healthy donor (30 nM). SOCE of CD34+ cells of *CALR* mutated patients (228.5 nM±26.1) was slightly reduced compared to *JAK2*-mutated patients (370.2±174.6) and lower than SOCE of a healthy subject (130 nM).
Summary and Conclusions: Our results suggest that *CALR* mutation in CD34+ cells of patients with PMF is associated with i) a reduction in basal Ca^{2+} levels compared to *JAK2* mutated cells, but an increase compared to healthy CD34+ cells; ii) an increased amount of free Ca^{2+} in the ER compared to *JAK2*-mutated and healthy CD34+ cells and, iii) a decreased SOCE compared to healthy CD34+ cells and, at a lower extent, compared to *JAK2*-mutated CD34+ cells. Taken together, these results seem to suggest that *CALR* mutation could be implicated in the myeloproliferative phenotype that characterizes PMF patients carrying CALR mutations by interfering with the signaling role of Ca^{2+}.

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VEGF-INDUCED CA2+ SIGNALS ARE DERANGED IN ENDOTHELIAL PROGENITOR CELLS FROM PATIENTS WITH MYELOFIBROSIS CARRYING JAK2 MUTATIONS

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Background: Recent studies have unveiled that calreticulin mutations may occur in patients with myelofibrosis who are wild type for Janus kinase 2 gene (JAK2). This phenotype might have an impact on intracellular Ca^{2+} signalling, which serves as a versatile messenger system in virtually all cell types. However, parallel work from our group has demonstrated that circulating endothelial progenitor cells (EPCs) from JAK2-mutated patients undergo a dramatic remodelling of the Ca^{2+} toolkit (Dragoni *et al.*, PloS One, in press). There is a significantly higher amount of free Ca^{2+} in the endoplasmic reticulum of these cells, whereas both inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) and store-operated Ca^{2+} entry (SOCE) are up-regulated. SOCE is the most important pathway for Ca^{2+} inflow in healthy EPCs, controlling VEGF-induced proliferation, tubulogenesis and NF-kB-dependent gene expression. However, the pharmacological inhibition of this pathway did not interfere with proliferation of EPCs isolated from JAK2-mutated patients and seeded in the presence of an expansion medium containing VEGF, bFGF and EGF.
Aims: The present investigation aimed at assessing whether VEGF evokes intracellular Ca^{2+} oscillations in EPCs derived from patients carrying JAK2 mutation and whether they are remodelled as compared to control cells.
Methods: Epifluorescence microscopy was employed to monitor and record intracellular Ca^{2+} signals in EPCs loaded with the Ca^{2+}-sensitive fluorochrome Fura-2/AM (2 μM; 30 min). VEGF was applied at 10 ng/ml.
Results: VEGF-induced asynchronous oscillations in $[Ca^{2+}]_i$ in EPCs of JAK2-mutated patients; this pattern of signalling is similar to that displayed by con-

trol cells. The amplitude of VEGF-evoked Ca^{2+} transients remained constant throughout the stimulation; however, their amplitude was significantly ($p<0.05$) reduced as compared to healthy cells. Subsequent wavelet analysis revealed that the oscillatory activity in EPCs from JAK2-mutated patients was significantly ($p<0.05$) less robust relative to control cells. The pharmacological inhibition of phospholipase Cg (PLCg)/$InsP_3Rs$ pathway abolished the onset of the Ca^{2+} response. The same result was obtained by removing external Ca^{2+}, thereby indicating that Ca^{2+} inflow is essential to trigger the signal. This finding is strikingly different from healthy cells, which require Ca^{2+} influx only to maintain, not to initiate, the Ca^{2+} train. SOCE blockade with BTP-2 reduced the number of VEGF-induced Ca^{2+} oscillations in EPCs from JAK2-mutated patients, but did not prevent them. La^{3+} and Gd^{3+}, in turn, suppressed the onset of the Ca^{2+} train; this suggested that the Ca^{2+} entry pathway triggering the Ca^{2+} response to VEGF is sensitive to these trivalent cations, but not to BTP-2. This pathway could be gated by 1-Oleoyl-2-acetyl-glycerol, a membrane permeable DAG analogue, that activates an influx of Ca^{2+} blocked by Gd^{3+} and La^{3+}, but unaffected by BTP-2, in EPCs from JAK2-mutated cells, but not in healthy cells.
Summary and Conclusions: The Ca^{2+} machinery shaping the Ca^{2+} response to VEGF is remodelled in EPCs from patients carrying JAK2 mutation. The oscillatory activity is less robust as compared to control cells despite for the higher expression of $InsP_3Rs$ and SOCE. The oscillatory signal is initiated by an OAG-mediated influx of Ca^{2+}, which in turns elicits the interplay between $InsP_3$-mediated Ca^{2+} release and SOCE. Future studies will have to assess why this mode of Ca^{2+} signal is not able to deliver a pro-angiogenic stimulus to these cells.

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INTEGRATED GENOMIC ANALYSIS ILLUSTRATES THE CENTRAL ROLE OF JAK-STAT PATHWAY ACTIVATION IN MYELOPROLIFERATIVE NEOPLASM PATHOGENESIS

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Background: The Philadelphia chromosome-negative myeloproliferative neoplasm (MPNs) are clonal hematopoietic disorders consisting of polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). To date, genomic studies have identified somatic alterations in the majority of MPN patients, including *JAK2* mutations in the majority of MPN patients and *CALR* mutations in *JAK2* mutant-negative MPN patients. However, the role of JAK-STAT pathway activation in different MPNs, particularly in patients without *JAK2* mutations, has not been definitively delineated. Further, the impact of *JAK2V617F* allele burden as well as the impact of different somatic alterations, including mutations in epigenetic regulators, such as *TET2* or *ASXL1*, on transcriptional output has not been extensively evaluated. Given the relative genetic heterogeneity of the MPNs, evaluation of the transcriptional output found in the MPNs could have broad therapeutic and biologic implications.
Aims: To characterize the transcriptional output of patients with MPNs with and without *JAK2* mutations.
Methods: We performed gene expression profiling using Affymetrix oligonucleotide microarrays in granulocytes from normal individuals and a cohort of well-characterized patients with chronic MPNs and integrated this data with detailed molecular characterization in order to understand how transcriptional output in MPN cells relates to the clinical phenotype and molecular genotype of MPN patients.
Results: We observed that a transcriptional signature consistent with activated *JAK2* signaling is seen in all MPN patients regardless of clinical phenotype or mutational status (Figure 1). In addition, the activated *JAK2* signature was present in patients with somatic *CALR* mutations. Conversely, GSEA revealed significant enrichment of the *CALR*-mutant MPN gene signature in *JAK2*V617F-mutant MPN patients relative to normal individuals consistent with a shared mechanism of transformation by *JAK2* and *CALR* mutations. In terms of patients with *JAK2*V617F mutations, patients with homozygous *JAK2*V617F mutations were characterized by a distinctive transcriptional profile compared to patients with heterozygous *JAK2* mutations. The genes most highly differentially expressed in MPN patients with homozygous *JAK2*V617F mutation included *JAK2* itself as well as *CD177*/*PRV1*. We also identified a distinct transcriptional signature of *TET2* mutations in MPN patent samples consisting of of 61 significantly differentially expressed genes in *TET2* mutant *versus* wildtype MPN patients (FDR<0.05).
Summary and Conclusions: Our data indicate that MPN patients, regardless of diagnosis or *JAK2* mutational status are characterized by a distinct gene expression signature with upregulation of JAK-STAT target genes, demonstrating the central importance of the JAK-STAT pathway in MPN pathogenesis. These findings imply that therapeutic interventions aimed at the JAK-STAT pathway may have efficacy regardless of the observed genotype and are likely to continue to represent an important therapeutic strategy in MPNs. As well, other genomic alterations, such as changes in the *JAK2*V617F allele burden as well as the presence of other mutational events such as *TET2* mutations, can alter the gene expression signature, and potentially phenotype, of the MPNs. As such, the wide array of genomic alterations that co-occur with *JAK2*/*MPL*/*CALR* mutations. likely play a role in the phenotypic heterogeneity observed across the MPNs. Further analysis of these alterations using human samples and animal models is required.

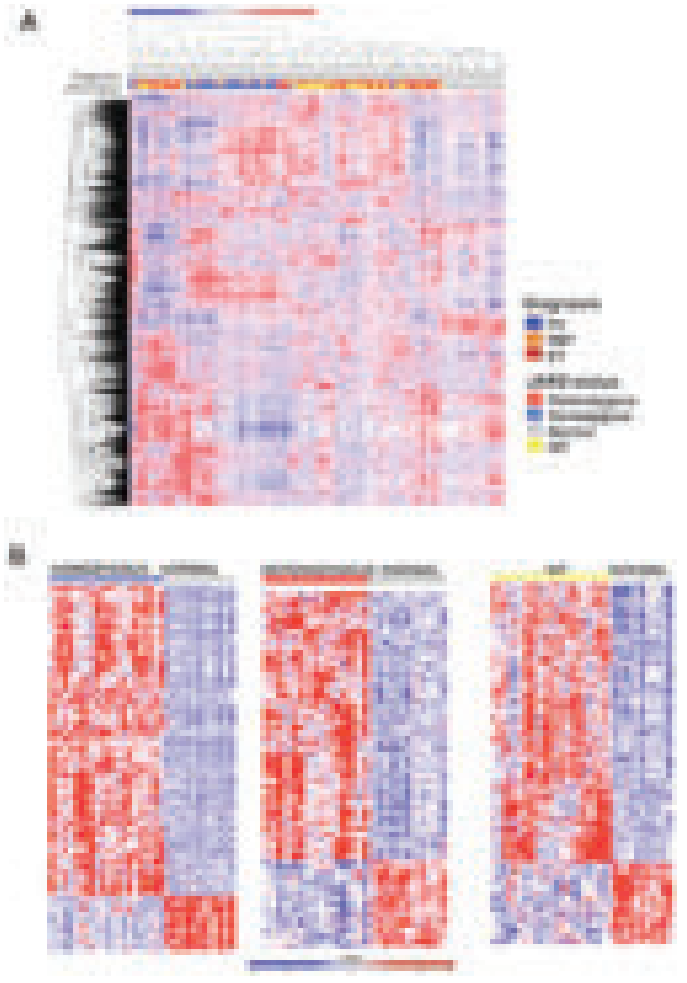

Figure 1.

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LOW RATE OF CALR MUTATIONS IN REFRACTORY ANAEMIA WITH RING SIDEROBLASTS AND MARKED THROMBOCYTOSIS

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Background: Refractory Anaemia with Ring Sideroblasts (RARS) and marked Thrombocytosis (RARS-T) is a myeloproliferative (MPN)/myelodysplastic (MDS) neoplasm with the *JAK2*^{V617F} mutation present in half of all and *MPL*^{W515} in few RARS-T cases. Recently somatic mutations in the Calreticulin (CALR) gene have been reported in a high proportion of *JAK2*- and *MPL*-non mutated MPN cases
Aims: The purpose of our study was to analyse the frequency of *CALR* mutations on a large RARS-T and RARS cohort and understand whether *CALR* mutations may also be responsible for thrombocytosis in this disease.
Methods: One hundred and twenty four samples including 95 RARS-T and 29 RARS from seven European centres in three European countries were collected and tested

Results: A total of 124 cases with MDS-RS (95 RARS-T and 29 RARS including 62 males and 62 females) were recorded in the study, which is, to our best knowledge, the largest series of MDS-RS studied for *CALR* mutations. Median age at diagnosis was 74 and 73 years for the RARS-T and RARS cohort, respectively. *SF3B1* mutation was noted in 24 (82.7%) RARS and 83 (88.3%) RARS-T, confirming that this mutation is highly represented in MDS-RS.A *JAK2*^{V617F} mutation was noted in 47 of the 95 RARS-T patients (49.4%) but none in RARS patients. A *CALR* mutation was noted in only one (1%) RARS-T and one (3.4%) RARS patient, each. The *CALR* mutation found in the RARS-T patient was a 10 bp (1129-1138) deletion located in the exon 9 which is not the most frequent reported *CARL* mutation. Surprisingly, this patient presented also a *JAK2*^{V617F} mutation with a low allele burden (4%) and a *SF3B1* mutation. The mutational status of this patient has been confirmed in two independent laboratories. He patient has been treated with Hydroxyurea since 2010 with a good tolerance. The latest full blood count showed haemoglobin 104 g/L, platelet count 584 G/L and leucocytes 4.3 G/L.Among the 29 RARS, we also identified a single case with *CALR* mutation. This unique *CALR*-positive RARS case was a 70-year old man who did not have elevated platelet count but rather a thrombocytopenia, and carried a *SF3B1* mutation. No *JAK2*^{V617F}, *MPL* or cytogenetic abnormality was noted in this patient.
Summary and Conclusions: *CALR* mutations are rare in MDS with RS and do not explain the myeloproliferation in the *JAK2*^{V617F}- and *MPL*-negative RARS-T cases. In addition, we report the first case of coexisting *CALR* and *JAK2*^{V617F} mutations in RARS-T.

P392

GATA1 IS OVEREXPRESSED IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND IT IS NORMALIZED BY ANAGRELIDE TREATMENT.

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Background: GATA1 is the founding member of the GATA transcription factor family and is essential for cell maturation and differentiation within the erythroid and megakaryocytic lineages. GATA1 is a pleotropic transcription factor, whose expression is essential in these lineages; its function depends upon its ability to bind to both DNA and protein partners. Disturbance of either of these functions causes severe hematopoietic dysfunction and can result in blood disorders, such as thrombocytopenia, anaemia and even leukaemia. Ectopic expression of GATA1 in the murine myeloid cell line M1 induces c-Mpl appearance and megakaryocyte (MK) differentiation. The same phenomenon has been showed to occur in hematopoietic stem cells; in that forced increased expression of GATA1 blocked self-renewal and induced the exclusive generation of MegE lineages. Several studies, have suggested a connection between GATA1 and myeloproliferative neoplasia (MPN). We previously reported, high GATA1 transcript levels are found in the bone marrow of patients with ET and PV, independent of the JAK2 V617F mutation. However, over expression of GATA1 is not seen in other MPN. Anagrelide (ANA) has been proven to be an effective drug in reducing platelet count and thrombotic risk in management of ET and PMF patients. However, the mechanisms by which this drug induces this effect is still unclear. Recently Erusalimsky and colleges have reported that ANA results in down-modulation of GATA1 and its co-factor FOG1 in MK during *in vitro* differentiation.
Aims: In this study, we analysed the expression of GATA1 in peripheral blood (PB) samples from 30 patients with ET and compared the levels of expression before and after treatment with common cytoreductive agents such as hydroxyurea (HU) and ANA.
Results: We confirmed the data obtained in BM, with a significant up-regulation of GATA1 in ET compared to controls. When we measured the expression of GATA1 before and during treatment with ANA, there was a significant reduction of the GATA1 expression at 3 and 6 months of therapy, concomitantly with a reduction in platelets (PLT) counts. Interestingly, this was not equally observed in patients treated with HU, where despite reduction in PLT counts, the GATA1 levels rose. Furthermore, when we analysed patients on combination treatment ANA+HU, GATA1 expression reduced only when patients were taking ANA. When ANA treatment was stopped and the patient continued only on HU, GATA1 levels rose again.
Summary and Conclusions: This data suggests a direct effect of ANA on GATA1 expression. GATA1 may represent a generic molecular marker in ET and a possible additional diagnostic criteria in thrombocytosis. GATA1 overexpression is independent from JAK2 mutations, and responds specifically to ANA therapy suggesting a role in monitoring therapy response.

P393

IMPORTANCE OF CALR MUTATIONS IN THE DIAGNOSIS OF PRIMARY MYELOFIBROSIS

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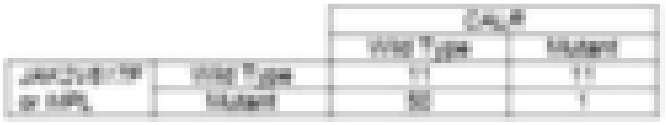
Background: In the last World Health Organization (WHO) classification, somatic mutations had been incorporated into diagnostic criteria for primary myelofibrosis (PMF). *JAK2V617F* and *MPL* mutations have been reported in 50-70% of patients with PMF, being 30-50% of patients the ones who didn't have any of these molecular markers. Recently it has been discovered mutations in calreticulin (*CALR*) gene which occur in 80-88% of PMF patients not having *JAK2V617F* or *MPL* mutations.
Aims: The aim of this study was to analyze the incidence and prognostic relevance of *CALR* mutational status in serial diagnosed patients with PMF who had not received previous treatment.
Methods: A total of 73 consecutive PMF patients were included (median age 69; 45 males) with fulfilling the WHO criteria valid at the moment of their diagnosis. This study was conducted in accordance with the Declaration of Helsinki. Genomic DNA was extracted from bone marrow or peripheral blood samples using the QIAamp DNA Blood mini kit (Qiagen). All samples were coded and assayed blindly for the *JAK2V617F, MPL* and *CALR* mutations. To detect the presence or absence of *JAK2V617F* mutation, an Allele-Specific PCR using TaqMan allelic discrimination was used. We used Sanger sequencing to detect MPL exon 10 mutations. And we screen for insertion and deletion mutations in *CALR* with 6-FAM labeled primers spanning exon 9. We confirm and describe *CALR* mutations type with Sanger sequencing. Laboratory parameters (red blood cell indexes, leukocyte and platelet counts) and clinical data (constitutional symptoms, complications and progression) were collected. **Results:** A total of 46/73 (63%) patients were *JAK2V617F* positive and from the 27 *JAK2V617F* negative, 5 had *MPL* mutations (18.5%). Twelve patients out of 73 (16.5%) had a *CALR* mutation (8 Type1, 1 Type2 and 3 which weren't any of the 36 described ones). One patient harbors both *JAK2V617F* and *CALR* mutation, the other eleven patients were *JAK2V617F* and *MPL* negative (50% of double negative patients) (Table 1). In global, 85% of patients carried a *JAK2V617F, MPL* or *CALR* mutation.
CALR mutation did not correlate with the presence of constitutional symptoms, complications (thrombosis, hemorrhage, transformation) or overall survival.

Table 1. *JAK2V617F, MPL* or *CALR* mutation distribution in a cohort of 73 PMF.



Summary and Conclusions: The study of *JAK2V617F, MPL* and *CALR* mutations demonstrate a clonal maker in 85% of PMF patients. Even it is described that CALR mutations are mutually exclusive with *JAK2V617F* or *MPL* mutations, we have found one patient who harbor both mutations. We will need more patients with PMF to corroborate if there is any clinical difference between those patients who have CALR mutations and the wild-type ones.

P394

THE SOMATIC MUTATION RATE IS INCREASED IN PATIENTS WITH CLASSICAL MYELOPROLIFERATIVE NEOPLASMS WHO HAVE A SECOND PRIMARY TUMOR

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Background: Classical chronic myeloproliferative neoplasms (MPNs) are associated with one of a set of specific somatic mutations, the most common of which is in the *JAK2* gene. Patients with MPN have an increased risk to develop acute myeloid leukemia (AML): whether they have also an increased risk to develop non-hematological malignancies is still controversial. Since MPNs are relatively rare, it has been suggested that an increased rate of somatic mutation may underlie the *JAK2* mutation as well as evolution to AML.
Aims: To investigate the role of the somatic mutation rate (μ) in classical MPNs.
Methods: Since measuring μ is rather labor-intensive, in this study we have measured instead the frequency of peripheral blood granulocytes carrying inactivating mutations in the X-linked gene *PIG-A,* whose product is required for glycosyl-phosphatidylinositol-anchored (GPI) proteins to become surface bound. We have shown previously that *PIG-A* is a good sentinel gene for this purpose, and that the frequency (*f*) of *PIG-A* mutant cells may be a good surrogate of μ (Rondelli *et al*. *PlosOne*. *2013*, 8:e54046). Five million granulocytes isolated from each patient were stained with a mixture of moAbs that recognize 3 different GPI-linked proteins (CD59, CD55, CD24), plus one pan-leukocyte marker (anti CD45-APC) and one granulocyte marker (anti-CD11b-FITC). *f* values were determined by flow cytometry.

Results: We have measured *f* in 102 patients with MPN, including 36 with polycythemia vera (PV), 23 with essential thrombocythemia (ET), 25 with primary myelofibrosis (PMF), 18 with post-PV/post-ET MF. Overall the proportion of those with a JAK2(V617F) mutation was 88%. The median value of *f* in these patients was not different from that of healthy controls ($5.5x10^{-6}$ *vs*. $4.9x10^{-6}$). We found no correlation between the value of *f* and the allele burden of *JAK2*(V617F) (R^2=0.0002). However, *f* was higher than $14.5x10^{-6}$ (the value that defines the 90th percentile in healthy controls) in 24.5% of patients with MPN, a proportion significantly higher than in controls (*P*<0.006). Moreover, in a subset of 18 patients who in addition to MPN had at least one primary tumor (2 had lymphomas and 16 had one of several types of epithelial tumors), the median value of *f* was 13.8 $x10^{-6}$: *i.e.,* significantly higher than in healthy controls (Mann Whitney test, *P*<0.005) (Figure 1).

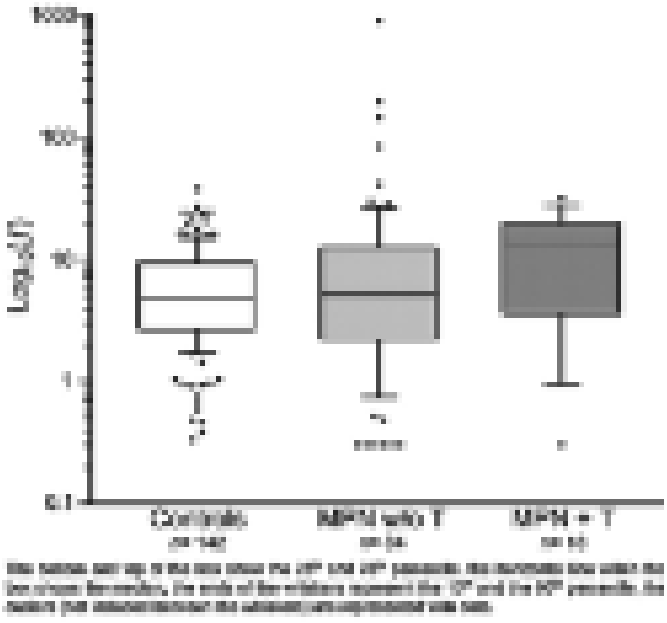

Figure 1. Box and whisker plot of mutant frequency (f) in controls, in patients affected by MPNs (MPN+T) and without (MPN w/o T) a second tumor.

Summary and Conclusions: 1) Although we cannot automatically extrapolate from our sentinel gene *PIG-A* to all other genes, we find no evidence that the *JAK2*(V617F) mutation In MPNs arises through genetic instability. 2) There was no significant difference in *f* values between MPN patients with or without a *JAK2* mutation, and no correlation between *f* and *JAK2*(V617F) allele burden: thus, *JAK2* mutation does not cause genetic instability. 3) Patients who have MPN and another malignancy are examples of multiple primary tumors. To the best of our knowledge, for the first time these data provide direct evidence that an intrinsically higher rate of somatic mutation plays a role in causing multiple primary tumors in subjects who did not otherwise appear to be 'cancer-prone'.

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MOUSE LEUKEMIC TRANSFORMATION IN PMF XENOGRAFT MODEL REVEALS PARACRINE REGULATION IN DISEASE PROGRESSION

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Background: Primary Myelofibrosis (PMF) is a myeloproliferating neoplasm characterized by profound bone marrow microenvironment disruption that subsequently leads to fibrosis and AML transition. Despite the identification of abnormal myeloid types during disease development, the complex cellular interactions supporting their expansion in the context of chronic myeloproliferation and disease evolution remains elusive.
Aims: In our studies to identify the PMF neoplastic stem cells in a xenotransplantation mouse model, we observed the peculiar emergence of mouse origin myeloid leukemia.
Results: Analysis of murine leukemic clones revealed multiple clonal integrations of ecotropic endogenous retrovirus (eERV). In addition to the single eERV provirus located on chromosome 11 (*Emv30*) in the NOD genome, up to five *de novo* germline eERV integrations were observed in mice from four independent NSG mouse colonies. Analysis of the *Emv30* provirus and the replicating eERV demonstrated that neither mutations nor recombination events were necessary for virus replication or AML induction. However, acquired pathogenicity could be attributed to PMF-mediated paracrine stimulation of the myeloid compartment, observed in spleen and bone marrow of xenografted mice. These proliferating cells serve as targets for retroviral transformation, as evidenced by integration in the *Evi1* locus – a hotspot for retroviral-induced myeloid leukemia.
Summary and Conclusions: In conclusion, this study corroborates a critical role of paracrine stimulation in PMF disease progression, underlines the importance of target cell numbers in ERV pathogenicity, and mandates awareness of ERV-replication in interpreting results from severely immunodeficient NOD mice.

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CYTOGENETIC INVESTIGATION OF G-CSF-STIMULATED PERIPHERAL BLOOD IN PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: The role of karyotyping in primary myelofibrosis (PMF) is not so well established, comparing with chronic myeloid leukemia, due to technical difficulties in obtaining of sufficient amount of bone marrow (BM) aspirate for cytogenetic investigation in many patients. The unfavorable cytogenetic changes in BM for PMF include complex karyotype or sole or two abnormalities that include +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-, or 11q23. However, the pathological process in PMF is not limited to BM. Some immature hemopoetic progenitor and stem cells affected with the disease relocate into the peripheral blood. Further improvements of the suitable diagnostic approaches are needed for better understanding of the PMF development and progression.
Aims: The **aim** of the study was to investigate the karyotype of the peripheral blood cells from myeloid strain of hematopoesis in patients with PMF.
Methods: The cytogenetic study was carried out for the peripheral blood from 18 patients with symptomatic PMF. In the group were 9 males and 9 females. Six patients weren't treated previously, the others received hydroxyurea. The cytostatic agent was stopped for 72 h before the sample collection. The peripheral blood cells were cultivated for 24h with recombinant human granulocyte-colony stimulating factor (G-CSF). Cytogenetic analysis was performed using G-method of differential staining. 15-20 metaphases were analyzed for every sample.
Results: The mitotic activity in the investigated samples in the presence of G-CSF was high enough to produce adequate number of metaphases for routine cytogenetic analysis in PMF patients, even if the bone marrow aspiration was unsuccessful. Chromosomal abnormalities were revealed in the blood of 8 (44.4%) patients (4 males and 4 females). Other patients had normal karyotype. All patients with cytogenetical abnormalities had mosaic karyotype with 2 or more clones. The spectrum of karyotypic changes included trisomy of chromosome 8, structural rearrangements of chromosome 1, deletions of 5q and 20q, and monosomies, especially of chromosomes 5, 7, 19 and 21. The rearranged chromosome 1 appeared in extra copies in one sample. The hypodiploid karyotypes were notable findings. In 2 patients tetraploid clones were revealed.
Summary and Conclusions: The profile of the abnormalities is partially comparable with data obtained from BM in other studies. A wide spectrum of cytogenetic abnormalities and mosaicism may indicate a significant contribution of pathological myeloid precursors and stem cells to the evolution of the disease and to the development of therapy resistance. The abnormal polyploid clones revealed in the peripheral blood of PMF patients could be probably produced by the blast cells (like polyploid cancer cells in solid tumors), because the polyploid megakaryocytes in endomitosis were unlikely to appear in the peripheral blood. The further studies are needed to establish the role of karyotyping of the peripheral blood and the exact cytogenetical abnormalities relevant in the diagnostics and monitoring of PMF.

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CHARACTERIZATION OF MPL-MUTATED MYELOID NEOPLASMS: A REVIEW OF 224 MPL+ CASES

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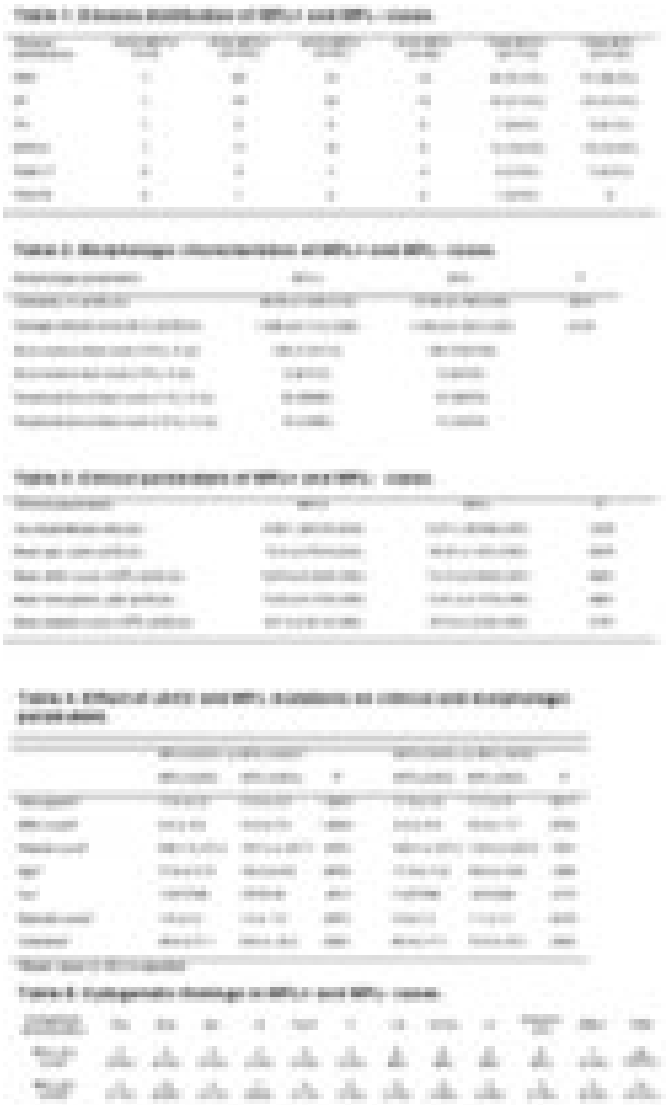
Background: Mutations within the myeloproliferative leukemia virus (*MPL*) gene have been identified in some myeloid neoplasms; however, MPL+ myeloid neoplasms have not been well characterized.
Aims: This study investigated the disease distribution, morphology, cytogenetic abnormalities, and select clinical features of MPL+ myeloid neoplasms in select cases.
Methods: 224 MPL+ myeloid neoplasms in blood or bone marrow from our case files from January 2008 to February 2012 and randomly selected 300

MPL-, BCR/ABL- myeloid neoplasms were retrived. Disease distribution, morphology, cytogenetic abnormalities, and select clinical features of MPL+ myeloid neoplasms were compared among select MPL+/JAK2-, MPL-/JAK2+, and JAK2-/MPL- cases.

Results: Results showed that MPL mutations occur in myeloid neoplasms including primary myelofibrosis; essential thrombocytosis; myeloproliferative neoplasm (MPN), unclassifiable; polycythemia vera (PV); refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T); and myeloid neoplasms associated with *PDGFB* rearrangement. Compared to MPL-, MPL+ cases are associated with lower hemoglobin, lower WBC counts, older age, higher reticulin scores, and lower bone marrow cellularity. Compared to MPL-/JAK2+, MPL+/JAK2- cases are associated with lower hemoglobin, lower WBC counts, lower bone marrow cellularity, but no significant differences in reticulin scores or patient age. Compared to MPL-/JAK2-, MPL+/JAK2- cases show significantly higher reticulin scores, but no statistical differences in hemoglobin, WBC counts, patient age, or bone marrow cellularity. MPL and JAK2 V617F mutations can coexist (7.9%); however, MPL mutations and BCR/ABL appear mutually exclusive. Overall incidence of cytogenetic abnormalities was not significantly different in cases with or without MPL mutations.



Summary and Conclusions: In this study, MPL positivity alone does not appear to result in a distinct entity.

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DEREGULATION OF MICRORNA146B-1 IS ASSOCIATED WITH MYELOFIBROSIS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: The myeloproliferative neoplasm (MPN) is a clonal hematopoietic disorder, which is characterized by extensive proliferation of multilineage blood cells and variable fibrosis. MicroRNAs (miRNAs) function as gene repressors by binding to the target messenger RNAs and which deregulation has been suggested to contribute to myeloproliferative neoplasm pathogenesis. Through miRNA profiling studies about MPN, some of miRNAs showed deregulated expression patterns associated with specific MPN subtypes or clinical presentations.

Aims: Our goal was to evaluate the expression of selected miRNAs previously described to be associated with primary myelofibrosis (PMF), in order to establish those miRNAs as potential markers for differentiating MPN subtypes or as fibrosis-associated markers.

Methods: Total 39 bone marrow samples of MPNs, 15 polycythemia vera (PV), 22 essential thrombocythemia (ET) and 5 PMF, and 15 normal controls which are not presenting fibrosis were included. Using stem loop-primed reverse transcription and quantitative real-time polymerase chain reaction, the expression of miRNAs (miR150-1, miR146b-1, miR31-1, miR95-2 and miR28-1) was evaluated from paraffin-embedded bone marrow biopsy. RNU6-2_11 was used as endogenous control for relative quantification. Information for *JAK2* V617F mutation, hematologic indexes and fibrosis grade was collected from medical records.

Results: Among miR150-1, miR146b-1, miR31-1, miR95-2 and miR28-1, the expression of miR146b-1 and miR150-1 were higher in MPNs compared to normal controls (*P*=0.022, 0.003, respectively). However, no significant differences were found among MPN subtypes. The expression of miR146b-1 was significantly associated with fibrosis grade. MPNs with severe fibrosis showed significantly higher expression of miR146b-1 than MPNs with moderate or mild fibrosis (*P*=0.015). There was no association between expression of miRNAs and J*AK2* V617F mutation or hematologic indexes such as WBC, platelet count, hemoglobin level or frequencies of complications.

Summary and Conclusions: We suggest that miR14b-1 and miR150-1 are upregulated in MPN patients and especially miR146b-1 is a potential fibrosis-associated factor. For evaluation of the contribution of miR146b-1 deregulation to fibrosis pathogenesis, study for larger number of patients is needed.

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HIGH THROUGHPUT SCREENING ON PERIFPERHAL BLOOD CD34 CELLS WITH A FLOW CYTOMETRY AUTOMATED PLATFORM (EXVITECH), TO IDENTIFY SENSITIVITY TO RUXOLITINIB IN MYELOFIBROSIS PATIENTS

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Background: Ruxolitinib is a potent JAK1/JAK2 inhibitor that has demonstrated improved survival, rapid decreased of splenomegaly in myelofibrosis (MF) patients; however, some of them do not have an adequate response, and identifying patients with a good response would enable personalizing its treatment.

Aims: To design an ex-vivo model, based on flow cytometry, to identify sensitivity to Ruxolutinib of CD34+ cells from peripheral blood and correlate it to their clinical evolution.

Methods: We have studied secondary or primary MF patients (n=12), and then we have compared three ex-vivo models to test Ruxolitinib sensitivity in patient samples from peripheral blood. Briefly, in the first model, mononuclear cells isolated from peripheral blood were cultured in Methocult TM GF_H4535 with 20 ng/ml IL-3, and 50 ng/ml SCF. After 2 weeks incubation, cells were plated at 15.000 per well in 96-well plates in increasing concentrations of Ruxolitinib. After incubation, at either 24-48-72 hours, cells were labelled with Annexin V and CD13, and analysed by the automated multiparametric flow cytometry platform ExviTech. In the second model, peripheral blood was treated directly with increasing concentration of Ruxolitinib. After 48-72 hr incubation, cells were analysed using Annexin V and CD34 to monitoring the sensitivity of CD34+ cells. In the third model we used the classic method; mononucleated cells were cultured in methylcellulose directly with increasing concentrations of Ruxolitinib for 12 to 14 days.

Results: In 9 out of 10 patient samples we could identify a population of 1.5±1.5% CD34+ cells in peripheral blood enough to study sensitivity to Ruxolitinib with the ExviTech platform. Freshly isolated CD34+ cells directly incubated with drug (model 2) were more sensitive to Ruxolitinib than cells treated after methylcellulose culture. Using this novel model 2, we identified 4 patients out of 7 with a high sensitivity to Ruxolutinib (Emax=92.5±10.7 *vs* Emax=23.8±11.8). However, in the model 1 using differentiated cells after 15 days of culture only 2 out of 8 samples were moderately sensitive to Ruxolitinib (Emax=49.39± 15.49). Nevertheless, in the third model, all samples were quite sensitive to Ruxolitinib, appearing very similar without appropriate stratification; they had an Emax=9.9±17.2. The correlation between the first and third models using 15 day of culture was not significant (p>0.5), but it showed a trend (Figure 1A). Model 2, incubating the drug in the freshly extracted cells, labelling CD34+ cells, seems to correlate with clinical response (decrease spleen size) of patient treated with Ruxolitinib efficacy (Emax), which measures the% cells left alive after incubation with the drug (Figure 1B). The more effi-

cacious Ruxolitinib is in depleting CD34+ cells in the patient samples, the more that the spleen volume decreases. Interestingly, mutated CALR CD34+ cells appear to be less sensitive (Emax=89.54±27.45) to Ruxolitinib than mutated JAK2V617F (Emax=29.7±38.0) (Figure 1C).

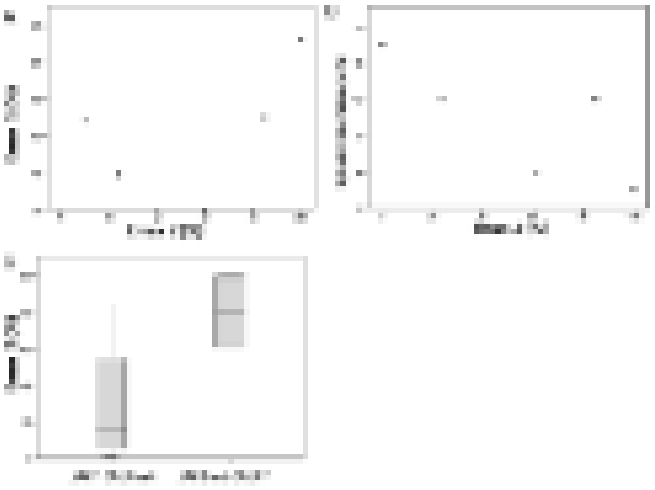

Figure 1. Comparison between model 1 Emax (Emax1) (Ruxolitinib treatment after 15 days in methylcellulose culture) and model 2 Emax (Emax2) Ruxolitinib treatment of fresh blood and CD34+ cell analysis). B) Clinical correlation between spleen decrease after 3 month of Ruxolitinib patient treatment, with model 2 Emax. C) Correlation between Emax2 and presence of mutated JAK2 (JAK2*) or mutated CALR (CALR*).

Summary and Conclusions: Study of the sensitivity to Ruxolitinib in CD34+ cells from peripheral Blood is feasible in MF patients; results are obtained in less than 72h and hence potentially prior to treatment. The best *ex vivo* model is the direct assay on freshly extracted CD34+ cells with minimal manipulation. It correlates better with Ruxolitinib clinical response in terms of spleen volume, and with the presence of JAK2 and CALR mutations. It seems that CD34+ cells are more sensitive to Ruxolitinib than the method of isolating and culturing cells for 15 days to differentiate.

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HEMATOPOIETIC PROGENITOR CELLS EXPRESSING VE-CADHERIN ARE DETECTABLE IN THE SPLEEN OF PATIENTS WITH MYELOFIBROSIS AND ARE IN CLOSE CONTACT WITH MICROVESSELS

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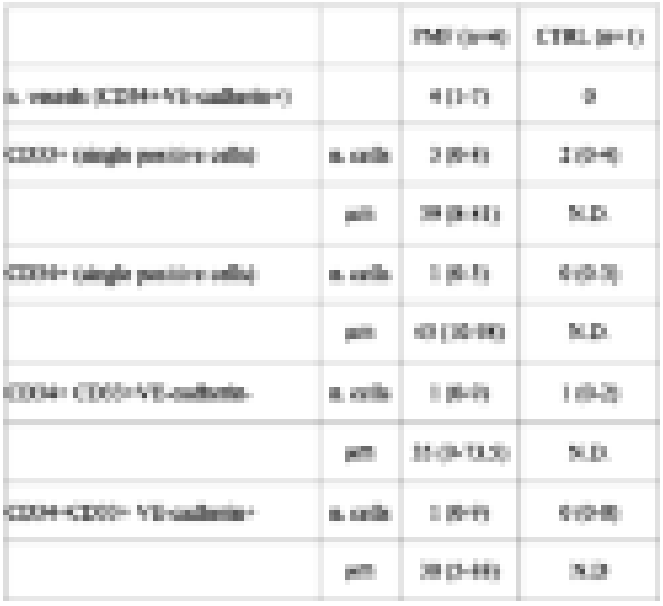
Background: Increased microvessel density contributes to abnormal bone marrow and spleen microenvironment in myelofibrosis (MF). In a previous work, we provided evidence that endothelial cells from either the spleen or the splenic vein of patients with MF frequently share the same genetic abnormality with the hematopoietic malignant cells: the *JAK2*V617F mutation (Rosti *et al.* Blood 2013). The existence of a common progenitor to endothelial and hematopoietic cells in which the V617F mutation occurs could explain the involvement of V617F-endothelial cells in the process of endotheliopoiesis and endothelium renewal. An alternative hypothesis for explaining the presence of the mutation in splenic endothelial cells and splenic vein is that the MF stem cell represents the cell giving rise to mutated endothelia. This event, which has been described in glioblastoma (Soda Y *et al.*, PNAS 2011) and in neuroblastoma (Pezzolo A *et al.*, J Clin Oncol 2007), would assimilate the spleen of patients with MF to a solid tumor and would justify the extension of the phenomenon to the microvessels and to the large vessels of the spleen.
Aims: To investigate the presence of transitional cells expressing both myeloid and endothelial cell markers in the spleen of patients with MF.
Methods: Spleens were collected from 4 patients with MF, who received splenectomy for transfusion-dependent anemia and/or relieve symptoms of massive splenomegaly, and a healthy subject who underwent spleen surgery after traumatic damage (CTRL). Fresh spleen samples were embedded in OCT, snap-frozen, and stored in liquid nitrogen. Spleen sections (4 µm) were stained with antibodies directed towards CD33 (myeloid lineage cells marker), CD34 (hematopoietic and endothelial progenitor cells marker), and VE-cadherin (endothelial marker). The images were obtained by confocal laser scanning microscopy (Olympus Fluoview FV10i, 60x objective) and processed by IMAGE J software. We evaluated 10 fields for each spleen section, measured the number of different cell subsets and their distance from the nearest vessel (in µm) (median/ten fields/patient). The results are shown as median/mean.
Results: The number of CD33+, CD34+, and CD34+CD33+ cells was higher in MF patients than in CTRLs. When we investigated the presence of cells expressing both hematopoietic and endothelial surface markers (CD34+CD33+VE-cadherin+), we found these cells in the spleen of all 4 MF patients (Table 1) and they were most likely to be found 40-50 µm from the nearest vessel structure. Besides, approximately 36% of the CD34+CD33+VE-cadherin+ cells were found within 20 µm of a vessel structure in MF spleens. On the contrary, CD34+CD33+VE-cadherin+ were never observed in the CTRL spleen.

Table 1.



Summary and Conclusions: Cells co-expressing both myeloid and endothelial markers can be identified in the spleen of patients with MF but not in a spleen from a normal subject. These "transitional cells", expressing the CD34, CD33 and VE-cadherin antigens, are located at a lower distance from vessels with respect to other cellular populations, supporting the hypothesis that they could contribute, through a transdifferentiation mechanism, to the formation of mutated endothelium.

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EVALUATION OF THE ASSOCIATION BETWEEN JAK2 46/1 HAPLOTYPE AND MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasm (MPN) is a hematologic malignancy characterized by chronic excessive production of one or more lineage of blood cells, and increased risk of secondary leukemic transformation. Recently, the G allele on *JAK2* rs10974944 which is part of the *JAK2* 46/1 haplotype has been suggested as a susceptibility factor of MPN development.
Aims: Our aim was to verify the relationship of *JAK2* 46/1 haplotype with development of MPNs in Korean population. Additionally, we evaluated the association between several clinicohematologic features and *JAK2* 46/1 haplotype to know how it works in MPN.
Methods: We selected 151 patients who had been diagnosed MPNs, including polycythaemia vera (PV, 35 patients), essential thrombocythaemia (ET, 108 patients), and primary myelofibrosis (PMF, 8 patients). Fifty healthy individuals were selected as normal control. The genotype of rs10974944 was analysed by direct sequencing.
Results: We found that the frequency of G allele was significantly higher in MPN patients (OR, 2.213; 95% CI 1.152~4.250, P<0.05) than in controls. The *JAK2* V617F mutation was higher in the G homo allele than in others (OR, 5.018; 95% CI 1.112~22.650, P<0.05) in studied population. In PV, patients which contain G allele (GG and GC) had higher WBC than those contain CC allele. ET patients contain GG/GC allele showed lower platelet count than those with CC allele. In *JAK2* V617F (+) ET patients, G allele was associated with more frequent organomegaly.

Summary and Conclusions: In aggrement with previous reports, the presence G allele of *JAK2* rs10974944 SNP is a possible oncogenic predisposing factor in MPN development. Moreover, at least partially, G allele has clinicohematologic effects in PV and ET.

P402

THE MULTIKINASE INHIBITOR R763/AS703569 BLOCKS STAT5 ACTIVITY AND INDUCES APOPTOSIS IN NEOPLASTIC HUMAN MAST CELLS

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Background: Systemic mastocytosis (SM) is a myeloid neoplasm defined by excessive proliferation and accumulation of neoplastic mast cells (MC) in one or more internal organs. Most patients with SM express a D816V-mutated variant of *KIT*, which confers resistance against several tyrosine kinase inhibitors (TKI) including imatinib. Apart from KIT, a number of additional kinase targets and signal transduction molecules, like Akt, Btk or STAT5, play a major role in growth and expansion of neoplastic MC in advanced SM. R763/AS703569 is a multi-targeted drug known to interact with a number of important signalling molecules such as Aurora kinase-A and -B, KIT or Btk.
Aims: We tested the effects of R763/AS703569 on growth, cell cycle-progression and survival of the human MC leukemia cell lines HMC-1.1 (lacking *KIT* D816V) and HMC-1.2 (expressing *KIT* D816V) as well as the canine mastocytoma cell lines C2 (harbouring a *KIT* ITD in exon 11) and NI-1 (exhibiting a duplication in exon 8 and a deletion in exon 10 of *KIT*).
Results: R763/AS703569 was found to counteract proliferation of both HMC-1 subclones with higher IC_{50} values obtained in HMC-1.1 cells (5-50 nM) than in HMC-1.2 cells (1-10 nM). We were also able to show that R763/AS703569 induces growth arrest in primary neoplastic MC in most (11/15) patients, with IC_{50} values ranging between 0.001 and 0.5 μM. In addition, R763/AS703569 was also able to inhibit growth of C2 cells (IC_{50}: 1-5 nM) and NI-1 cells (IC_{50}: 50-100 nM) in a dose-dependent manner. Furthermore R763/AS703569 induced a strong G2/M arrest in all cell lines tested. The R763/AS703569-induced growth inhibition was accompanied by apoptosis as evidenced by TUNEL assay, light microscopy and detection of cleaved caspase 3. As assessed by Western blotting, R763/AS703569 was found to inhibit the phosphorylation of several key kinase targets, including KIT, Btk, Akt and Aurora kinase-A in HMC-1 cells. In addition, R763/AS703569 was found to block the activation of STAT5 in both HMC-1 subclones. Interestingly, R763/AS703569-induced deactivation of STAT5 was seen within a few minutes and it occurred at very low drug concentrations (<10 nM), whereas the R763/AS703569-induced deactivation of KIT, Btk, Akt and Aurora kinase-A occurred between 100 and 1,000 nM. Based on the unique target spectrum and major drug-effects observed, we asked whether R763/AS703569 and other targeted drugs would exert synergistic anti-neoplastic effects. In order to address this question, drug combination experiments were performed. We found that R763/AS703569 cooperates with the multikinase inhibitors PKC412 (midostaurin) and dasatinib in producing growth inhibition and apoptosis in neoplastic MC.
Summary and Conclusions: Together, R763/AS703569 is a novel promising multi-kinase- and STAT5 blocker that overrides drug resistance in KIT D816V-transformed neoplastic MC. Whether R763/AS703569 is also able to block neoplastic cell growth *in vivo* in patients with advanced SM remains to be determined in clinical trials.

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RESULTS FROM A 3.5-YEAR UPDATE OF COMFORT-II, A PHASE 3 STUDY COMPARING RUXOLITINIB (RUX) WITH BEST AVAILABLE THERAPY (BAT) FOR THE TREATMENT OF MYELOFIBROSIS

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Background: Ruxolitinib (RUX), a potent JAK1/JAK2 inhibitor, has shown rapid and durable improvements in MF-related splenomegaly, symptoms, and quality of life measures in the phase 3 COMFORT studies. RUX therapy remains well tolerated and has been associated with a significant survival advantage at 2 and 3 years of follow-up in COMFORT-II.
Aims: This 3.5-year analysis of COMFORT-II presents updated efficacy and safety findings and longer-term survival data, including results from some patients (pts) for whom survival information was not previously collected.
Methods: COMFORT-II is a randomized (2:1), open-label study comparing RUX (15 or 20 mg bid based on baseline (BL) platelet count [100-200 or >200 × 10^9/L, respectively]) *vs* best available therapy (BAT) in pts with MF (N=219). Study findings are based on an intention-to-treat (ITT) analysis. An increase in spleen volume >25% over on-study nadir that was no longer a ≥35% reduction from BL was considered a loss of response. The original informed consent and case report form did not include follow-up of pts for survival after they had discontinued from the study, and only on amending was this information collected. Overall, 41 pts did not have follow-up information; 28 pts prior to the amendment. The Kaplan-Meier method was used to estimate overall survival (OS); analysis of OS has been updated with longer follow-up and to include additional survival information for 15 of 41 pts who previously lacked follow-up information (RUX, n=5; BAT, n=10).
Results: The median duration of RUX treatment in pts originally randomized to RUX (N=146) was 2.6 years. In the 73 pts originally randomized to BAT, 62% of pts (45/73) crossed over to RUX after a median of 66 weeks, with 40% (18/45) still ongoing in the extension phase. These pts have 1.2 years median duration of RUX treatment. Discontinuations were reported in 63% of RUX pts (92/146) primarily because of adverse events (AEs; 20%) and disease progression (18%). All BAT pts discontinued from the BAT arm. Of those who crossed over to receive RUX, 60% (27/45) discontinued; 18% (8/45) discontinued due to AEs. In total, 52% of RUX pts achieved a ≥35% reduction from BL spleen volume at least once during treatment with loss of response noted in 45% (34 pts) to date. No new or unexpected AEs were observed with longer follow-up. Since prior analysis (Cervantes *Blood* 2013), new grade 3/4 AEs included anemia (RUX, n=1), thrombocytopenia (RUX and RUX+extension phase, 1 pt each), pneumonia (RUX crossover, n=1), and acute renal failure (RUX, n=1). Overall AEs after treatment discontinuation were not different than AEs on treatment and no new patterns of AEs emerged. Overall, 27% (n=40) and 40% (n=30) of pts in the RUX and BAT arms, respectively, died during the study. Deaths that occurred on treatment or within 28 days after discontinuation were reported in 10% (n=14) of RUX pts and 8% (n=6) of BAT pts (2 deaths after RUX crossover). In the ITT analysis, RUX was associated with a 42% reduction in the risk of death compared with BAT (HR=0.58, 95% CI 0.36-0.93); the median OS has not yet been reached. The probability of survival at 3.5 years was 71% and 54% in the RUX and BAT arm, respectively (log-rank *P*=.02; Figure 1).

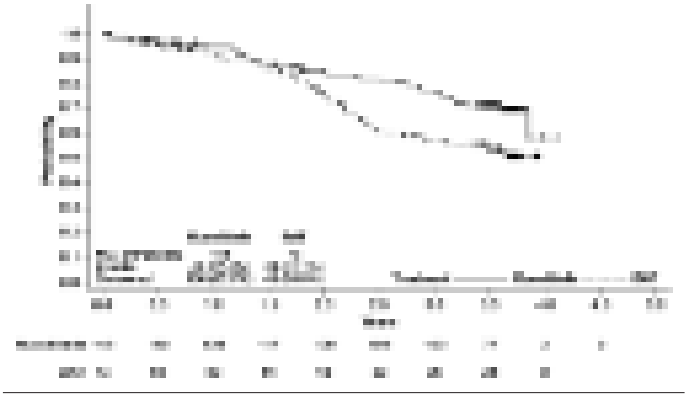

Figure 1. Overall survival in COMFORT-II (3.5 years).

Summary and Conclusions: RUX treatment continues to provide a survival advantage consistent with that observed in previous analyses. Inclusion of pts initially lost to follow-up results in separation of survival curves sooner than previously reported (78 *vs* 96 wk). The survival advantage is suggested to reflect improvements of outcomes in RUX pts and worsening of BAT pts. Because of the large number of pts who crossed over from the BAT arm to receive RUX, this OS analysis remains a conservative estimate of the effect of ruxolitinib treatment on survival.

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OUTCOME OF JAK2/MPL/CALR TRIPLE NEGATIVE PATIENTS WITH MYELOFIBROSIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Calreticulin (CALR) mutations have been identified in JAK2 and MPL wildtype patients with essential thrombocythemia and myelofibrosis, leaving a small proportion of patients without one of the three canonical mutations (triple negative patients). Triple negative patients with myelofibrosis not undergoing alloHSCT have been associated with poor outcome (Tefferi *et al.* 2014).
Aims: To evaluate clinical characteristics and outcome of myelofibrosis patients with or without *CALR*, *JAK2* or *MPL* mutations who underwent allogeneic hematopoietic stem cell transplantation (alloHSCT).
Methods: 133 patients with WHO defined primary myelofibrosis (n=97, 73%) or post-ET/post-PV myelofibrosis (n=36, 27%) underwent alloHSCT. Bone marrow or peripheral blood samples were obtained before transplantation. *JAK2, MPL*, and *CALR* were amplified by PCR and were directly sequenced. Mutations were confirmed in an independent experiment.
Results: Median age at transplant was 57 years (range 18-75). The majority of patients (n=90, 67%) were transplanted with a matched donor (29 from related and 61 from unrelated donors). Fourty-three patients (32%) received a mismatched unrelated allograft. The conditioning regimen was of reduced intensity in all but one patient. Fourty-five patients died by the time of last follow-up (34%). Median follow-up was 4.04 years after transplantation. Twenty nine patients (22%) were triple negative, 71 (53%) had JAK2 mutations, 28 (21%) had CALR and 5 (4%) had MPL mutations. Comparing triple negative patients with JAK2/CALR/MPL mutated patients there were no significant differences for patient, disease or transplant characteristics except a higher frequency of male patients in the group of triple negative patients (72% *vs* 50%, P=.03). Univariate analysis disclosed significant overall survival differences between triple negative, JAK2/MPL-mutated or CALR mutated patients (4-year OS 48.3% *vs* 65.8% *vs* 85.7%, respectively, P=.015, Figure 1). Cumulative incidence of relapse (CIR) was not significantly different in triple negative patients compared to JAK2/MPL or CALR mutated patients (4-year CIR 32% *vs* 23% *vs* 12% respectively, P=.70), while NRM was significantly higher in the triple negative patients compared to JAK2/MPL or CALR mutated patients (4-year NRM 50% *vs* 30% *vs* 8% respectively, P=.013). In multivariate analysis triple negative patients had significantly shorter OS and higher NRM compared to patients with any of the three CALR, JAK2, or MPL mutations (HR 2.1, 95%CI 1.12-4.02, P=.02; NRM: HR 2.2, 95%CI 1.10-4.71 P=.026). Of all other patient, disease and transplant characteristics only age above the median age and female donor sex predicted shorter OS after alloHSCT in multivariate analysis. Age above the median age remained as independent predictor of a higher risk for NRM. Stage III/IV acute GvHD was found in 20.4% and 15.4% of triple negative and patients with any mutation, respectively. Extensive chronic GvHD was found in 21.7% and 10.8% of triple negative and patients with any mutation, respectively.

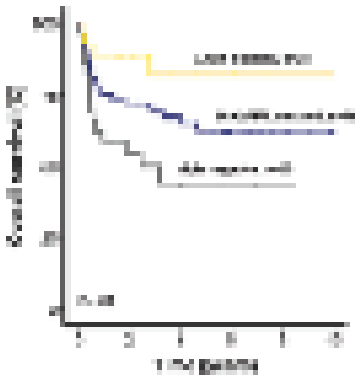

Figure 1.

Summary and Conclusions: Triple negative patients have a shorter overall survival after alloHSCT and a higher rate of NRM in comparison to patients carrying CALR, JAK2, or MPL mutations, possibly related to higher rates of more severe GvHD. However, alloHSCT can still induce long-term remissions in a significant proportion of triple negative patients. CALR mutation testing should be included in the work up of myelofibrosis patients undergoing alloHSCT as it can separate patients with favorable and less favorable prognosis.

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CHANGES IN BONE MARROW MORPHOLOGY IN PATIENTS WITH MYELOFIBROSIS TREATED FOR UP TO 5 YEARS WITH EITHER RUXOLITINIB OR BEST AVAILABLE THERAPY

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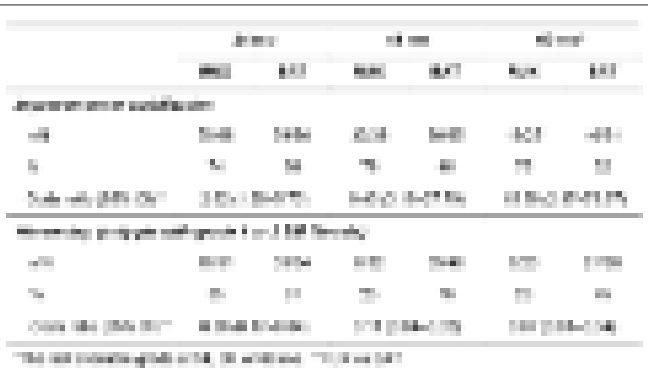
Background: Myelofibrosis (MF) is a life-shortening MPN characterized by progressive bone marrow (BM) fibrosis, extramedullary hematopoiesis, and debilitating symptoms. Conventional therapy for MF, even if prolonged, does not improve BM fibrosis.
Aims: This analysis evaluated the effects of up to 5 years therapy with ruxolitinib (RUX), a JAK1/JAK2 inhibitor, on BM fibrosis in a cohort of pts with MF from a phase 1/2 study (NCT00509899) and compared them with data from a cohort treated with best available therapy (BAT).
Methods: RUX-treated pts had primary MF (PMF), post-PV MF, or post-ET MF and were studied at the MD Anderson Cancer Center. BM biopsies were obtained at baseline (BL) and at 24 months (mo) (68 pts), 48 mo (38 pts), 54 mo (23 pts), 60 mo (10 pts), or 66 mo (4 pts) of RUX. For the BAT cohort, BM biopsies were prospectively collected from 192 pts with PMF treated with BAT (45% hydroxyurea, 8% interferon-α, 25% assorted therapies, 22% supportive-only therapy) for 24 mo (98 pts), 48 mo (65 pts), 54 mo (16 pts), 60 mo (9 pts), or 66 mo (6 pts). Samples from the BAT cohort were matched to those from the RUX cohort for BL BM morphology. Grades of reticulin fibrosis (WHO), collagen deposition, and osteosclerosis based on the ELN criteria were determined by consensus after independent blinded review by 3 pathologists (HMK/JT/CBR). Samples were categorized as showing improvement, stabilization, or worsening from BL in BM fibrosis grade. Odds ratios were determined by logistic regression. Separate analyses were conducted to explore associations of changes in (a) BM fibrosis grade or (b) the abundance of megakaryocytes (MEG) and select stromal cell populations (plasma cells, macrophages) and MEG dysplasia with changes in hemoglobin (Hb), platelet count, WBC count, and palpable spleen length (SPL) at 24 mo. Due to sample size limitations, these analyses were descriptive in nature.
Results: Compared with the RUX cohort, pts in the BAT cohort overall had lower IPSS risk (high-risk: RUX, 59%; BAT, 15%) and less splenomegaly (mean SPL: RUX, 18.8 cm; BAT, 3.6 cm) at BL. There were no statistically significant differences in percentage distribution of BM fibrosis grade (*P*=0.3298, Cochran-Mantel-Haenszel test). In the RUX cohort, at BL, 60% of pts had ≥90% hematopoietic cellularity; 22%, 53%, and 25% had BM fibrosis grade 1, 2, and 3, respectively; collagen accumulation and osteosclerosis was seen in 47% (18% grade 2/3) and 50% (19% grade 2/3) of pts, respectively. The odds of improved or stabilized BM fibrosis after 24, 48, and 60 mo were greater with RUX than BAT, whereas the odds of worsening were reduced with RUX *vs* BAT (Table 1). Improvement or stabilization of BM fibrosis with RUX was concordant with the direction of changes in both collagen deposition and osteosclerosis. A reduction in BM fibrosis grade at 24 mo coincided with mild reduction in Hb and WBC median values at 24 mo, with the latter being known JAK2 inhibition effects. Pts with an increased frequency of BM macrophages exhibited a higher median Hb, while a decreased number of stromal plasma cells was associated with an increase in Hb (both at 24 mo). Additional correlations between morphology changes with biomarker alterations are ongoing.

Table 1.



Summary and Conclusions: These results show that long-term RUX therapy, unlike BAT, may forestall or reverse BM fibrosis. Complex relationships between changes in fibrosis and clinicolaboratory parameters are possibly consistent with a JAK-mediated effect. Interrelationships of BM cell population alterations with clinicolaboratory changes denote a possible RUX effect on BM stroma biology. Collectively, these data suggest that sustained JAK inhibition may be disease-modifying in MF.

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SURVIVAL IN PATIENTS WITH FAMILIAL AND SPORADIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPNs) are chronic hematological malignancies associated with an elevated risk of thromboembolic events, transformation to acute myeloid leukemia, and a shortened life expectancy compared to the general population. MPNs show a familial clustering with a 5- to 7-fold elevated risk of MPNs among first-degree relatives of MPN patients compared to relatives of matched controls. There are however no studies comparing survival in patients with familial *versus* sporadic MPN.
Aims: To elucidate possible differences in survival in familial compared to sporadic MPN patients in a large population-based study.
Methods: Patients diagnosed with MPNs between 1958 and 2005 were identified through the Swedish Cancer Register and our national MPN network consisting of all hematology and oncology centers in Sweden. Time of death was obtained from the Cause of Death Register with follow-up to December 31, 2007. MPN patients who had at least one linkable first-degree relative with any MPN in the Swedish Multigenerational Register were included in the study. Cox regression was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) of death from any cause in familial compared to sporadic MPN patients. Survival was estimated by the Kaplan-Meier method.
Results: A total of 8,122 MPN patients with a linkable first-degree relative were included. Ninety-two (1.1%) MPN patients had a first-degree relative with any MPN. We found no differences in overall survival in patients with familial MPN compared to sporadic MPN, HR=1.1 (95% CI 0.9-1.5; p=0.39; Figure 1). In stratified analyses, there were no significant differences in outcome between male MPN patients (HR=1.2, 95% CI 0.7-1.9), female MPN patients (HR=1.1, 95% CI 0.7-1.8) or between MPN patients aged <70 year (HR=0.8 95% CI 0.5-1.2) and ≥70 years (HR=1.2, 95% 0.7-2.1) when familial MPN patients were compared to the corresponding sporadic MPN patients groups. In addition, in stratified analyses of patients with different MPN subtypes (polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF) and MPN unclassifiable (MPN-U)), there was no difference in survival in patients with or without a family history of MPN. The HRs in familial MPN patients with PV, ET, MPN, MPN-U were HR=1.2 (95% CI 0.8-1.9), HR=1.0 (95% CI 0.4-2.4), HR=0.4 (95% CI 0.03-5.1), and HR=1.3 (95% CI 0.43-5.1), respectively when compared to sporadic MPN patients of the corresponding subtype.

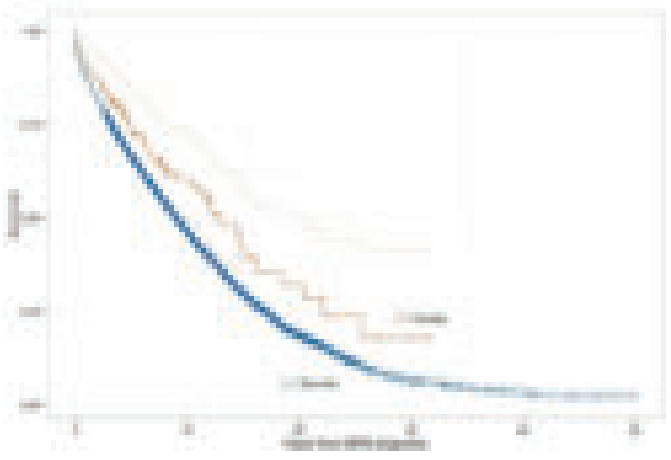

Figure 1. Kaplan-Meier curve of overall survival over time in familial and sporadic MPN patients with 95% confidence intervals.

Summary and Conclusions: In this large population-based on more than 8,000 patients, we found survival to be similar in patients with familial and sporadic MPNs. Our findings do not suggest a more aggressive disease course in familial MPNs. We recommend that family history of MPN should be incorporated in the clinical work-up of MPN patients. However until we gain more insight regarding the potential differences in genetic background and in rate of complications, clinical management should not differ between familial and sporadic MPN patients.

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SPLANCNIC VEIN THROMBOSIS ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASMS. A STUDY OF THE IWG-MRT IN 494 SUBJECTS.

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Background: Philadelphia-negative Myeloproliferative Neoplasms (MPN) include Polycythemia Vera (PV), Essential Thrombocythemia (ET), Myelofibrosis both Primary (PMF) and secondary to PV and ET (PPV-, PET-MF) as well as unclassified MPN (U-MPN). An increased risk of venous thrombosis in unusual sites, ie splanchnic vessels (SVT), is particularly associated with MPN. SVT can lead to complications such as portal hypertension, esophageal and gastric varices, ascites and hepatic failure. A recent meta-analysis reported that a MPN is the underlying cause of portal vein thrombosis in 31.5% and of Budd Chiari syndrome in 40.9% of cases (Smalberg, 2012); however analysis of disease characteristics and outcome has been hampered by heterogeneity of available patients' cohorts comprising relatively small number of cases.
Aims: We conducted a retrospective multicenter study collecting clinical and biological data of patients (pts) with SVT associated with WHO2008-diagnosed MPN, with the aim to describe patients' characteristics, trends and prognostic factors that may have implications for clinical practice.
Methods: Data were collected from 16 international hematologic centers in the framework of IWG-MRT.
Results: A total of 494 cases of portal, splenic or mesenteric vein thrombosis (75.2%) or Budd Chiari syndrome (24.8%) associated with MPN were collected. Current analysis refers to 475/494 cases, and final data will be presented at EHA meeting. Frequency of MPN associated subtype was 38.1% ET (n=181), 34.9% PV (n=166), 16.2% MF (n=77), 10.8% U-MPN (n=51). Median follow-up 87.9 mo (range 0.5-430); female 61.3% (n=292; P<0.0001 *vs* male); median age at MPN diagnosis (dg) 44.4 y (range 12-90), significantly younger than non-SVT associated MPN. In 229 cases (48%) MPN and SVT dg were coincident, while in 104 (22%) SVT occurred before MPN dg (median 40 mo, range 5-335) and in 129 (27%) during MPN follow up (median 79 mo, range 5-394). Biological featured included JAK2V617F mutation present in 99% PV, 84.7% ET, 88.1% PMF and 92.9% U-MPN pts, while erythropoietin-independent colonies (EEC) were present at diagnosis in 80/110 evaluated cases (72.7%), 38/47 PV (84.4%), 32/45 ET (71.1%), 8/11 PMF (72.7%) and 2/7 U-MPN (28.6%). A concurrent thrombophilic status was found in 38.9% of cases. Therapy after SVT included anticoagulation in 77% of pts, antiaggregant therapy in 23.5% and both in 1.5%; 68.8% of pts received cytotoxic drugs, 11.4% were treated with trans jugular portosystemic shunt. No differences in survival were noted with these approaches. Beta blockers was used in 48.5% of pts and correlated with improved survival (p=0.041). At last follow up 70/473 pts (14.8%) died; causes of death are evolution to AL (16.4%), other cancers (14.5%), disease progression without AL (12.7%), SVT (10.9%), hepatic failure and venous thrombosis other than SVT (9.1% each), heart failure and arterial thrombosis (7.3% each), hemorrhage (5.5%), renal failure and infection (3.6% each). After 10 y follow up 8/166 PV (5%), 14/181 ET (8%), 14/77 PMF (18%) and 1/51 U-MPN (1.96%) pts died (p<0.01). Survival was significantly affected by occurrence of thrombosis other than SVT (p<0.0001), that occurred in 35.8% of pts but not by recurrence in splanchnic vessels (p=0.068).
Summary and Conclusions: This large study describes characteristics, therapeutic options and outcome of SVT associated with MPN, pointing to an overall good prognosis compared with non-SVT associated MPN and identified thrombosis in districts other than splancnic district as the leading cause of death, suggesting the need to potentiate antithrombotic therapy.

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CLINICAL CHARACTERISTICS IN MYELOPROLIFERATIVE NEOPLASM WITH CALRETICULIN MUTATIONS

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Background: Somatic insertions/deletions in the calreticulin (CALR) gene were recently identified in the background of JAK2 V617F negative myeloproliferative neoplasm (MPN).
Aims: The aim of our study was to investigate the frequency and clinical characteristics of CALR mutation-positive MPN in a cohort of Hungarian patients.
Methods: A combination of allele-specific PCR (JAK2-V617F, MPL), quantitative TaqMan-assay (JAK2-V617F), high resolution melting (MPL), fragment-sizing (CALR) and Sanger-sequencing (CALR, MPL) was applied to identify the driver mutation in 215 polycythemia vera (PV), 289 essential thrombocytosis (ET) and 99 primary myelofibrosis (PMF) patients.
Results: PV patients all carried the JAK2 V617F mutation. In the ET cohort, the frequency of V617Fmut was 53.3% (n=154), CALRmut 33.2% (n=96), MPLmut 3.1% (n=9), while 30 patients (10.4%) were JAK2-CALR-MPL mutation negative. Similar distribution of the above mentioned somatic mutations was observed in PMF patients [56.6% V617Fmut (n=56), 25.3% CALRmut (n=25), 7.0% MPLmut (n=7), and 11.1% triple-negative (n=11)]. Comparing CALRmut ET-patients to V617Fmut ET patients, younger age at disease onset (53 *vs.* 61 years, p=0.03), higher platelet count (981 *vs.* 778 G/L; p<0.001), lower hemoglobin (131 *vs.* 147 g/L; p<0.001) and lower white blood cell count (9 *vs.* 10 G/L; p=0.034) was observed. Venous thrombosis (7.1 *vs.*17.6%, p=0.03), arterial thrombosis (9.4 *vs.* 14.4%, p=0.3) or hemorrhage (4.7 *vs.* 9.2%, p=0.3) occurred less frequently in CALRmut compared to V617Fmut ET patients, resulting in lower risk for vascular complications (17.6 *vs.* 35.9%, p=0.003). On the other hand, post-ET myelofibrosis was more frequent in CALRmut patients compared to V617Fmut ET patients (14.9 *vs.* 5.9%, p=0.03), while leukemic-transformation occurred with the same frequency (3.4 *vs.* 2.6%). The type of driver mutation did not influence overall survival in the ET cohort. Among MF patients, younger age at disease onset (55 *vs.* 69 years, p=0.002) and higher platelet count (535 *vs.* 250 G/L; p=0.001) were observed in the CALRmut subgroup compared to JAK2mut patients. Leukemic-transformation was more frequent among the triple negative cases (36.4%; 4/11) compared to V617Fmut (9.3%; 5/54, p=0.038) or CALRmut cases (13.6%; 3/22, p=0.2). CALRmut PMF-patients showed better overall survival compared to JAK2mut PMF-patients (p=0.04) and to triple-negative PMF-cases (p=0.01). In multivariate analyzes, carriership of CALRmut was characterized by HR=0.13, (0.012-0.739, p=0.021) compared to triple negatives. The ratio of type 2 to type 1 CALR mutation was smaller in PMF compared to ET (p=0.05). In ET, CALR mutation load was higher than JAK2 mutation load (p<0.001). CALR mutation load rarely exceeded 50%, but gradually increased in advanced MPN-stages.
Summary and Conclusions: In the present study, we confirmed in an independent cohort that, CALRmut MPN associated with distinct clinical characteristics and extended the available observations to relationships between CALR mutation type, load and clinical outcome.

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JAK2 V617F ALLELE BURDEN AND VASCULAR COMPLICATION OCCURRENCE RISK IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Patients with Philadelphia-negative myeloproliferative neoplasms (Ph^-, MPNs) are at increased risk of thromboembolic and hemorrhagic complications. Although the presence of *JAK2* V617F mutation is deemed an important diagnostic criterion for MPNs and is considered as a new thrombosis risk factor beside commonly recognized: age over 60 years and previous history of thrombosis, it remains ambiguous and conflicting in the few previous studies, whether its quantity influences the occurrence of vascular complications in MPNs patients.
Aims: The aim of the study was to establish the influence of *JAK2* V617F presence and allele burden on the risk of vascular complications in MPNs patients and assess its possible application in the risk stratification for vascular complications in patients with Ph^-, MPN.
Methods: Analysis was performed in a cohort of 186 patients diagnosed with polycythemia vera (53), essential thrombocythemia (114), primary myelofibrosis (11) and unclassified MPN (8). Assessment of *JAK2* V617F-positivity was conducted with amplification-refractory mutation system polymerase chain reaction (ARMS-PCR). The type and prevalence of any vascular complications clinically proven and/or confirmed by diagnostic imaging in the studied group over a median follow-up of 29.5 months (range: 0-306), based on a retrospective analysis of patients medical files. The risk of vascular complications in 126 *JAK2* V617F-positive patients was analyzed with respect to allele burden, defined as the ratio of mutated *JAK2* V617F to both *JAK2* V617F and wild-type *JAK2* V617F, assessed with allele-specific 'real-time' quantitative polymerase chain reaction (AS RQ-PCR).
Results: Overall prevalence of any vascular complications was 42.5%. Arterial thrombosis occurred in 22%, venous thromboembolism (VTE) in 11.8%, bleeding episodes in 23.7% of patients. Individuals harboring *JAK2* V617F mutation, regardless of MPN type, were at higher risk of venous thromboembolism (OR=5.5, 95% CI: 1.2-24.5, P=0.006). *JAK2* allele burden higher than 25% identified patients with 7.4-fold increased risk of VTE (95% CI: 1.6-34.7, P=0.004). Nevertheless, no significant increase in the risk of bleeding complications was found in this group in comparison with V617F-negative population OR=0.8, 95% CI: 0.3-2.1, P=0.8. The number of venous thromboembolic events progressively increased in patients along with the increase in *JAK2* V617F allele burden (P=0.0014 using Chi-squared test for trend). Patients with the result >75% of mutant allele burden were also at 4.5-fold higher risk for arterial thrombosis (95%CI: 1.4-13.8, P=0.01). The prognostic value of the mutant allele burden for predicting VTE was confirmed by ROC curve analysis. The best proposed cut-off value for the increase of venous thromboembolism risk was 27% of mutant allele burden with sensitivity of 60% and specificity of 57.5% (Figure 1).

Figure 1. Effects of the *JAK2* V617F mutant allele burden on the risk of vascular complications in patients with Ph MPNs.

Summary and Conclusions: The group of patients with allele burden higher than 25%, as they are at increased risk of VTE and at no increased risk of bleeding, may benefit the most from vigilant monitoring and appropriate prophylaxis against vascular events. Patients with a *JAK2* V617F allele burden higher than 75% require additional management to prevent arterial thrombosis. The inclusion of *JAK2* V617F mutant allele burden in new risk stratifications seems to be justified and requires controlled prospective trials.

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RUXOLITINIB PLUS PANOBINOSTAT IN PATIENTS WITH PRIMARY MYELOFIBROSIS, POST–POLYCYTHEMIA VERA MYELOFIBROSIS OR POST–ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS: A PHASE 1B DOSE-FINDING STUDY

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Background: Myelofibrosis (MF) is a clonal myeloproliferative neoplasm characterized by dysregulation of the Janus kinase (JAK) pathway, resulting in bone marrow fibrosis, splenomegaly, and debilitating constitutional symptoms. Ruxolitinib (RUX), a potent JAK1/JAK2 inhibitor, demonstrated superiority in spleen volume reduction, symptom improvement, and survival compared with the control arm in the phase 3 COMFORT-I and COMFORT-II studies. Panobinostat (PAN) is a potent pan-deacetylase inhibitor (pan-DACi) that inhibits JAK signaling through disruption of the JAK2 protein chaperone heat shock protein 90. The combination of RUX and PAN demonstrated synergistic anti-MF activity in preclinical studies. In phase 1/2 studies, PAN has shown splenomegaly reduction and improvement of marrow fibrosis. The updated results of a phase 1b study of the combination of RUX and PAN in patients (pts) with MF are presented here.

Aims: The primary objective was determination of the recommended phase 2 dose (RP2D) and/or maximum tolerated dose. Secondary objectives included safety, efficacy, and pharmacokinetics.
Methods: Eligible pts had intermediate-1–, -2–, or high-risk MF by International Prognostic Scoring System criteria, with palpable splenomegaly (≥5 cm below the costal margin). The treatment schedule was RUX (5-15 mg) twice daily (bid) every day and PAN (10-25 mg) once daily 3 times per week (tiw; days 2, 4, and 6) every other week (qow) in a 28-day cycle. The primary objective was determination of the recommended phase 2 dose (RP2D) and/or maximum tolerated dose. Dose escalation was guided by a Bayesian logistic regression model with overdose control based on cycle 1 dose-limiting toxicities (DLTs) and additional safety findings. Following identification of the RP2D, additional pts were enrolled and treated at this dose.
Results: Preliminary data presented here are based on a cutoff date of July 31, 2013. A total of 48 pts were enrolled (38 escalation phase and 10 expansion phase). Three DLTs were observed in the escalation phase (grade 4 thrombocytopenia [n=2], grade 3 nausea [n=1]). Preliminary RP2D was identified at RUX 15 mg bid every day and PAN 25 mg tiw qow. In the escalation phase, grade 3/4 adverse events (AEs) regardless of causality included anemia (42%), thrombocytopenia (21%), abdominal pain (8%), and diarrhea (8%), and AEs led to discontinuation in 21% of pts. Anemia (8%) was the most common serious AE in the escalation phase. Grade 3/4 AEs in the expansion phase included anemia (20%) and asthenia (10%). Preliminary activity in the dose-escalation phase was demonstrated by ≥50% decrease in palpable spleen length at any time in 76% of pts, with 50% of pts demonstrating 100% (nonpalpable spleen) response (Figure 1). In the expansion phase, all 4 evaluable pts demonstrated a best spleen response of 100% (nonpalpable spleen).

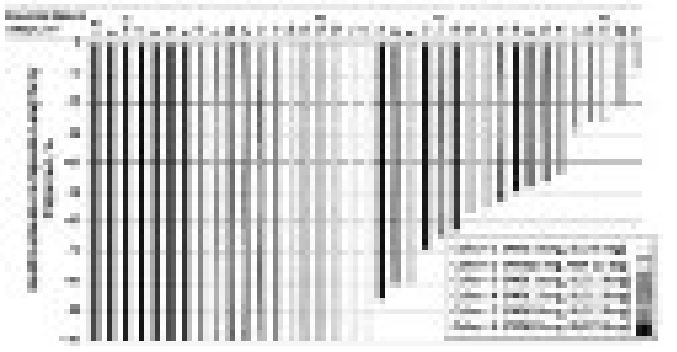

Figure 1.

Summary and Conclusions: The combination of the JAK1/JAK2 inhibitor RUX and the pan-DACi PAN demonstrated a tolerable safety profile with encouraging efficacy as demonstrated by spleen responses in patients with intermediate- and high-risk MF. Patients are currently being treated in the expansion phase at the RP2D. Updated safety and efficacy data from the expansion phase will be presented.

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UTILITY OF BONE MARROW HISTOLOGY AND IMMUNOHISTOCHEMISTRY IN THE DIAGNOSTIC WORK-UP OF EOSINOPHILIA OF UNKNOWN SIGNIFICANCE

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Background: Clonal eosinophilia is associated with disparate myeloid neoplasms and the presence of tyrosine kinase fusion genes of which *FIP1L1-PDGFRA* is by far the most frequent. In contrast, sustained non-clonal eosinophilia with organ involvement is associated with autoimmune disorders (reactive hypereosinophilia, HE_R), *e.g.* eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome) or as a diagnosis of exclusion, hypereosinophilic syndrome (HES). Irrespective of the final diagnosis, the majority (>80%) of patients with non-clonal eosinophilia achieve durable complete remissions on corticosteroid-based immunosuppression. In cases without a clear diagnosis, a rapid response to corticosteroids is therefore considered as useful marker for almost definite exclusion of clonal eosinophilia. Bone marrow (BM) histology is considered an essential tool in the diagnostic work-up of hypereosinophilia of unknown significance (HE_{US}) and the subsequent differentiation between clonal or non-clonal origin.
Aims: Within the "German Registry on Disorders of Eosinophils and Mast Cells", we therefore retrospectively analysed initially and routinely evaluated histopathological und immunohistochemical BM characteristics of 116 patients who were subsequently identified as *FIP1L1-PDGFRA* positive (n=56) or corticosteroid-responsive HE_R/HES (n=60). The latter group was chosen to maximally exclude the possibility of hidden clonality.
Results: Useful pointers towards a diagnosis of *FIP1L1-PDGFRA* positive disorders were BM hypercellularity (45/56, 80% *vs.* 23/53, 43%; p<0.05) and increased numbers of (CD34-positive) blasts (11/56, 20% *vs.* 2/60, 3%; p<0.05) whereas relative numbers of eosinophils (43%, range 5-80 *vs.* 26%, range 5-30; n.s.) were not informative. Additional features of *FIP1L1-PDGFRA* positivity were significant fibrosis (25/35, 71% *vs.* 1/23, 4%; p<0.05), increased numbers of loosely scattered mast cells (23/33, 70% *vs.* 7/23, 30%; tryptase/CD117 positive, 17/17, 100% *vs.* 4/12, 33%; p<0.05) and aberrant expression of CD25 on mast cells (11/18, 61% *vs.* 2/13, 15%; p<0.05). However, these stainings were in fact only performed in ≤50% of patients (fibrosis 58/116, 50%; tryptase/CD117 29/116, 25%; CD25 31/116, 27%). In *FIP1L1-PDGFRA* positive disease, clonal eosinophilia was suggested in 36/56 (64%) and non-clonal eosinophilia in 13/56 (23%) of cases, while no final diagnosis was made in 7/56 (13%) cases. Preliminary diagnoses towards clonal eosinophilia included chronic eosinophilic leukemia (CEL, n=15), systemic mastocytosis (SM, n=6), chronic myeloid leukemia (CML, n=5) and myeloid neoplasm unclassified in chronic or blast phase (MPN_u, n=10). In HE_R/HES, non-clonal eosinophilia was suggested in 43/60 (72%) and clonal eosinophilia in 7/60 (12%) of cases, while no final diagnosis was made in 10/60 (16%) patients. The overall sensitivity for reliable diagnosis of clonal or non-clonal eosinophilia was 68% (79/116).
Summary and Conclusions: We therefore conclude that 1) numbers of eosinophils in BM are not useful to differentiate between clonal and non-clonal eosinophilia, 2) the most informative stains that fit in with standard work-flows include CD34 for blast cells, tryptase/CD117/CD25 for mast cells and Gomori´s stain for fibrosis, 3) a broad range of myeloid neoplasms (*e.g.* CEL, SM, MPN_u) are associated with suspected clonal eosinophilia, and 4) appropriate use of available and potentially new markers in combination with reference pathology in experienced centers should allow to improve the currently limited diagnostic value of routine BM examinations in the diagnostic work-up of HE_{US}.

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INFLUENCE OF IL-28B AND IL-6 GENETIC POLYMORPHISM ON IFN-ALFA TREATMENT OUTCOME IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL TROMBOCYTHEMIA

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Background: In Polycythemia Vera (PV) and Essential Trombocythemia (ET), Interferon-α (IFN-α) is a therapeutic option with a potential of inducing hematological and molecular response. However, the treatment may be limited due to a lack of efficacy and a multitude of adverse side effects. Genetic polymorphism in the region of the Interleukin-28B (*IL-28B)* gene on chromosome 19 was recently found to be strongly predictive of the responsiveness to dual therapy with Peg-IFN-α and Ribavirin in Hepatitis C virus (HCV) genotype 1 patients. Using genome-wide association studies (GWAS), favorable single nucleotide polymorphisms (SNP) was identified, in particular the rs12979860 CC, rs8099917 TT and rs12980275 AA genotypes (gt), showing increased sustained virological response (SVR) rates with IFN-α treatment. Interleukin-6 (*IL-6*) is a proinflammatory cytokine with a documented role in disease progression of several neoplasms. The G-174C polymorphism of the *IL-6* gene controls serum levels of *IL-6*, with the G allele found to have a higher transcriptional level than the variant C allele and the presence of the C allele resulting in lower *IL-6* levels.
Aims: The outcome of IFN-α treatment may be related to inborn variations in genes involved in inflammation. We address whether polymorphism in the genes of *IL-28B* and *IL-6* influence the outcome of IFN-α treatment in PV and ET.
Methods: In total, 90 patients with PV (n=47) or ET (n=43), currently or previously treated with IFN-α, were included. Prior to IFN-α, 28 patients had been treated with hydroxyurea. Hematological response was evaluated using the European Leukemia Net (ELN) criteria from 2008. Written informed consent was obtained from all patiens. DNA was extracted from whole blood, and genotyping of the four SNPs, rs12979860, rs8099917, rs12980275 in the *IL-28B* gene locus and G-174C in the *IL-6* gene locus was performed by Taqman analysis.

Results: Complete response (CR) was achieved in 28 patients with PV and 30 patients with ET. Partial (PR) or no response (NR) was noted in 19 patients with PV and in 13 patients with ET. The median lenght of IFN-α treatment was 35 months. In the group of PV patients presenting with the favorable *IL-28B* rs12979860 CC gt, 78% responded with CR compared to 46% in the group with CT+TT (p=0,018). A similar response was seen in the combined group of PV and ET with 79% responding with CR in comparison to 54% (p=0,016). The results with regard to *IL28B* rs12980275 AA/AG+GG were similar to the above reported of rs12979860 CC/CT+TT, supposedly due to a well described linkage disequilibrium. The *IL-28B* rs8099917 gt TT/TG+GG did not show a significant association in any group. The combined group of carriers of the CC gt of *IL-6* G-174C had an inferior outcome with CR in 41% compared to carriers of CG or GG gt with CR in 69% (p=0,048). In subgroup analyses, the result for PV (p=0,047) was significant, but not the result for ET (p=0,426) (Table 1).

Table 1. Hematological response (ELN-criteria 2008) during INF-α treatment with regard to IL-28B and IL-6 genotypes.

Summary and Conclusions: Patients with the CC gt of *IL-28B* rs12979860, the AA gt of *IL-28B* rs12980275 and the non-CC gt of *IL-6* G-174C achieve CR more frequently compared to patients carrying the variant alleles. These differences were statistically significant in PV and in the PV+ET group, but not for ET alone. Thus, despite the heterogeneity of the patient cohort, we found evidence suggesting that inborn variations in *IL-28B* and *IL-6* gt influences the hematological response to IFN-α treatment.

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DYNAMICS OF CALR MUTANT ALLELE BURDEN IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

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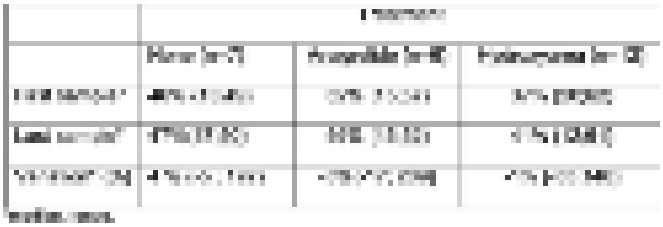
Background: Mutations in the calreticulin gene (*CALR*) have been recently described in around 49-84% essential thrombocythemia (ET) and 67-88% primary myelofibrosis (PMF) patients, which lack *JAK2* and *MPL* mutations. However, there is limited information regarding the dynamics of *CALR* mutant allele burden over time in the absence or presence of cytoreductive treatment.
Aims: To analyze the dynamics of *CALR* mutant allele burden over time in a cohort of ET patients.
Methods: From a whole cohort of 220 ET patients diagnosed in a single institution, we analyzed the presence of *CALR* mutations in 74 patients lacking mutations in the *JAK2* (p.V617F) and the *MPL* (exon 10) genes. The mutational analysis of exon 9 of the *CALR* gene was performed in DNA extracted from purified granulocytes by PCR, using a 6-carboxyfluorescein labeled reverse primer, followed by fragment analysis in a Genetic Analyzer 3500DX (Applied Biosystems). The *CALR* mutated allele burden was calculated as the percentage of the peak area of the mutated allele/peak area of the total (wild type+mutated) *CALR*. Variation in the *CALR* mutated allele burden was calculated as follows: (*CALR* mutant in last sample- *CALR* mutant in first sample)/ *CALR* mutant in first samplex100.
Results: *CALR* mutations were detected in 33/74 (45%) patients negative for *JAK2* p.V617F and *MPL* mutations. The cohort of *CALR* positive patients comprised 10 males and 23 females with a median age of 59 years (range 23-93). The mutations detected were: del 52bp (type 1 mutation) in 14/33 cases (42.4%), insertion of 5bp (type 2 mutation) in 12/33 patients (36.4%) and the remaining 7 were other types of mutations (21.2%), including 3 cases in which an insertion or deletion of 1 bp was detected. The median value of *CALR* mutant allele burden in the first sample available was 44.28% (range 13-62%). In 25 patients sequential samples could be analyzed, 7 cases received no treatment, 6 cases received anagrelide and 12 received hydroxyurea (HU) (Table 1). The median time between the first and last sample was 8 years (range 1-12 years). No significant differences were observed among *CALR* mutant allele burden in untreated and treated patients or between anagrelide or HU treatment. Nevertheless, in 2 untreated patients an increase higher than 10% in the *CALR* allelic ratio was observed during follow-up, and in 5/12 patients receiving HU a decrease higher than 15% was observed over time.

Table 1.



Summary and Conclusions: *CALR* mutant allele burden remains stable over time in untreated and cytoreductive-treated ET patients, although in some individual cases a significant variation can be observed.
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RESPONSE TO RUXOLITINIB IN PATIENTS WITH INTERMEDIATE-1, INTERMEDIATE-2 AND HIGH-RISK MYELOFIBROSIS: RESULTS OF THE UK ROBUST TRIAL

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Background: Ruxolitinib, a small molecule inhibitor of Janus Kinase 1 and 2 (JAK1/2), down-regulates the JAK/STAT pathway which is overactive in myelofibrosis (MF). MF is characterized by bone marrow fibrosis, splenomegaly and reduced health-related quality of life (HRQoL). Ruxolitinib demonstrated a statistically significant reduction in splenomegaly compared with placebo or best available treatment in phase 3 trials (COMFORT-1 and -2, respectively) in patients with intermediate-2 and high-risk disease, according to the International Prognostic Scoring System (IPSS).
Aims: ROBUST, a UK single-arm, open-label phase 2 trial aimed to assess response to ruxolitinib in patients with MF (including patients with intermediate-1 disease plus splenomegaly) using a novel composite endpoint, treatment success, defined as a spleen and/or symptom response and to assess the impact of treatment on HRQoL using the EQ-5D.
Methods: Adults with primary MF, post-polycythemia vera MF and post-essential thrombocythemia MF (World Health Organization criteria) were enrolled in this open-label, multicentre UK study. Patients had intermediate-1, intermediate-2 and high-risk disease (according to the IPSS) with or without prior treatment (excluding JAK2 inhibitors). Patients initiated treatment with ruxolitinib 15 or 20mg twice daily according to baseline platelet levels and the dose could be increased in patients with inadequate responses. Treatment was continued until progression, unacceptable toxicity, death or withdrawal. Spleen length (assessed by palpation), total symptom score (TSS, assessed with the MF Symptom Assessment Form out of 100, MF-SAF) and HRQoL (assessed with the EQ-5D; 1.00 as best health) were measured at baseline and weeks 4, 8 (spleen length only), 12, 24 and 48. The primary composite endpoint, treatment success, was defined as the proportion of patients achieving a 50% reduction in spleen length and/or 50% decrease in TSS at 48 weeks. This final analysis presents data at 48 weeks.
Results: A total of 48 patients (intermediate-1:n=14; intermediate-2:n=13; high:n=21) were included. Mean±SD age was 69.1±10.4 years and 43.8% of patients were female. At baseline, patients had Eastern Cooperative Oncology Group (ECOG) scores of 0 (52.1%), 1 (35.4%) or 2 (12.5%), mean±SD spleen length was 13.5±7.3cm, mean±SD TSS was 16.3±11.9 and mean±SE EQ-5D was 0.72±0.03. Reductions in spleen length and improvements in symptoms scores from baseline to week 48 were seen in all IPSS groups (Table 1). At week 48, 35 and 18 of 48 patients were evaluable for spleen length and TSS, respectively. From baseline, mean spleen length decreased by 5.8±7.0cm and mean TSS improved by 7.8±10.0. In the total population at week 48, 39.6% of patients had a ≥50% decrease in spleen length, 20.8% had a ≥50% reduction in TSS and treat-

ment success was seen in 50.0% of patients. Changes from baseline in splenomegaly outcomes and treatment success were similar in all IPSS groups. Mean platelets levels decreased by approximately 40% from baseline to week 4 and then stabilized. Mean hemoglobin levels decreased from baseline by 10% to week 12, attenuating to 5% by week 48. Adverse events and 48 week EQ-5D data will be available for presentation at the meeting.

Table 1. Summary of efficacy endpoints at 48 weeks.

Summary and Conclusions: Our results demonstrate that MF is associated with a significant symptom burden and adversely affects HRQoL. Ruxolitinib was shown to reduce symptoms and splenomegaly. In our study, treatment success, a composite endpoint combining objective and subjective measures, was seen in all IPSS groups including in over half of patients with intermediate-1 disease.

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TREATMENT OF PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS WITH PEGYLATED INTERFERON ALPHA-2A: A PROSPECTIVE MONOCENTER COHORT STUDY.

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Background: In patients with myeloproliferative neoplasms (MPN), pegylated interferon alpha-2a (PegIFN2a) has been reported to be effective and better tolerated than non-pegylated interferon alpha-2b (IFN2b), and able to induce a reduction of the JAK2 V617F allelic burden up to a complete molecular response. Moreover, the absence of leukemogenic potential renders PegIFN2a a preferential agent in young patients. However, published experience is limited (Kiladjian JJ *et al.*, Blood 2008; Quintas-Cardama A *et al.*, JCO 2009; Gowin K *et al.*, Haematologica 2012).
Aims: To assess in patients with MPN the safety of PegIFN2a and the hematological and molecular response.
Methods: Fifty-four pts. (M/F 22/32, median age 56 yrs, range 35-84) with polycythemia vera (PV, n=22), essential thrombocythemia (ET, n=31), myelofibrosis (MF, n=1) treated with PegIFN2a were prospectively evaluated from July, 2010 to January, 2014. JAK2 V617F-positive patients were preferentially recruited, so that all patients but one with ET had the mutation. Hematological response was assessed according to the ELN criteria (Barosi G *et al.*, Blood 2009). The JAK2 V617F allelic burden was tested before starting treatment and then every 3 months; patients were evaluable for molecular response if quantitated on at least two different occasions.
Results: Twenty-two pts. (median age 50.5, range 35-67) received PegIFN2a as a first line treatment, and 32 after discontinuation of previous treatment because of no response (n=3), hydroxyurea [HU]-related mucocutaneous toxicity (n=12) or fever (n=1), IFN2b-related adverse effects (n=4), concern of leukemogenic potential of HU or pipobroman in younger patients (n=12). The median dosage was initially 90 mcg/ week (range 67-180), and after adjustement for adverse effects and response 90 mcg/week (range 22-270). The median duration of treatment was 37 weeks (range 4-188) in PV, and 28 weeks (range 4-32) in ET; 20 pts. (37%, PV=9, ET=11) were treated >1 year. Hematological response was complete in 13 pts. (24%, PV=5, ET=8) and partial in 26 (48%, PV=8, ET=18); 14 (26%, (PV=9, ET=5)) had no response. Sixteen pts. had splenomegaly, and 5 had a median reduction of 4 cm (range 2-6). The baseline median JAK2 V617F allelic burden was 75.5% in 18 evaluable PV pts. (range 16-100) and decreased to 57.5% (median 3-100) after median 48 weeks (range 20-188) of treatment; 6 pts. (33%) had a decrease >50%, but none achieved a complete molecular response. In 21 evaluable ET pts. the median JAK2 V617F allelic burden was unmodified (27%, range 7-100) in respect to the baseline (27%, range 6-98). Adverse events occurred in 31 pts. (57%), mainly flu-like symptoms (n=17, 31%), thyroiditis (n=8, 15%), itching (n=8, 15%), elevated liver enzymes (n=3, 5%), diarrhea (n=1, 2%), and 20 (37%) had to discontinue treatment. In our cohort 31 pts. had a previous thrombosis (57%); during treatment 5 pts. (9%) had a recurrence (mesenteric vein thrombosis n=1, deep venous thrombosis n=1, TIA n=1, ischemic stroke n=1) or a first event (myocardial infarction n=1), in spite of adequate primary or secondary antithrombotic prophylaxis.
Summary and Conclusions: PegIFN2a was confirmed to be effective in inducing hematological response in MPN patients. A partial molecular response was observed in at least one-third of PV patients, but not in ET patients. However, the rate of discontinuation due to severe adverse effects was higher than previously reported. Finally, cytoreduction obtained with PegIFN2a showed limited efficacy in preventing thrombosis.

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SPECIFIC DETECTION OF CALR-MUTATED MYELOPROLIFERATIVE NEOPLASMS BY IMMUNOSTAINING

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Background: Mutations of *CALR*, the gene encoding the endoplasmic reticulum Ca2+ binding chaperon calreticulin, were recently discovered in *JAK2* and *MPL* wild type (wt) patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF); thus identifying *CALR* mutations is important for diagnosing these diseases. *CALR* mutations are heterogeneous and involve the whole exon 9, but all lead to a novel common protein C-terminus.
Aims: We sought to develop a diagnostic tool for identifying *CALR* mutated patients based on the recognition of the novel C-terminus sequence of the protein.
Methods: To this end, we developed a polyclonal antibody directed against a common 17-mer C-terminal peptide from the mutated calreticulin. Purified antibody was used to perform immunostaining on bone marrow (BM) biopsies of PV, ET and PMF patients who provided an informed written consent. Calreticulin expression levels were measured by gene expression array analysis in CD34-positive cells, isolated from BM of healthy donors, induced to erythroid, myeloid and megakaryocytic unilineage differentiation. Genotyping for *JAK2* and *MPL* was performed by Real Time quantitative PCR and mutations in *CALR* were assessed by bidirectional Sanger sequencing.
Results: The antibody specificity was supported by results of western blotting in granulocytes of *CALR* mutated patients. By immunolabelling we show that the antibody specifically labelled patients harboring different types of *CALR* mutations with no staining in subjects with non-hematologic disorders, *JAK2* and *MPL* MPN mutated patients and triple negative ET and PMF. The staining was localized mainly in the megakaryocytes (mk) with a comparable mean percentage of labelling depending on *CALR* mutation type, respectively 91% (range 74-98%) for *CALR*del52, 92% (range 88-98%) for *CALR*ins5 and 87%/98% for the 2 *CALR*indel mutated patients. Conversely myeloid and erythroid cells showed a faint staining suggesting a preferential overexpression of calreticulin in mk lineage. This was indeed supported by finding that calreticulin mRNA level in normal hematopoietic cells was significantly upregulated in megakaryocyte differentiation (fold change 3.4) compared with erythroid and granulocyte (fold change respectively 0.3, 0.6). Using an antibody agaist wt calreticulin, a similar preferential staining of megakaryoyctes was observed in control BM sections.
Summary and Conclusions: Taking advantage of the unique common novel protein due to *CALR* mutations, we developed a polyclonal antibody specifically directed against the mutated protein that selectively labelled cells in BM of *CALR* mutated patients, unlike in normal subject and ET and PMF patients harboring *JAK2* and *MPL* mutations. Therefore, immunostaining using mutated *CALR*-antibody represents a specific, cost-effective and easy diagnostic tool for molecular classification of ET and PMF patients, eventually suitable for small hematology units where sequencing is not available. The almost exclusive staining of the mk lineage, with 80-100% of the cells being labelled, suggests a prevalence of mutated clone over normal megakaryocytopoiesis in *CALR* mutated patients. We also show for the first time that calreticulin is preferentially expressed in cells belonging to the mk lineage, making the case for the specific association of CALR mutations with disorders characterized by extensive involvement of Mk.

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THE ROLE OF POLYCOMB PROTEINS IN NON-HODGKIN B CELL LYMPHOMA: FROM PRECLINICAL MODELS TO TARGET THERAPY

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Background: Polycomb group (PcG) proteins are recruited within two main Polycomb Repressive Complexes (PRC), PRC1 and PRC2, to promote transcriptional repression of target genes. Within PRC2, EZH2 is responsible for histone H3 lysine-27 trimethylation. EZH2 is highly expressed in B cells recruited into the germinal center (GC) reaction during a T-cell dependent immune response. Importantly, *EZH2* gain-of-function mutations are frequently selected in Diffuse Large B-cell lymphomas and Follicular Lymphomas arising from GC B cells, suggesting a possible contribution of the mutant PcG protein to lymphomagenesis. PRC2 has been proposed to act in concert with PRC1, which is responsible through the E3 ligases Ring1a/Ring1b proteins to catalyze lysine-119 monoubiquitylation on Histone H2A. The contribution of EZH2 and the Ring1 proteins to GC B cell responses and B cell lymphomagenesis has remained, till recently, largely unexplored.
Aims: Our work aimed to dissect the *in vivo* function of PRC1/PRC2 in GC B cell biology. Moreover, we investigated the mechanisms through which the Polycomb axis could possibly contribute to the establishment and maintenance of the B cell malignant phenotype in different types of Non-Hodgkin lymphoma (NHL).
Methods: We combined mouse conditional gene targeting studies to high throughput genomic analyses to reveal the molecular targets and mechanism of action of the Polycomb axis in GC B cells. Moreover, conditional gene inactivation studies and highly specific small molecule inhibitors were employed to establish the role and mechanism of action of Polycomb proteins Ezh2 and Ezh1 in the maintenance of different types of NHL.
Results: Inactivation of PRC1 and PRC2 function in GC B cells resulted in marked apoptosis, poor antibody responses and significantly fewer high-affinity memory B cells. Ezh2 sustained the survival of GC B cells protecting cells against genotoxic damage induced by Activation Induced cytidine Deaminase (AID). Genome wide identification of Polycomb targets in GC B cells revealed a significant enrichment for genes whose repression is necessary to sustain GC B cell function and prevent terminal differentiation. Data, to be presented, will reveal the molecular pathways underlying the contribution of PRC1/PRC2 to GC B cell responses and to the maintenance of the malignant B cell phenotype in NHL.
Summary and Conclusions: Our results reveal that key components of Polycomb repressive complexes represent central regulators of GC B cell function. Moreover, using a combination of gene and pharmacological inactivation of Polycomb proteins in NHL, we unravel possible molecular pathways through which the Polycomb axis sustains the transformed B cell phenotype. Finally, we report the identification of genetic mutations that predict response *versus* resistance of NHL cells to EZH2 small molecule inhibitors.

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MICROARRAY ANALYSIS OF CHROMOSOME 11Q IN A GROUP OF AGGRESSIVE LYMPHOMA: THE PARTIAL DUPLICATION OF 11Q AS A CYTOGENETIC MARKER OF SUBSET OF THE GREY ZONE BETWEEN BURKITT'S (BL) AND DIFFUSE LARGE B-C

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Background: Aggressive B-cell lymphomas are clinically and pathologically diverse and reflect multiple pathways of transformation. Most of BL and DLBCL lymphomas can be accurately classified on the basis on clinical data, morphology, immunophenotype and cytogenetic changes. Translocations involving *MYC* oncogene are the molecular hallmark of BL. Most cases involve the *IGH* gene on chromosome 14. Less commonly, the light-chain genes on chromosomes 2 and 22 are involved in the translocation. Generally BL karyotypes are simple with secondary aberrations being singular events. In DLBCL karyotypes are more complex. Chromosomal translocations affecting the band 3q27 with *BCL6* gene are the most common. Other frequent genetic changes include presence of *BCL2* or *MYC* rearrangements. Diagnostically difficult are aggressive B-cell lymphomas with intermediate features between BL and DLBCL. These unclassifiable cases do not form a separate or uniform entity. Here we present a subset of intermediate BL/DLBCL which contains cases with BL morphology but atypical immunophenotype and specific genetic features: presence of chromosomal marker dup11q, lack of *MYC* rearrangement (6 from 7 cases), as well as *BCL2* and *BCL6* rearrangements (all cases).
Aims: The aim of study was detailed description of the marker dup(11q) in a subset of *MYC* negative BL/DLBCL.
Methods: Haematological Cancer+SNP Array (8x60K) CytoSure, classical cytogenetic and FISH with *BCL2, BCL6, MYC, IGH, CEP11,CCND1, ATM, MLL* probes.
Results:
All patients showed simple karyotypes with dup(11). Duplicated region was variable in size and ranged from 71,72Mb to 11,91Mb in chromosomal localization from dup(11)(q24 q12) to dup(11)(q22.2q23.3), respectively. In 5 from 7 patients additional multiplication inside duplicated region (11q23.3) was detected. This multiplication was also various in size from 2,99Mb to 14,2 Mb. In all cases in the minimal region of 11q duplication the *MLL* gene was located. FISH with CEP11 and MLL, ATM and CCND1 probes confirmed duplication and multiplication regions. Additionally, relative position of FISH probe signals revealed inversion of the 11q duplicated region in 5/7 cases. In 6 of 7 cases terminal deletion del(11)(q24.1) was revealed. In one case deletion of 11q24.3 was biallelic. The size of this small region was 774,52Kb and covered five genes: *ETS1, FLI1, KCNJ1, KCNJ5, TP53AIP1.*
Summary and Conclusions: A subgroup of the *MYC* negative intermediate BL/DLBCL showed specific chromosomal marker dup11q. Marker is characterized by repetitively occurring: inversion of the duplicated material, selective amplification of the region 11q23.3 and the simultaneous loss (mono-or biallelic) of a small, telomeric segment with 11q24.1 breakpoint. Further detailed characterization of changes in the marker 11q will be aimed at pre-define genes that potentially involved in the pathogenesis of the group of lymphoma.

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THE B CELL ANTIGEN RECEPTOR IS CRITICAL FOR BURKITT LYMPHOMA GROWTH VIA GSK3B REGULATION

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Background: Mature B-lymphocytes depend on expression of a functional B cell antigen-receptor (BCR) for survival. It has been proposed that development and maintenance of Burkitt lymphoma (BL), an aggressive malignancy originating from mature B cells, depends on signaling competence of the BCR. Circumstantial evidences support this hypothesis: 1) Ig/MYC translocations preserve Ig loci carrying functional rearrangements; 2) exome sequencing data identified recurrent mutations in genes regulating BCR function. Nevertheless, to date, a conclusive genetic proof supporting the role of the BCR in BL maintenance is still missing.
Aims: We assessed the effect(s) of genetic ablation of the BCR on an aggressive form of c-MYC driven mature B cell malignancy resembling human BL.
Methods: To elucidate the contribution of the BCR in BL maintenance, we used the l-MYC mouse tumor model in which acute ablation of the BCR was induced in tumor B cells via Cre/loxP technology. We employed both *in vitro* and *in vivo* approaches to study the effects of BCR inactivation on tumor growth. Biochemical studies where coupled to whole transcriptome analyses and use of small molecule inhibitors to functionally dissect the molecular pathways controlled by the BCR in c-MYC driven B lymphoma cells.
Results: Acute ablation of the BCR in primary B lymphoma cells isolated from the l-MYC tumor led to the rapid disappearance of tumor cells both *in vitro* and *in vivo* after transplantation into immunoproficient recipients. Importantly, these effects were observed when BCR-deficient lymphoma cells were under competition with their BCR^+ counterparts. We identify GSK3b as a critical modulator of the response of tumor cells to tonic BCR signaling. BCR expression was essential to support PI3 kinase activity and protected tumor cells from mTOR inhibition. Tonic BCR signaling controlled over 400 genes in c-MYC driven B lymphoma cells, mostly through GSK3b modulation. Data to be presented will summarize the major gene categories controlled by the BCR in lymphoma cells. Importantly, we identified lymphomas that had acquired resistance to BCR inactivation, thus becoming BCR-independent. Comparative genome hybridization analysis allowed us to identify a limited set of secondary genetic aberrations that were selectively associated with acquired resistance to BCR loss. Experiments to be presented have addressed the role of specific genetic determinants in enabling lymphoma cells to overcome BCR dependency.
Summary and Conclusions: Our study provides genetic proof that tonic BCR signaling is essential for survival and proliferation of c-MYC transformed aggressive B cell lymphomas. BCR exerts its function primarily through the modulation of GSK3b activity. Our data indicate that BCR-negative tumors succumb in the presence of their BCR^+ counterparts. Therefore, current therapies aimed to interfere with BCR signaling in lymphomas risk to favor the outgrowth of BCR-negative tumors. The identification of genes that render c-MYC driven lymphomas resistant to BCR loss provides the framework for future studies aimed to test their role as novel therapeutic targets.

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CONCOMITANT INHIBITION OF EZH1 AND EZH2 IMPAIRS GROWTH OF C-MYC DRIVEN B CELL LYMPHOMAS

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Background: Non-Hodgkin B-cell lymphomas (NHL) originate in most cases from B cells recruited into the germinal center (GC) reaction during a T cell dependent immune response. Enhancer of Zeste Homolog 2 (EZH2) is a Polycomb group (PcG) protein that catalyzes Histone H3 lysine-27 trimethylation (H3K27me3) within Polycomb Repressive Complex 2 (PRC2), leading to transcriptional repression of target genes. EZH2 is highly expressed in GC B cells and frequently deregulated in NHL of GC origin, including Burkitt lymphoma (BL). The role of EZH2 in maintenance and progression of c-MYC driven B cell lymphomas is largely unknown. This question is of clinical relevance as highly-specific small molecule inhibitors against EZH2 have been proposed as novel NHL therapeutics.
Aims: Our work investigated the role of EZH2 in the growth of primary c-MYC driven B cell lymphomas.
Methods: We engineered the l-MYC Burkitt lymphoma (BL) mouse model to enable conditional inactivation of the *Ezh2* gene through the Cre/loxP recombination system. We performed *in vitro* and *in vivo* analysis to study the effects of acute inactivation of EZH2 methyltransferase activity on growth of primary lymphomas. Genome wide H3K27me3 and EZH2 profiling combined with transcriptome analysis were employed to identify direct and indirect targets of EZH2 in lymphoma B cells and to reveal the transcriptional changes occurring in PcG mutant tumors following *Ezh2* inactivation. These analysis were complemented by a series of studies aimed to investigate the therapeutic efficacy of a recently described EZH1 and EZH2 double inhibitor, Unc1999 in the treatment of aggressive l-MYC primary lymphomas and human BLs.
Results: Genetic *Ezh2* inactivation was induced in primary B cell lymphomas isolated from l-MYC transgenic mice. Ezh2 inhibition led to global reduction of H3K27me3 levels. Surprisingly, loss of EZH2 function showed minor effects on tumor growth. To test whether the close EZH2 paralog EZH1, had replaced EZH2 catalytic function in PcG mutant lymphomas, we treated l-MYC primary lymphomas with the EZH1/2 small molecule inhibitor UNC1999. Strikingly, concomitant inhibition of EZH1/2, led to a significant impairment in lymphoma growth. Gene expression and H3K27me3 ChIP-seq analyses allowed the elucidation of the possible molecular pathways through which the Ezh proteins sustain the growth of aggressive c-MYC driven B cell lymphomas.
Summary and Conclusions: Our results identify EZH1 and EZH2 as important epigenetic regulators of the growth of c-MYC driven mature B cell lymphomas. The necessity to interfere with both EZH1 and EZH2 function to counteract lymphoma growth highlights the need for further preclinical studies to define the therapeutic efficacy of EZH2-only inhibitors in the cure of Diffuse Large B cell lymphomas and Follicular lymphoma. Finally, our data provide experimental evidence that PRC2 small molecule inhibitors may represent a valid therapeutic approach also for NHL subtypes including BL, which lack *EZH2* gain-of-function mutations.

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CLONAL EVOLUTION IN BURKITT LYMPHOMA/LEUKEMIA

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Background: Typical (pediatric) Burkitt lymphoma/leukemia (BL/leukemia) is, in contrast to other *MYC*-translocation positive lymphomas, characterized by an overall low genomic complexity and favorable prognosis with only few relapses. However, the patients who do show relapse or disease progression have a very dismal prognosis. As sequential cytogenetic studies are rarely performed still little is known about the underlying genetics of disease progression and relapse in BL/leukemia.
Aims: (i) To characterize the karyotype complexity, (ii) analyze the cytogenetic evolution and (iii) investigate the secondary cytogenetic changes (SCCs) in BL/leukemia in which sequential cytogenetic studies have been performed.
Methods: From all BL/leukemia samples retrieved at our institution from 1997 to 2013, karyotypes assessed by R-banding and fluorescence *in situ* hybridization (FISH) of six samples of BL/leukemia with sequential cytogenetic data were available. In addition, the published cytogenetic literature was explored using the Mitelman database as comprehensive cytogenetic resource. For each karyotype a complexity score (CS) was calculated by counting the number of numerical and structural chromosomal aberrations. Karyotypes were considered complex (complex karyotype, CK) if showing ≥3 aberrations (including *IG-MYC*). For evaluation of numerical and structural chromosome abnormalities karyotypes were converted with the Cydas software package.
Results: The six cases of BL/L with sequential cytogenetic data available included five pairs of initial diagnosis and sequential samples (one sequential sample n=4; two sequential samples n=1) and another patient was studied at time of first relapse, blast crisis and secondary relapse. A search of the Mitelman database and the literature yielded additionally 18 pairs, including two patients with more than one follow up sample. All samples were positive for the *IG-MYC* translocation and consisted of a t(8;14)(q24;q32)/*IGH-MYC* in 20/24 cases (83%) and *IGK/L-MYC* in 4/24 (17%). The initial diagnostic samples showed a complexity score of 2 (which is similar to other primary BL cases). Secondary investigations had a complexity score of 5 and third investigations a complexity score of 9. In fact, for the four patients with multiple follow-up samples available a mostly linear increase of karyotype complexity could be observed. Complex karyotypes were more frequently observed in sequential samples than in primary samples. Furthermore, we found strong evidence for clonal evolution since 19/24 of the sequential samples contained abnormalities already present at diagnosis with new acquired aberrations. Frequently observed SCCs at diagnosis and sequential samples included (partial) gains of chromosome 1q and 7q as well as (partial) losses of 13q, all being more frequent in the latter group. In contrast, loss of 17p and trisomy 21 were exclusively present in secondary cytogenetic samples, pointing to a role in disease progression. Interestingly, two cases acquired additional *IG*-translocations (involving other genes than *MYC*) during clonal evolution, hereby posing the question whether or not these cases should be considered double-hit lymphomas.
Summary and Conclusions: By analyzing paired BL/Leukemia samples, we found increasing karyotype complexity, specific SCCs, and clonal evolution that might reflect the underlying mechanism of disease progression or relapse and the poor outcome of such cases.
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P422

ACTIVATED SUMOYLATION MACHINERY IS A HALLMARK OF BURKITT LYMPHOMA WITH NEW THERAPEUTIC IMPLICATIONS

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Background: Myc oncoproteins (c-Myc, N-Myc and L-Myc) are transcription factors that regulate cell growth, cell division and metabolism under physiologic conditions. Myc overexpression is a hallmark of cancer, present in most advanced tumors, and associated with poor prognosis. We have previously shown that Myc overexpression results in specific cancer cell liabilities, *e.g.* during cell cycle progression, that constitute therapeutic targets for synthetic lethality approaches. Small Ubiquitin-like Modifier (SUMO) proteins covalently bind to other proteins to modify their function. SUMOylation is involved in various cellular processes including transcription and cell cycle progression.
Aims: To assess the role of SUMOylation in Myc induced lymphoma and other cancer and to identify potential new treatment strategies.
Methods: Transcriptome and protein analysis for expression of components and regulators of the SUMOylation machinery in human Burkitt lymphoma, murine Myc-induced lymphomas and control cells was applied. Lymphoma cells were treated with inhibitors of SUMOylation and the effects were validated with stable knock down of essential SUMOylation components.
Results: Hierarchical cluster analysis comparing RNA expression data in murine normal control, pre-malignant and lymphoma Eμ-*Myc* B cells identified a Myc-induced SUMOylation-related gene expression signature. This signature was present in pre-malignant and Eμ-*Myc* lymphoma cells and involved the upregulation of various critical components of the SUMOylation machinery, including the E1 ligases SAE1 and SAE2, the E2 ligase Ube2i and the E3 ligases Ranbp2 and PIAS2. Moreover this translated into elevated protein expression of the whole SUMOylation pathway and ubiquitous hyper-SUMOylation of proteins in Eμ-*Myc* lymphoma cells. For cross-species validation we analyzed human gene expression data and found that the Myc-induced regulation of SUMOylation-associated genes was also present in human *IG/MYC* Burkitt lymphomas, in contrast to Non-*IG/MYC* B-cell lymphomas. Interestingly immunhistochemical staining for SUMO2/3 revealed hyper-SUMOylation in patient samples of Burkitt lymphoma compared to DLBCL, MCL, and FL. What is more analysis of ChIP-on-chip experiments showed direct binding of Myc to regulatory genomic regions of almost all SUMOylation regulators (SUMO2, SUMO3 and E1, E2 and E3 ligases). The characteristic of cancer cells to depend on certain intact physiologic mechanisms is known as non-oncogene addiction. Since SUMOylation of proteins is involved in essential metabolic, survival and proliferation pathways we reasoned that intact SUMOylation is a non oncogenic pathway that Myc-driven cells rely upon. We thus hypothesized that Myc-dependent cells could be specifically susceptible when interfering with SUMOylation by pharmacological means. Eμ-*Myc* lymphoma cells were highly sensitive to the SUMOylation inhibitors ginkolic acid and anarcardic acid and a second generation SUMOylation inhibitor 2-D08. In particular, inhibition of SUMOylation lead to cell cycle arrest, polyploidy, and subsequent cell death. When validating these effects with specific knock down constructs for SAE1 and SAE2, crucial mediators of SUMOylation, the identical phenotype was

observed. This therapeutic effect was Myc-specific as shown by use of genetically defined cell lines and conditional Myc-overexpression systems. Specifically human Burkitt lymphoma cell lines were strikingly more sensitive to inhibition of SUMOylation than non-Myc-transformed lymphoma samples.
Summary and Conclusions: Taken together, we provide correlative and experimental evidence that the Myc-associated expression of genes involved in SUMOylation is a hallmark of Burkitt's lymphoma and constitutes a non oncogenic pathway which is therapeutically exploitable in lymphoma and other Myc-driven cancers.

P423

CD19-TARGETING LIPOSOMES CONTAINING RAPAMYCIN INDUCE AUTOPHAGIC CELL DEATH IN BURKITT'S LYMPHOMA CELLS

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Background: Patients with Burkitt's lymphoma often have poor outcomes in spite of intensive chemotherapy. The conventional chemotherapeutic agents induce apoptotic and/or necrotic cell death in lymphoma cells. When lymphoma cells are resistant to apoptotic and/or necrotic cell death signaling, induction of different type of cell death, autophagy will be another choice of treatment. An inhibitor of mammalian target of rapamaycin (mTOR), rapamycin has been a representative autophagy-inducing agent, which recent clinical studies have proven to be efficacious against mantle cell lymphoma. On the other hand, CD19-targeting liposomal delivery is an attractive strategy to raise the efficacy of chemotherapeutic agents against B-cell lymphoma as previously demonstrated using doxorubicin.
Aims: In the present study, we first investigated the susceptibility of Burkitt's lymphoma cells against mTOR inhibitors. Second, the effectiveness of CD19-targeting delivery of rapamycin to Burkitt's lymphoma cells was examined.
Methods: Rapamycin was encapsulated by anionic liposome, GLYCOLIPO and conjugated with anti-CD19 antibody (CD19-GL-Rap) or anti-CD2 antibody (CD2-GL-Rap) as control. A fluorescent probe Cy5.5 was also liposomized in the same way (CD19 or CD2-GL-Cy5.5). A Burkitt's lymphoma cell line, SKW6.4 was cultured for over 70-passages with low concentration of doxorubicin, which named SKW-DOX, a doxorubicin-resistant subline. CD19 positive cells were collected using magnetic beads from peripheral blood in leukemic phase or malignant pleural effusion of patients with Burkitt's lymphoma.
Results: Burkitt's lymphoma cell lines, SKW6.4, Raji and Namalwa were all susceptible to rapamycin *in vitro*. The most susceptible cell line, SKW6.4 was selected for further examination. The other mTOR inhibitors, temsirolimus and everolimus had also enough cytotoxic activity against SKW6.4 cells. Then, the CD19-targeting delivery was investigated using CD19 or CD2-GL-Cy5.5. We confirmed beforehand that SKW6.4 cells highly expressed CD19 and had no CD2 on their surface. CD19-GL-Cy5.5 was more effectively uptaken into SKW6.4 cells than CD2-GL-Cy5.5, examined by both flow cytometry and fluorescence microscopy *in vitro*. Even when the cells were inoculated subcutaneously into immunodeficient non-obese diabetic (NOD) severe combined immune-deficient (scid) mice, intravenously administered CD19-GL-Cy5.5 has made subcutaneous tumor fluorescent brighter than CD2-GL-Cy5.5, detected by IVIS system. We next examined the efficacy of CD19 or CD2-GL-Rap. CD19-GL-Rap had a greater cytocidal effect than CD2-GL-Rap *in vitro*. The specific toxicity of CD19-GL-Rap was cancelled by the pretreatment of the cells with neutralizing anti-CD19 antibody. A molecular marker of autophagy, LC3-II was detected by Western blotting in CD19-GL-Rap-treated cells. The cytotoxic activity of CD19-GL-Rap was not reduced by a pan-caspse inhibitor, Z-VAD-FMK or antioxidant, N-atetyl L-cysteine. Not only Burkitt's lymphoma cells collected from our patients but also doxorubicin-resistant SKW6.4 subline, SKW-DOX cells were also susceptible to CD19-GL-Rap. The survival period of mice treated with intravenous CD19-GL-Rap was longer than those with CD2-GL-Rap after the intraperitoneal inoculation of SKW6.4 cells.
Summary and Conclusions: CD19-targeting liposomes containing rapamycin could be a promising treatment inducing autophagic cell death against Burkitt's lymphoma.

P424

TARGETING THE TYROSINE KINASE RECEPTORS ROR1 AND AXL (RTKS) IN BURKITT'S LYMPHOMA

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Background: Burkitt's lymphoma (BL) is an aggressive malignancy of B-cell origin and classified into 3 subtypes including endemic, sporadic and immunodeficiency-associated variants. The main transforming event is translocation of the MYC oncogene to one of the immunoglobulin (Ig) heavy or light chain loci. There is a great need to develop novel drug for targeted therapy of cancers, including BL. ROR1 and AXL receptor tyrosine kinases (RTKs) have emerged as interesting molecules for targeted cancer therapy. Both these RTKs have important roles during embryogenesis and are down-regulated in adult human tissues. Both RTKs have been shown to be overexpressed in hematological malignancies and solid tumors. SiRNA targeting these RTKs in CLL cells induced apoptosis.
Aims: To investigate the expression of ROR1 and AXL RTKs in the BL cell lines (LUKAS, NAMALVA and RAMOS). The cytotoxic activity of two novel small-molecule tyrosine kinase inhibitors (TKI) targeting ROR1 and AXL tyrosine kinase domain and the effects on downstream signaling molecules were studied.
Methods: ROR1 and AXL expression were determined by flowcytometry and Western blot (WB). TKI KAN439365 (Kancera AB, Stockholm, Sweden) targeting ROR1 and a commercially available AXL RTK inhibitor (Selleckchem, Houston, TX, USA) were used to treat the BL cell lines. Cytotoxic effects were determined by MTT and Annexin-V/PI assays. PARP and caspase 3 cleavage as well as total and phosphorylated ROR1, Src, PI3K, AKT, mTOR and CREB were analysed by WB.
Results: LUKAS and NAMALVA cells expressed ROR1 as determined by flowcytometry and WB. However, RAMOS cells did not show surface expression of ROR1, but ROR1 was detectable by WB (indicating expression of the cytoplasmic TK domain). ROR1 was phosphorylated in all cell lines. All cell lines but not LUKAS expressed AXL both by flowcytometry and WB. After 18 h of incubation with 1, 5 and 10 μM of the inhibitors, 40-90% of cells were dead. Cell death was preceeded by ROR1 and AXL TKIs induced dephosphorylation of ROR1 and AXL respectively (<2h) as well as of SRC, PI3K, AKT, mTOR, and CREB. ROR1 TKI did not dephosphorylate AXL and AXL TKI did not dephosphorylate ROR1.
Summary and Conclusions: ROR1 and AXL RTKs are overexpressed in Burkitt's lymphoma and are of importance for the survival. Although AXL RTK was not expressed in LUKAS cells, the AXL inhibitor induced apoptosis suggesting other mechanisms of cell killing than mediated by the AXL receptor. ROR1 activation through PI3K/AKT/mTOR pathway might provide a survival signal for BL cells. This is the first report of two novel classes of drugs targeting ROR1 and AXL (ROR1 and AXL-TKI) in BL malignancy. Further characterization of ROR1 and AXL TKI in other primary hematological cells and cell lines should be of interest to establish their potential therapeutic value.

P425

HIGH EXPRESSION OF MIRNA-199A_1 AND MIRNA-497_1 IS ASSOCIATED WITH BETTER OVERALL SURVIVAL IN AGGRESSIVE NON-HODGKIN´S LYMPHOMA

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Background: Micro-RNAs (miRNAs) are short non-coding single-strand RNA molecules that regulate gene expression at the post-transcriptional level. They are involved in cell development, differentiation, apoptosis, and proliferation. They can either function as tumor suppressor genes or oncogenes in various important pathways. The expression of specific micro RNAs has been identified to correlate with tumor prognosis.
Aims: Since the role of microRNAs in aggressive lymphomas is not precisely elucidated, we aimed to investigate the expression of various microRNAs and evaluate their impact on overall survival of aggressive Non-Hodgkin lymphoma (NHL) patients.
Methods: For microRNA expression analysis we performed real-time PCR on 78 samples, including 42 diffuse large B-cell lymphoma (15 germinal center B, 20 non germinal center B, 7 unclassified), 21 follicular lymphoma grade III, and 15 controls, including 7 peripheral B-cells, 4 germinal-center B-cells, and 4 lymphoma cell lines (Raji, SUDHL4, Karpas, Ly8). Expression levels of 11 microRNAs, including miR15b-2, miR16-1*, miR16-2, miR16-2*, miR27a, miR27a*, miR98-1, miR103a, miR185, miR199a-1, and miR497-1 were measured and correlated with clinical data. Preliminary data showed that these microRNAs may play an important role in lymphoma genesis.
Results: Mir16-1*, miR16-2*, miR27a, miR103a miR185, miR199a-1, and miR497-1 were at least 6 fold differentially expressed in lymphoma samples and controls ($p<0.01$). Highest overexpression was found in miR27a (30 fold overexpression), miR199a-1 (200 fold overexpression) and miR497-1 (80 fold overexpression). An additional Kaplan Meier analysis revealed that a higher expression level of miR497-1 and miR199a-1 is significantly associated with better overall survival in patients with aggressive lymphomas ($p=0.042$, $p=0.007$).
Summary and Conclusions: Our data indicate that various microRNAs are overexpressed in aggressive NHL and may contribute to lymphoma genesis. Furthermore we could demonstrate that high expression levels of miR497-1 and miR199a-1 are associated with better overall survival. To detect possible target genes of these microRNAs further functional characterization is warranted.

P426

LOSS OF BCL2L12 PROTEIN EXPRESSION PREDICTS RELAPSE IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB PLUS CONVENTIONAL CHEMOTHERAPY (CYCLOPHOSPHAMIDE, DOXORUBICIN, VINCRISTINE, AN

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common histological subtype of non-Hodgkin lymphoma (NHL) corresponding to 30-40% of cases in adults. Although classified as a single entity by the World Health Organization (WHO), DLBCL is characterized by significant heterogeneity with variation in molecular pathogenesis, clinical behavior, response to therapy and long term survival. BCL2 family of proteins is the hallmark of apoptosis regulation. Recently, new members of BCL2 family were discovered and found differentially expressed in many types of cancer, including hematologic malignancies. BCL2L12 is a recently identified gene belonging to the BCL2 family. The expression status of BCL2L12 in patients with DLBCL has not been examined yet.
Aims: The aim of the present study was the investigation of protein expression levels of BCL2L12 by immunohistochemistry in patients with DLBCL and its relation to established prognostic factors and survival.
Results: We studied 130 DLBCL patients treated with R-CHOP (68 males and 62 females), with a median age of 67 years (range: 20–95) at the time of diagnosis. High BCL2L12 protein expression was associated with Ki-67 immunopositivity (P=0.009), while low BCL2L12 protein index was related to absence of B-symptoms (P=0.028). Kaplan-Meier survival analysis revealed that low *BCL2L12* protein index predicts short-term relapse in DLBCL patients treated with R-CHOP (P=0.039). In addition, high BCL2L12 protein expression is a favorable biomarker in DLBCL, predicting prolonged overall survival (P=0.041). Most importantly, its prognostic potential regarding overall survival of R-CHOP–treated DLBCL patients was found to be independent of IPI risk and cell-of-origin classification (P=0.048), as shown by multivariate Cox regression analysis.
Summary and Conclusions: Expression of the apoptosis-related protein BCL2L12 is associated with presence of B-symptoms in DLBCL patients treated with R-CHOP and appears as a favorable molecular biomarker in this disease.

P427

NR4A3-MEDIATED APOPTOSIS SUPPRESSES LYMPHOMAGENESIS IN A XENOGRAFT MOUSE MODEL

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Background: Recently, we described a significant down-regulation of NR4A1 (Nur77) and NR4A3 (Nor-1) -two members of the orphan nuclear receptors acting together as critical tumor suppressor genes in acute myeloid leukemia- in aggressive lymphoma (Deutsch *et al.,* Blood 2014). NR4A1 over-expression proved its pro-apoptotic function in aggressive lymphoma cells and its lymphoma suppressive properties *in vivo* was demonstrated in a xenograft mouse model.
Aims: Since the role of down-regulated NR4A3 in aggressive lymphomas is unknown, we aimed to investigate the function of NR4A3 in lymphoid malignancies.
Methods: For functional characterization NR4A3 was over-expressed in a SuDHL4 lymphoma cell line by using an inducible lentiviral construct followed by various apoptotic assays and followed by a xenograft mouse experiment.
Results: Induction of NR4A3 expression led to a significantly higher proportion of induced SuDHL4 cells undergoing apoptosis as demonstrated by DNA cleavage, Annexin V staining and increased caspase 3-7 activity suggesting a functional redundancy to NR4A1 in aggressive lymphoma. To test the tumor suppressor function of NR4A3 *in vivo*, the stably transduced SuDHL4 lymphoma cell line was further investigated in the NOD scid γ (NSG) mouse model. Induction of NR4A3 in SuDHL4 abrogated tumor growth in the NSG mice, in contrast to vector control- and uninduced SuDHL4 cells, which formed massive tumors.
Summary and Conclusions: Our data suggest that NR4A3 has a pro-apoptotic function in aggressive lymphoma and define that NR4A3 together with NR4A1 function as novel tumor supressor involved in aggressive lymphoma development.

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PHENOTYPIC AND FUNCTIONAL LONG-TERM DYNAMICS OF PERIPHERAL BLOOD NATURAL KILLER CELL COMPARTMENT IN DIFFUSE-LARGE-B-CELL LYMPHOMA PATIENTS BEFORE AND AFTER R-CHOP-BASED IMMUNOCHEMOTHERAPY

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Background: Natural Killer (NK) cells represent an important component of tumor immune surveillance, and play a key role in rituximab-dependent killing of lymphoma cells through an antibody-dependent cellular cytotoxicity (ADCC) mechanism. Since NK functions might significantly contribute to the success of rituximab-based therapies, we focused our work on monitoring changes of NK subsets *in vivo*, during immunochemotherapy.
Aims: To assess the phenotypic and functional asset of peripheral blood NK cell subsets in newly diagnosed Diffuse-large-B-cell-lymphoma (DLBCL) patients, and to evaluate its acute and long-term modifications upon R-CHOP regimen.
Methods: PBMC from 32 DLBCL patients and 27 healthy, age- and sex-matched controls were analyzed for: 1) the percentage (over PBMC) of $CD56^{dim}$, $CD56^{bright}$, and CD16+ NK cell subsets, measured by multi-parameter flow cytometric (FACS) analysis; 2) the functional capability of NK cell subsets: *i.e.* the frequency of IFN γ-expressing cells and cytotoxic granule-containing (granzyme B+, GrzB+) cells, evaluated by intracellular staining and FACS analysis; and 3) "natural" and CD16-dependent NK cytotoxic functions, quantified by ^{51}Cr release assay. Patients' PBMC were analyzed at diagnosis, at mid-therapy, and at different time points up to 12 months after the end of R-CHOP therapy. All subjects gave informed consent; the study was approved by the institutional Ethical Committee.
Results: The NK cell compartment of DLBCL patients at diagnosis was phenotypically and functionally altered, in comparison to healthy controls: 1) patients' PBMC showed an absolute NK cell count not different from controls, while the percentage of $CD56^{dim}$ and CD16+ NK cell subsets (over lymphocytes) were higher than controls; 2) the frequency of GrzB+ cells was markedly increased in $CD56^{dim}$, $CD56^{bright}$, and CD16+ NK cell subsets; 3) "natural" and CD16-dependent NK cytotoxic activities, as well as the percentage of IFN γ-producing NK cells upon *in vitro* stimulation were not different compared to healthy subjects. At mid-treatment, 1) the frequency of CD16+ NK cells (over lymphocytes), 2) the percentage of NKG2D+ cells in CD16+ and $CD56^{dim}$ NK subsets, and 3) "natural" and CD16-dependent NK cytotoxic activities were significantly reduced. These impairments persisted up to 1 month after R-CHOP, and recovered stably by 3 months after the end of treatment (last analysis: 12 months after R-CHOP). At odds with the above results, 1) the percentage of GrzB+ cells remained elevated in $CD56^{dim}$, $CD56^{bright}$, and CD16+ NK subsets till 3 months after therapy, and returned to normal by 6 months after therapy, and 2) the frequency of IFN γ+ NK cells did not show any significant variation, at any time point.
Summary and Conclusions: 1) The systemic NK compartment at disease onset showed a phenotypic and functional disturbance, resembling a chronic activation state; 2) The marked therapy-induced reduction of "natural" and CD16-dependent NK cytotoxic activities was accompanied by the down modulation of CD16 and NKG2D activating receptors, and was not associated with a gross impairment of NK cell lytic potential and IFN γ production. This suggests that the therapy-driven continuous stimulation through CD16 receptor could be involved in a prolonged NK cell functional impairment *in vivo*. Since impaired NK cytotoxic functions during immunochemotherapy might affect NK cell-mediated, rituximab-dependent ADCC, our observations may be relevant for the improvement of therapeutic strategies.
M.C. Cox and S. Battella equally contributed to the study.

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CONSTITUTIVE ACTIVATION OF THE DNA DAMAGE RESPONSE PATHWAY IS A NOVEL THERAPEUTIC TARGET IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) frequently shows large numbers of genomic aberrations, G1/S checkpoint dysfunction, which are features of instable genomes, and c-MYC overexpression.
Aims: The recent finding that MYC driven cancers such as Burkitt lymphoma (BL) are sensitive to inhibition of the DNA damage response (DDR) pathway, prompted us to investigate the role of DDR pathway as therapeutic target in DLBCL.
Methods: We analysed by immunohistochemistry the expression levels of active DDR pathway components in 99 consecutive DLBCL patients treated at our insti-

tution, with available complete clinical data. 44 Hodgkin lymphoma (HL) cases, 22 BL, 46 indolent B-cell lymphoma cases and 3 normal tonsils were analysed as a control. The cutoff for positivity was set at 30% of cells expressing the protein of interest. The checkpoint kinase (CHK1/2) inhibitor PF-0477736 was used as a tool to evaluate the role of aberrant DDR signalling in DLBCL pathogenesis and the efficacy of DDR inhibition in DLBCL cell lines and primary cells.
Results: We show that the DDR pathway is aberrantly active in aggressive lymphoma cell lines and primary tissues (DLBCL and BL), whereas indolent B-cell lymphoma subtypes show very low expression of active DDR components. In fact constitutive CHK1 and CDC25c phosphorylation were detected in 38 and 40% of DLBCL cases respectively, and about half (47%) of DLBCL cases displayed constitutive expression of histone H2AX phosphorylated at serine 139 (γH2AX), a marker of DNA damage and genomic instability. Less than 15% of indolent lymphomas showed significant pCHK1, pCDC25 or γH2AX expression. γH2AX expression was found to be an independent negative prognostic predictor in DLBCL patients treated with chemoimmunotherapy, being able to identify patients with poor prognosis even in the low-intermediate international prognostic index (IPI 0-2) subgroup. 5-year survival was 41% for γH2AX positive *vs* 70% for γH2AX negative patients. We observed a significant association between γH2AX expression, DDR activation (defined as constitutive CHK1/CHK2 phosphorylation), c-MYC expression levels, and p53 overexpression, suggesting an intimate relationship between genomic instability, DDR activation, G1/S checkpoint dysfunction and c-MYC expression in DLBCL: triple positive cases (n=17) for c-MYC, γH2AX and DDR activation (pCHK1/2+) were characterized by a significantly higher expression of p53, (cutoff of 50%, which is an accepted marker of *TP53* mutations), compared to triple negative cases (n=25) (53% *vs* 20% respectively, p=0.04). We also demonstrate that both activated B-cell (ABC) and germinal center B-cell (GCB)-derived DLBCL cell lines (SUDHL-4, SUDHL-6, BJAB, HBL-1, TMD8, U2932) are very sensitive to the CHK1/2 inhibitor PF-0477736, with IC50s ranging from 9 to 220 nM at 48 hours. Accordingly PF-0477736 induced cell death at nanomolar concentrations in DLBCL primary cells. PF-0477736 determined inhibition of the phosphorylation of downstream target CDC25c coupled with a rapid increase in γH2AX phosphorylation, consistent with DNA damage accumulation, followed by apoptosis (Annexin V/PI staining). PARP cleavage was detected by western blot as soon as after 24 hours of incubation in all cell lines (Figure 1).

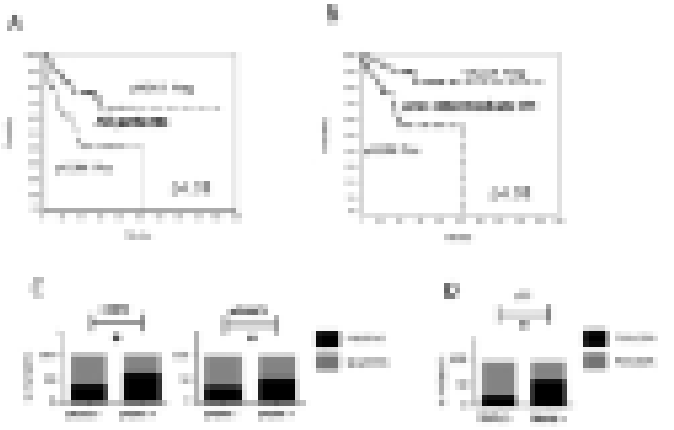

Figure 1.

Summary and Conclusions: These data demonstrate that the DDR pathway is aberrantly active in about half of DLBCL cases and may be involved in resistance to DNA damaging agents, as constitutive γH2AX expression correlates with poor prognosis following conventional chemoimmunotherapy. Pharmacologic inhibition of DDR activation through targeting of CHK kinases may represent a new promising therapeutic strategy in γH2AX positive DLBCL.

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FOXO1 TRANSCRIPTION FACTOR MEDIATES TOXICITY OF SYK INHIBITION IN DLBCL.

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Background: In normal B lymphocytes, B-cell receptor (BCR)-induced activation of PI3K-AKT kinases and subsequent phosphorylation of FOXO1 is a critical effector of pro-survival signalling mediated by the BCR. In murine models, conditional deletion of FOXO1 protected quiescent peripheral B cells from apoptosis mediated by inducible loss of the BCR, demonstrating that PI3K-AKT-FOXO1 axis plays a central role in B-cell homeostasis. Disruption of the BCR signal also leads to the apoptosis of BCR-dependent diffuse large B-cell lymphoma cells (DLBCL), the effect at least in part mediated by decreased AKT activity.
Aims: Since decreased AKT activity leads to decrease in FOXO1 phosphorylation and its subsequent activation, we hypothesized that FOXO1 might be an important effector of BCR inhibitor-mediated toxicity in human BCR-dependent lymphomas. For this reason, we examined the role of FOXO1 in BCR-dependent DLBCL cells treated with SYK inhibitor.
Methods: BCR-dependent DLBCL cell lines (DHL4, DHL6, Ly1, Ly7) were incubated with SYK inhibitor R406 (4 uM) and AKT phosphorylation, FOXO1 activation and transcriptional activity were assessed by phospho-specific flow cytometry, Western Blot and RT-PCR. FOXO1 activity was also assessed in DLBCL cells transduced with a constitutively active AKT mutant. Toxicity of active FOXO1 was assessed by transducing DLBCL cells with a constitutively nuclear FOXO1 mutant. Cellular proliferation, cell cycle changes and apoptosis were assessed by MTS proliferation assay, DNA content-based flow cytometry and Annexin V-PI staining, respectively. To investigate the role of FOXO1 for the activity of SYK inhibitor in DLBCLs, FOXO1 was silenced with shRNA and cellular proliferation and apoptosis were assessed by MTS assay and Annexin V-PI staining.
Results: In all tested cell lines, AKT and FOXO1 phosphorylations were sensitive to SYK inhibition and decreased after incubation with SYK inhibitor. Diminished FOXO1 phosphorylation resulted in its nuclear relocalization and induction of FOXO1-dependent expression of p27, BIM, TRAIL and GADD45A. In DHL4, DHL6 and Ly7 cells transduced with a constitutively active form of AKT, SYK inhibitor did not modulate FOXO1 phosphorylation and exhibited dramatically decreased toxicity. To assess whether the increased activity of FOXO1 is sufficient to induce apoptosis of DLBCL cells, we transduced DHL4 cells with a constitutively nuclear and transcriptionally active FOXO1-3A mutant. The mutant induced G1/S cell cycle arrest and triggered apoptosis, whereas wild-type FOXO1 did not change proliferation or cellular viability, demonstrating that FOXO1 activation is sufficient to induce apoptosis of DLBCL cells. Finally, we assessed the toxicity of SYK inhibitor in cells lacking FOXO1. DHL4 and Ly7 cells were stably transfected with FOXO1-targeting shRNA, decreasing FOXO1 protein level up to 91%. Compared to non-targeting control, cells with silenced FOXO1 exhibited 70% lower sensitivity to SYK inhibitor, as measured with proliferation and apoptosis assays (p<.0001). Importantly, the alteration in FOXO1 levels did not change the phosphorylation levels of SYK and AKT.
Summary and Conclusions: Taken together, our results demonstrate that in BCR-dependent cell lines, inhibition of SYK leads to decreased FOXO1 phosphorylation and induces expression of pro-apoptotic FOXO1 target genes. FOXO1 silencing decreases the sensitivity of DLBCL cell lines to SYK inhibitor, indicating that FOXO1 is an important effector of SYK inhibition in DLBCL.

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KINOME REPROGRAMMING IN DLBCL BY THE BTK-SPECIFIC INHIBITOR ONO-4059 HIGHLIGHTS SYNERGISTIC COMBINATIONS FOR CLINICAL APPLICATION

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Background: Although most patients with DLBCL are cured with chemoimmunotherapy, clinical outcomes remain poor for patients with relapsed/refractory (R/R) disease and new therapeutic approaches are needed. We and others have previously shown that BTK inhibitors (BTKi) including Ibrutinib (Imbruvica) and the specific BTKi ONO-4059 can have considerable single agent activity in R/R DLBCL of the Activated B-cell (ABC) phenotype (de Vos *et al.* EHA 2013, Rule *et al.* ASH, 2013). Nevertheless, most R/R DLBCL patients progress on single agent BTK therapy, suggesting the rapid development of resistance. Studies in other malignancies have shown unexpectedly rapid adaptive "reprogramming" of the kinome in response to specific inhibitors (Duncan *et al.*, 2012; Kampen *et al.*, 2013) but kinome reprogramming in DLBCL has not been reported.
Aims: We have sought to determine mechanisms of resistance in the BTKi sensitive ABC-DLBCL cell line TMD-8, in the hope that this might reveal interacting kinase pathways that potentially could be targeted concurrently to enhance efficacy.
Methods: TMD-8 is an ABC-DLBCL cell line, which undergoes rapid, classical apoptosis at nanomolar concentrations of ONO-4059 (EC50 9.5nM). We have previously shown that ONO-4059 synergises with BCL2 inhibitors in TMD-8 *via* upregulation of BIM, following dephosphorylation of ERK (Kozaki *et al.*, EHA 2013). To investigate mechanisms of resistance to BTKi in ABC-DLBCL and to identify interacting kinase pathways, we studied the effects of both short and long-term exposure of ONO-4059 on TMD-8. Secondly, we developed a ONO-4059 resistant TMD-8 cell line (TMD-8R, apoptosis EC50 >3000nM) by prolonged exposure to ONO-4059 over nine months and investigated kinome reprogramming by immunoblot and phosphokinase antibody arrays.

Results: ONO-4059 induced partial down-regulation of both pAKT and pERK1/2 within 4hrs treatment in the parental cell line TMD-8, but then with prolonged drug exposure (>24hrs) hyperactivation of both pAKT and pERK1/2 was observed in surviving cells. In contrast, pSTAT1 and pSTAT3 were down-regulated in both short and long-term exposures. Interestingly, TMD-8R showed comparable inhibition of pBTK on exposure to ONO-4059 but did not undergo apoptosis. TMD-8R showed no up-regulation of BIM and consequently the synergy between BTKi and BCL2 inhibitors was lost. Furthermore, similar hyperactivation of pAKT and pERK after ONO-4059 was observed in the TMD-8R. Activation of both pathways was confirmed functionally by the demonstration of strong synergy between PI3K inhibitors with BTKi in both TMD-8 and TMD-8R.
Summary and Conclusions: These data show that like other malignancies ABC-DLBCL may undergo rapid adaptive kinome reprogramming following exposure to BTKi. The mechanisms underlying this process are being investigated. Practically, activation of the PI3K/AKT pathway in response to BTK inhibition leads to the development of resistance to BTKi. Collectively, these data indicate that the combination of ONO-4059 with PI3K and perhaps BCL2 inhibitors would have maximal efficacy in this difficult to treat patient group.

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PRECLINICAL EVALUATION OF A NOVEL, HIGHLY POTENT AND SELECTIVE PI3K-DELTA INHIBITOR, CPL-302-201, FOR THE TREATMENT OF HEMATOLOGIC MALIGNANCIES

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Background: PI3Ks belong to the family of lipid kinases that play pivotal role in numerous intracellular signaling pathways. PI3Kα and PI3Kβ are ubiquitously expressed, while PI3Kδ and PI3Kγ are predominantly expressed in hematopoietic cells, although PI3Kγ can also be detected in endothelium, heart and brain. PI3Kδ is the key enzyme involved in BCR and chemokine receptors signaling critical for B cell activation, differentiation and migration. In B cell-derived hematologic malignancies PI3Kδ signaling pathway is engaged in the proliferation and survival of cancer cells. Thus, inhibition of PI3Kδ has been proposed as a new anti-neoplastic therapy in chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphomas. Recent clinical trials showed clinical benefit of Idelalisib (PI3Kδ inhibitor) for CLL patients, however some adverse events were reported. Therefore, there is an urgent need to develop more selective PI3Kδ inhibitors.
Aims: The aim of the study was to investigate the therapeutic potential of a novel PI3Kδ inhibitor CPL-302-201 in hematologic malignancies.
Methods: A novel PI3Kδ inhibitor, CPL-302-201, was developed using knowledge-based drug design approach. To determine the activity and the selectivity of the compound among class I of PI3K lipid kinases and selected protein kinases, ATP-based enzymatic assay was used. Additionally, its biological potency and selectivity was evaluated in a number of cell-based models by Western blot and cell viability assay, ATPlite®.
Results: Obtained results indicate that CPL-302-201 inhibits PI3Kδ kinase activity at low nanomolar concentrations (IC_{50}=6,6 nM) comparable to Idelalisib (IC_{50}=4,1 nM). However, CPL-302-201 exhibits outstanding selectivity over α, β and γ isoforms of PI3K (1890, 830 and 9000-fold, respectively) in comparison to Idelalisib (178, 100 and 27-fold, respectively). Moreover, CPL-302-201 does not inhibit protein kinases from the selectivity panel at the concentration of 1 μM. In cell-based viability assays CPL-302-201 inhibits non-Hodgkin lymphoma cell lines. Western blot analysis has shown that CPL-302-201 significantly inhibits phosphorylation of Akt in Raji cell line after anti-IgM stimulation (IC_{50}<50 nM). Additionally, cellular selectivity of the compound against PI3Kγ has been confirmed by showing no effect on Akt phosphorylation in RAW 264.7 cell line after C5a stimulation up to the concentration of 5μM. Currently, the pharmacokinetic properties of CPL-302-201 are being evaluated.
Summary and Conclusions: We have designed CPL-302-201 – a novel PI3Kδ inhibitor with high potency and outstanding selectivity in comparison to Idelalisib, both in the kinase- and cell-based assays. Preclinical data from non-Hodgkin lymphoma cell lines indicate the therapeutic potential of CPL-302-201 in hematologic malignancies. The activity and unique selectivity of CPL-302-201 qualifies it for the consideration as a drug with a wide therapeutic window and limited side effects in clinical use.

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NOVEL HISTONE DEACETYLASE INHIBITOR ST7612AA1 EXHIBITS EFFICACY IN PRE-CLINICAL MODELS OF MATURE B-CELL LYMPHOMAS

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Background: Treatment of patients with lymphomas that are resistant/refractory to standard chemotherapy is challenging and urgent. Recently, the important role of epigenetic pathways in the pathogenesis of lymphomas has been highlighted by the observations that several genes encoding epigenetic modifiers are selectively and prominently targeted by mutations and genomic aberrations. Histone deacetylase inhibitors (HDACi) are a class of molecules that have shown clinical efficacy in hematological malignancies.
Aims: Here, we have assessed the *in vitro* and *in vivo* activity of the γ-lactamamide thiol-derivative ST7612AA1 (Sigma Tau, Pomezia, IT), a novel HDACi, in lymphoma cell lines.
Methods: The used cell lines were derived from: diffuse large B-cell lymphoma (DLBCL, n=10), mantle cell lymphoma (n=4), splenic marginal zone lymphoma (n=3). Cells were exposed to increasing concentrations of ST7612AA1 and the MTT assay was performed after 72 hours to determine its effect on proliferation. Apoptosis was assessed by Annexin-V/7-AAD staining. Gene expression profiling (GEP) was done, in triplicate, with the Illumina HumanHT-12 Expression BeadChips on one activated B-cell (ABC) DLBCL (TMD8) and one germinal center B-cell (GCB) DLBCL (DOHH2) cell line treated with ST7612AA1 or DMSO for 8 hrs. Differential expression analysis was performed with LIMMA, GSEA and Metacore. DOHH2 cells were injected s.c. in NOD-SCID mice, then treated with ST7612AA1 (40mg/Kg, p.o.) or its vehicle control.
Results: The median IC50 was 394 nM (range, 46-2664) for all the cell lines without significant differences among histological subtypes or between GCB- and ABC-DLBCL: DLBCL-ABC 257 nM (101-805); DLBCL-GCB 636 nM (46-2664); MCL 433 nM (248-553); SMZL 119 nM (102-257). ST7612AA1 induced apoptosis in 3/6 DLBCL cell lines tested. ST7612AA1 presented both nuclear and cytoplasmic anti-deacetylase activity as demonstrated by Western blotting analysis showing increased levels of both acetylated histone H3 and acetyl-a-tubulin. GEP showed that ST7612AA1 affected important oncogenes and tumor suppressor genes. Among the most down-regulated genes there were TNFRSF17 (BCMA), MYC, IRAK1, MYD88, CCND2, BLK, CDK4, IKZF1, BID, while the up-regulated transcripts comprised CDKN2C (p18), CDKN1A (p21), CDKN2D (p19), HLA, CD69, and genes coding histones, MHC-I, MHC-II and metallothioneins. The down-regulated genes were enriched of MYC and E2F targets and members of the TLR/MYD88 pathway. The gene expression signature also revealed significant overlap with genes affected by other HDAC-i and the MTOR-inhibitor rapamycin. Finally, *in vivo* experiments demonstrated that ST7612AA1 determined a significant delay in tumor progression (P=0.0449).
Summary and Conclusions: ST7612AA1 showed promising activity in preclinical models of mature lymphomas and represent a new HDACi worth of further evaluation as single agents and in combination with other agents.

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DARATUMUMAB TREATMENT IN COMBINATION WITH CHOP OR R-CHOP RESULTS IN THE INHIBITION OR REGRESSION OF TUMORS IN PRECLINICAL MODELS OF NON-HODGKINS LYMPHOMA

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Background: Daratumumab is a human antibody that binds to CD38 on the cell surface and induces cell killing by multiple mechanisms including complement mediated cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell phagocytosis (ADCP) and apoptosis. Daratumumab is currently being tested in clinical trials to treat multiple myeloma.
Aims: To explore other hematological malignancies where daratumumab could be efficacious as an anti-tumor therapy, the activity of daratumumab was evaluated *in vitro* and *in vivo* utilizing Non-Hodgkin's Lymphoma (NHL) tumor cell lines and human primary samples.
Methods: The expression of CD38, CD 46, CD55 and CD59 on tumor cell lines was determined using flow cytometry. ADCC assays were carried out using tumor cell lines as target cells and human PBMC as effector cells in a ratio of 50:1. CDC assays were carried out using human serum at 10%. Primary tumor specimens were evaluated by immunohistochemistry (IHC) for CD38 expression. For *in vivo* models, a Burkitt's lymphoma (NAMALWA) or a Diffuse Large B-Cell Lymphoma (DLBCL) (SU-DHL-6) cells were injected subcutaneously in SCID mice and tumors were allowed to grow to about 150-200 mm^3. The animals were treated with daratumumab (10 mg/kg QWx3) or CHOP (QDx5) alone or in combination. For patient-derived DLBCL model, tumor fragments were grafted subcutaneously and the dosing was initiated when tumors reach 125-250 mm^3. The animals were treated with daratumumab alone (20 mg/kg QWx3), CHOP (QDx1), R-CHOP (QDx1) alone or a combination of daratumumab with CHOP or R-CHOP.
Results: Evaluation of 16 NHL cell lines for CD38 expression suggested that

CD38 expression levels varied among cell lines, but the majority (81%, n=13/16) had >1000 CD38 receptors per cell. Daratumumab induced >20% apoptosis in 11 out of 16 cell lines in the presence of a cross-linking agent. In tumor cell killing assays, daratumumab induced >25% ADCC in 7 out of 16 cell lines and >30% CDC in 6 out of 16 cell lines. While no linear correlation was observed between CD38 expression and the extent of ADCC and CDC, tumor cell lysis >10% was observed only in cell lines with >50,000 CD38 receptors/cell. The levels of complement inhibitory proteins (CIP) (CD46, CD55 and CD59) were evaluated to determine of these proteins affected CDC in response to daratumumab but no direct correlation was observed between CDC and CIP expression in these cell lines. In NAMALWA *in vivo* model, daratumumab in combination with CHOP showed significant tumor growth inhibition (47%, p <0.001) compared to the control group on day 26. In SU-DHL-6 model, daratumumab either alone or in combination with CHOP showed significant tumor growth inhibition (55% and 63%, respectively, p<0.01) by day 32. In a patient-derived DLBCL model with high (3+) CD38 expression in 80% of tumor cells by IHC, daratumumab in combination with CHOP or R-CHOP showed tumor regression and the tumors did not regrow when the treatment with daratumumab was stopped after 3 doses. The animals were tumor free at the end of the study (day 60) and showed long-term survival compared to daratumumab alone, CHOP alone or R-CHOP alone.
Summary and Conclusions: These data suggest that daratumumab inhibits growth of NHL tumors that express CD38 and may offer therapeutic benefit in the clinical setting of NHL.

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DIGITAL IMAGE ANALYSIS OF KI-67 FOR RISK STRATIFICATION OF PATIENTS WITH MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) accounts for 4-5% of non-Hodgkin lymphomas in Western countries and is incurable following conventional chemotherapy. MCL is molecularly characterized by the translocation t(11;14)(q13;q32) which leads to overexpression of the cell cycle regulator cyclin D1. The major predictive factor for survival from MCL is proliferation. Counting the fraction of Ki-67-positive cells (Ki-67 index) in 1000 tumor cells in immunostained sections is considered the "gold standard" to predict prognosis. The use of Ki-67-immunostaining for outcome prediction in routine diagnostics has been hampered by interobserver variability due to the lack of a standardized staining technique as well as to the semi-quantitative nature of the scoring performed in routine practice. In this study we applied digital image analysis (DIA) software to determine the proliferation rate in two cohorts of MCL patients and compared the results to manual counting of Ki-67-positive cells as well as to semi-quantitative scoring.
Aims: To develop a standardized software application for fast and objective scoring of Ki-67 in MCL for prognostic stratification in routine diagnostics.
Methods: Two patient cohorts were included in the analysis. In the first cohort, 62 patients included in two Nordic multicenter prospective phase-II studies-MCL2 (Geisler, CH. *et al.* Blood; 2008) and MCL3 (Kolstad, A. *et al.* Submitted), were used to train the software tool (Immunopath) for analysis of the Ki-67 index in each case. Semi-quantitative scores were available from the two studies. In the second cohort, 29 patients included in the LLMPP-study (Rosenwald, A. *et al.* Cancer Cell; 2003) were used to validate the scoring protocol established in the first patient cohort. Representative areas were marked on digital images for DIA and manual counting. In the first patient cohort, the Ki-67 index was counted manually (gold standard) and by DIA on identical digital images on minimum 1000 tumor cells, in addition to DIA of the whole representative tumor area in each case. In the second cohort, the Ki-67 index was counted manually (gold standard) in identical images by two independent observers, in addition to DIA of the whole representative tumor area in each case.
Results: When performing manual counting of Ki-67 index by two independent observers on digital images, the interobserver variability is reduced to a minimum (Spearman's rho=0.978, p <0.001). There was a strong correlation between manual counting and DIA on identical images (Spearman's rho=0.959, p <0.001) (Fig 1). When using Ki-67 index as a continuous parameter in a Cox regression analysis, Ki-67 index showed a statistically significant relevance for OS with both manual counting and DIA in both patient cohorts. There was a less convincing correlation between semi-quantitative scores and manual counts (Spearman's rho=0.807, p <0.001) and semi-quantitative scores were not significant for OS. The DIA showed a trend towards higher estimates of Ki-67 positive cells compared to the manual count (Figure 1).
Summary and Conclusions: Manual quantification of Ki-67 on digital images of immunostained slides practically eliminates interobserver variability. We obtained excellent correlation between manually and digitally quantified Ki-67 index. The Ki-67 index quantified by manual count as well as by DIA is associated with outcome in MCL. However, manual counting is time-consuming and thus not applicable to routine diagnostics. DIA on the other hand can easily be introduced in a routine diagnostic setting and improve risk stratification for MCL.

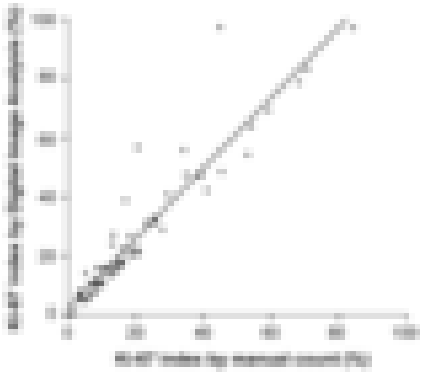

Figure 1.

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DETECTION OF SOX11 PROTEIN BY FLOW CYTOMETRY

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Background: Mantle cell lymphoma (MCL) is a rare, but distinct, B cell derived non-Hodgkin lymphoma (NHL), that accounts for 8% of all malignant lymphomas. It is CD20 and CD5 positive with variable expression of CD23. Thus, by flow cytometry a certain phenotypic overlap with B-cell chronic lymphocytic lymphoma (B-CLL) exist. However, MCL, in contrast to B-CLL, overexpress cyclin D1 by the t(11;14)(q13;32) translocation, juxtaposing the CCND1 gene to the IgH enhancer. This translocation is regarded as primary event in the evolution of MCL. Further, the neuronal transcription factor Sry-related high-mobility-group box 11 (SOX11) is found to be highly upregulated in MCL, independent of cyclin D1-status. Here, we investigated if SOX11 expression could be a flow cytometry marker to discriminate MCL from B-CLL.
Aims: The aim of this study was to verify the utility and feasibility of the new monoclonal antibody anti-SOX11 (MRQ-58) for flow cytometric analysis of primary MCL samples.
Methods: Basal protein expression of SOX11 was verified by western blot and flow cytometry using the commercially available mouse monoclonal anti-SOX11 antibody MRQ-58 in six MCL cell lines: Granta519, Rec1, JeKo1, Mino, Z138 and JVM2. Antibody specificity was verified by transient knockdown of SOX11 in Granta519 and Z138 cell lines and immunoreactivity in the nucleus was confirmed by confocal microscopy. SOX11 expression was investigated in 11 tumor samples of primary cyclin D1-positive MCL from 10 patients and 10 tumor samples of B-CLL by multicolor flow cytometry. In all samples SOX11 mRNA levels were also determined by qPCR.
Results: Immunoblotting for SOX11 showed that the antibody used detected a single band at the predicted size for SOX11 protein in SOX11 positive MCL cell lines, but did not bind to any protein in the SOX11 negative MCL cell line, JVM2. Similar results were obtained using flow cytometry: five SOX11 positive cell lines showed higher SOX11 fluorescence intensity compared to isotype control, whereas for JVM2 the SOX11 fluorescence intensity overlapped with the isotype control. Multicolor flow cytometry showed that all primary MCL tested were SOX11 positive with median fluorescence intensity (MFI), after subtraction to isotype control, in the range 12.63 to 87.26 SOX11 was not detected in any of the B-CLL cases by flow cytometry (MFI range -2.25 to 1.24). We correlated MFI to the Ct values obtained from the qPCR results (mean of triplicates). Since we did not relate SOX11 protein levels to any other protein but to the isotype control, we used equal amounts of cDNA within the samples set (the higher Ct the lower mRNA expression). We found that those two parameters correlated, Spearman correlation coefficient -0.69 (p=0.002).
Summary and Conclusions: We showed that SOX11 protein consistently could be detected and quantified by flow cytometry. Implementing detection of SOX11 in the routine diagnostic flow cytometry would be beneficial for accurate and reliable diagnosis of MCL, especially for rare atypical cases of MCL negative for CD5 or cyclin D1. SOX11 expression also helps distinguish MCL from other malignancies with similar phenotype, like B-CLL. Different from immunohistochemistry, analyzing SOX11 by flow cytometry will generate quantitative answers. Possibility of using SOX11 in a multi-color panel with other markers is an additional advantage. Although flow cytometry would not be able distinguish SOX11 nuclear positivity, it could improve diagnosis, as a complement to IHC.

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ABSOLUTE MONOCYTE AND LYMPHOCYTE COUNT IN FOLLICULAR LYMPHOMA: PROGNOSTIC ROLE IN SURVIVAL OUTCOME EVALUATION

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Background: The Follicular Lymphoma International Prognostic Index (FLIPI) still remains the most used model for risk stratification of patients with follicular lymphoma (FL).After rituximab introduction there was a meaningful improvement in progression free survival (PFS) and overall survival (OS) of FL, and a new prognostic score was considered necessary (FLIPI2). Furthermore several papers were published on prognostic value of lymphopenia and monocytosis in lymphomas, showing that these parameters are able to identify high risk cases. Absolute monocyte count (AMC) and absolute lymphocyte count (ALC) could be considered as surrogate markers: AMC for microenvironment supporting tumor growth and ALC as expression of immune status.
Aims: We performed an ancillary study from FOLL05 clinical trial with the aim to analyze, in patients treated for FL with regimens containing rituximab, the prognostic role of AMC and ALC in terms of PFS and OS. The FOLL05 study was a large prospective, randomized, multicenter protocol of Fondazione Italiana Linfomi, which enrolled from 2006 to 2010 a total of 531 eligible patients; last update was in December 2013. It was approved by ethics committees and required each patient to provide written informed consent.
Methods: Since the cut-offs for AMC and ALC reported in literature were mostly related to aggressive lymphomas, we assessed their reproducibility in FL, modeling AMC and ALC as continuous covariates with a Cox PH restricted cubic spline regression, adjusted by FLIPI and stratified by chemotherapy. The AMC or ALC value who cross the log(HR)=0 was chosen as cut-off. These methods confirm that the cut-offs of AMC >630/mm3 and ALC≤1000/mm3 are applicable also in FL selecting worst groups, as already observed in previous analysis on aggressive lymphomas. We assessed the importance of AMC and ALC predictor over 1000 bootstrap inclusion fraction (BIF) in Cox PH model.
Results: 431 out of 531 patients had complete data for AMC and ALC; median age at diagnosis was 56 years. With a median follow-up of 57 months the 5-years PFS was 57% (95%CI 52-62) and 5-years OS 90% (95%CI 87-93). One hundred seven (25%) patients had AMC >630/mm3. Monocytosis correlated with FLIPI score (P=0.012) while it was not associated with FLIPI2 and any type of chemotherapy. The 5-years PFS was 61% and 44% for AMC ≤630 and >630/mm3, respectively (P=0.0009, Figure 1a). Adjusted by FLIPI and stratified by chemotherapy the HR was 1.66 (95%CI 1.21-2.27) defining AMC >630/mm3 as a strong predictor since the BIF over 1000 bootstrap resamples was 84.8%. The 5-years OS was 93% and 81% for AMC ≤630 and >630/mm3, respectively (P=0.003, Figure 1b). Ninety one (21%) patients had ALC ≤1000/mm3, that was not correlated with FLIPI and chemotherapy, while it was associated with FLIPI2 (P=0.014). The 5-years PFS was 58% and 51% for ALC >1000 and ALC ≤1000/mm3, respectively (P=0.129, Figure 1c). Adjusted by FLIPI and stratified by chemotherapy the HR was 1.22 (95%CI 0.86-1.71) showing ALC ≤1000/mm3 as a very weak predictor since the BIF over 1000 bootstrap resamples was 21%.The 5-years OS was 90% and 93% for ALC >1000and ≤1000/mm3, respectively (P=0.957, Figure 1d).

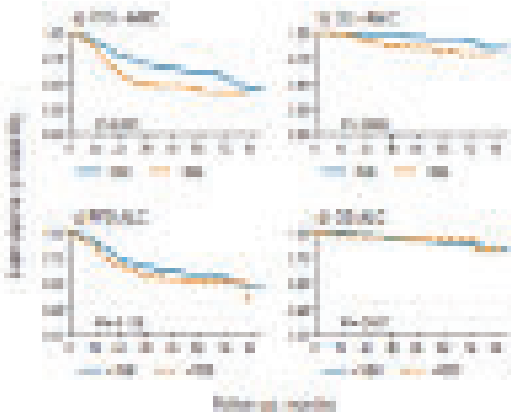

Figure 1. PFS and OS by AMC (a, b) and ALC (c, d) adjusted by FLIPI and stratified by chemotherapy.

Summary and Conclusions: Our large study, with data collected prospectively, showed that monocytosis is a strong negative predictive factor for survival outcome, in particular for PFS, while lymphopenia ≤1000/mm3 showed a weak predictive role.

P438

BRIEF RITUXIMAB, BENDAMUSTINE, MITOXANTRONE (R-BM) INDUCTION PLUS RITUXIMAB CONSOLIDATION IN ELDERLY *DE NOVO* ADVANCED STAGE FOLLICULAR LYMPHOMA: FINAL RESULTS OF A STUDY BY FONDAZIONE ITALIANA LINFOMI

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Background: a previous FIL trial (Vitolo *et al.*, JCO 2013) showed that a brief R-FND (Fludarabine, Mitoxantrone, Dexamethasone) induction chemoimmunotherapy followed by rituximab consolidation achieved high CR and PFS rates in elderly Follicular Lymphoma (FL) patients (pts); bendamustine is a well-tolerated and effective drug (Rummel *et al.*, Lancet 2013) but data on its use in combination with other cytotoxic agents are scant.
Aims: to evaluate overall response rate (ORR) of a brief chemoimmunotherapy with R-Bendamustine in combination with Mitoxantrone in elderly pts, reducing toxicity compared to the previous study.
Methods: 76 elderly *de novo* FL pts (age 65-80) were enrolled (Sept 2009-Nov 2011). Inclusion criteria were: grade I, II and IIIa; advanced (stage III/IV) or stage II disease requiring treatment; "FIT" pts according to comprehensive geriatric assessment (ADL, I-ADL, CIRS). Treatment plan was: 4 monthly courses of R-BM (375 mg/m^2 Rituximab day 1, 90 mg/m^2 Bendamustine days 1-2, 8 mg/m^2 Mitoxantrone day 1) followed by 4 weekly Rituximab infusions as consolidation; Rituximab maintenance was not planned because not licensed at that time in Italy. Polymerase chain reaction (PCR) for BCL2/IgH rearrangement was performed on bone marrow samples at diagnosis, after R-BM and at the end of consolidation.
Results: Median age was 71 years (range 65-79); 29 males, 47 females; WHO grading was as follows: I 18%, II 54% and IIIa 28%; 15% had advanced stage II disease, 30% stage III and 55% stage IV; 47% had BM involvement, 20% B symptoms and 7% leukemic dissemination; 59% patients had no comorbidity, 20% one and 21% ≥2 comorbidities. According to FLIPI pts were: low (L) 8%, intermediate (I) 32% and high (H) risk 60%. PCR analysis for BCL2/IgH rearrangement was carried out in 75 pts at diagnosis: 39 (51%) were Bcl-2 positive. Seventy (92%) pts completed the planned treatment: 50/70 (72%) in the planned time. Six pts did not complete the treatment: 1 for progressive disease, 4 for adverse events (2 haematological toxicities with prolonged neutropenia; 1 CMV colitis and 1 for infection and concomitant worsening of pre-existing oral pemfigo) and 1 patient for worsening of performance status. ORR to treatment was 94% with 59 (78%) complete remissions (CR) and 12 (16%) partial remissions (PR). Response to induction R-BM was as follow: 32 (42%) CR, 40 (53%) PR and 4 (5%) stable (SD) or progressive disease (PD); 28 (70%) of the 40 pts in PR after R-BM converted to CR following further Rituximab consolidation. At the end of treatment 25/39 (64%) of pts were PCR negative and most of them, 21/25 (84%), were also in CR. At a median follow-up of 31 months, 2-yrs OS rate was 95% and 2-yrs PFS rate was 88% (no difference in PFS according to FLIPI L+I *vs* H risk: 90% *vs* 87%). Overall, 5 deaths were recorded, one due to progressive disease. A total of 577 courses of R-BM and Rituximab were given and overall the regimen was well-tolerated in an outpatient setting. The most frequent severe toxicity (CTC grade 3-4) was neutropenia reported in 18% of the cycles, but only 6 cases of severe infection and 8 cases of neutropenic fever occurred; 68% of the pts received G-CSF support during treatment (Figure 1).

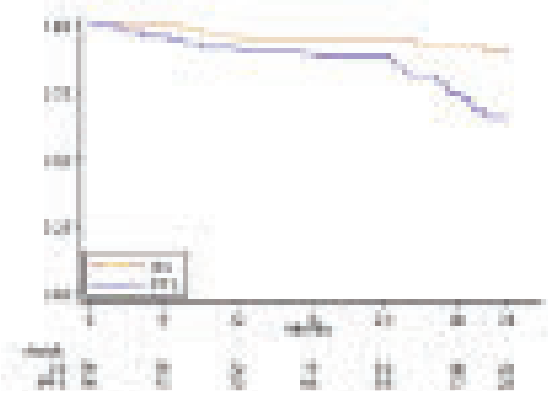

Figure 1.

Summary and Conclusions: the reduction of the amount of chemoimmunotherapy with a brief R-Bendamustine combined with Mitoxantrone regimen is an effective and safe treatment strategy in elderly FL pts with high CR and molecular remission rates and prolonged PFS.

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BENDAMUSTINE IS NOT ASSOCIATED WITH AN INCREASE IN INFECTIONS – SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Background: Bendamustine is a chemotherapeutic drug with structural similarities to both alkylating agents (nitrogen mustard derivative) and purine analogues (benzimidazole ring). Theoretically, due to its nucleoside-like properties it might be associated with more infections.Data in the literature are lacking regarding the infection-related adverse events of bendamustine-containing regimens.
Aims: We aimed to assess the risk of infections associated with bendamustine.
Methods: Systematic review and meta-analysis of all randomized controlled trials comparing bendamustine containing regimens (alone or combined with other chemotherapeutic agents and/or rituximab) to any other regimens. Trials evaluating bendamustine for any lymphoproliferative disorder or plasma cell dyscrasia were included. A comprehensive search of The Cochrane Library, MEDLINE, conference proceedings and references was conducted until February 2014. Two reviewers appraised the quality of trials and extracted data. Outcomes assessed were: any infections, grade 3-4 infections, fatal infections, grade 3-4 neutropenia and grade 3-4 lymphopenia. For dichotomous data, relative risks (RR) with 95% confidence intervals (CIs) were estimated and pooled. We used fixed effect model to pool data, unless there was significant heterogeneity, in which case we used the random effects model.
Results: Eight trials conducted between the years 2006 and 2013 and randomizing 2022 patients were included. We included 4 trials of patients with non-Hodgkin lymphoma (Rummel 2013, Rummel 2010, Herold 2006 and the Bright study 2013), 3 trials of CLL (Knauf 2009, Niederle 2013, LeBlond 2013), 1 trial of patients with multiple myeloma (Ponish 2006). The bendamustine arm included: bendamustine alone (2 trials), bendamustine-rituximab (BR) (4 trials), bendamustine, vincristine, prednisone (BOP) (1 trial), and bendamustine, prednisone (BP) (1 trial). The comparator arms in 6 of the trials included other alkylating agents: chlorambucil, R-chlorambucil, R -CHOP (in 2 trials), R-COP, COP, and melphalan-prednisone (MP). In 2 trials the comparator arm included fludarabine (alone or with rituximab). There was no statistically significant effect for bendamustine on the rate of any type of infection (RR 1.06 [95% CI 0.83, 1.34], 6 trials). There was no increase in the rate of grade 3-4 infections (RR 1.3 [95% CI 0.73, 2.3], 6 trials) or fatal infections (RR 0.69 [95% CI 0.30, 1.58], 3 trials). There was no difference in the rate of infections between trials that administered bendamustine as first line and trials that administered it for relapse. Data were too scarce to analyze by specific types of infections separately. There was no increase in the rate of grade 3-4 neutropenia in the bendamustine arm (RR 0.9 [95% CI 0.58, 1.42], 6 trials). This was true when the comparator was alkylating agent containing regimens (RR 0.87 [95% CI 0.52, 1.48], 4 trials) as well as fludarabine containing regimens (RR 1.02 [95% CI 0.54, 1.91], 2 trials). There was a significant increase in grade 3-4 lymphopenia in the bendamustine arm compared to the alkylating agent containing regimens arm (RR 1.95[95% CI 1.54, 2.47).
Summary and Conclusions: Our systematic review demonstrates no effect of bendamustine on the rate of infections when compared to either alkylating agents or fludarabine, despite an increase in lymphopenia. Thus, bendamustine remains a safe therapeutic option. The main drawback of this meta-analysis is the heterogeneity between malignancies and treatments.

P440

EXPRESSION OF CD200 AND CD148 IN LEUKEMIC B-CELL CHRONIC LYMPHOPROLIFERATIVE DISORDERS

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Background: B-cell chronic lymphoproliferative disorder (B-CLPD) is comprised of a wide range of B-cell derived malignancies. Characteristic morphology and immunohistochemistry studies are fundamental to the diagnosis of B-CLPD. Compared to traditional histopathological analysis, flow cytometric evaluation of tumor cell immunophenotyping is more reproducible and less time demanding. Although there are some established Immunophenotypic score systems, for example, Matutes score system for the diagnosis of chronic lymphocytic leukemia (CLL), differential diagnosis between these B-CLPD remains difficult in some cases. Thus, new reliable and sensitive biomarkers are urgently needed. Recently, CD200 and CD148 were found expressed in different stages during B cell development, which indicated possible discrimination value.
Aims: To access definite expression patterns of CD200 and CD148 in B-CLPD and its potential diagnostic value.
Methods: Flow cytometric analysis was performed on fresh PB/BM specimen. Specific antibodies conjugated with fluorescein were used with murine isotype control applied with each sample to set negative gate.
Results: We have evaluated the expression patterns of CD200 and CD148 in 376 cases of B-CLPD by flow cytometry. Our results shows that CD200 and CD148 expression patterns distinguishes different B-CLPD, and may have potential differential diagnostic value in B-CLPD, especially in CD5 positive B-CLPD, CLL, mantle cell lymphoma (MCL) and other CD5+ B-CLPD. CLL exhibited relatively uniform cell surface phenotypes of CD200 and CD148 with high positivity and mean fluorescence intensity (MFI) of CD200 and relatively low CD148 MFI; In contrast, MCL showed significantly different expression patterns of low CD200 and strong CD148 expression compared to CLL. There were significant differences of CD200 positivity (P<0.0001), CD200 MFI (P<0.0001), and CD148 MFI (P<0.0001) between CLL and MCL. And other B-CLPD have also showed diverse expression patterns of CD200 and CD148 (Figure 1). ROC and AUC analyses suggested that CD200 MFI yielded an AUC of 0.97 and CD148 MFI yielded an AUC of 0.90 in discriminating CLL from MCL. Furthermore, CD148MFI/CD200MFI >4.8 produced a sensitivity of 94.5% and a specificity of 100% in establishing the diagnosis of MCL when differential diagnosis between MCL and CLL is needed.

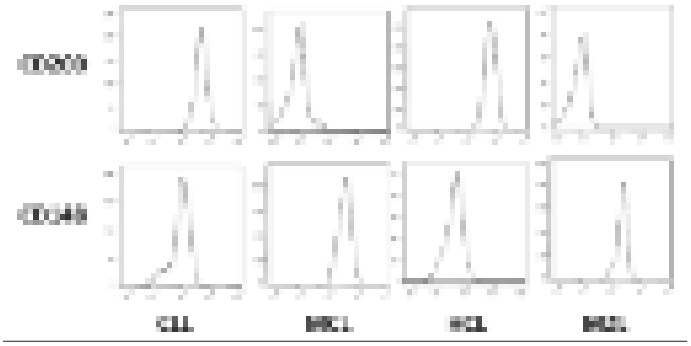

Figure 1.

Summary and Conclusions: We have therefore concluded that CD200 and CD148 expression patterns are totally different among B-CLPD, and the combination of CD200 and CD148 may have potential differential diagnostic value in leukemic B-CLPD, especially in CLL and MCL, and the introduction of CD200 and CD148 could be a powerful tool in B-CLPD diagnostic panel.

P441

ABEXINOSTAT (S78454) A HISTONE DEACETYLASE INHIBITOR, IN PATIENTS WITH REFRACTORY OR RELAPSED HODGKIN'S LYMPHOMA, NON-HODGKIN LYMPHOMA AND CHRONIC LYMPHOCYTIC LEUKAEMIA: PRELIMINARY PHASE II RESULTS

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Background: Histone deacetylase inhibitors (HDACi) have emerged as promising new anticancer agents with potent and specific activity in hematological malignancies. Abexinostat is a new hydroxymate-based pan-HDACi of class I and II. A phase I/II international, multicentre, open-label, non-randomized, non-comparative dose-escalation study determined the recommended dose (RP2D) with oral abexinostat: 80 mg BID (14 days on, 7 days off). Phase I results have shown that abexinostat has an acceptable safety profile and demonstrated activity on indolent lymphomas and Hodgkin's lymphoma, with 3 complete

responses (CR), 6 partial responses (PR) and 9 stable diseases (SD) in 29 patients treated and with baseline and post-baseline tumor evaluation: 62% had a clinical benefit.
Aims: The aim in this phase II part was to further evaluate the efficacy of abexinostat as a single-agent in patients with relapsed or refractory non-Hodgkin lymphoma (NHL) or chronic lymphocytic leukaemia (CLL) at the RP2D.
Methods: Five cohorts were open in parallel: follicular lymphoma (FL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), marginal zone B-cell lymphoma (MZL) or Waldenström macroglobulinaemia (WM)/lymphoplasmatic lymphoma and CLL/lymphocytic lymphoma. The primary endpoint was objective response rate (ORR: CR and PR). The responses were evaluated respectively with Cheson (2007), Hallek (2008) and Kimby (2006) criteria for NHL, CLL and WM. Safety, overall survival (OS) and progression-free survival (PFS) were secondary endpoints.
Results: Until the 1st of July 2013, 53 of 55 patients were treated and evaluable for tumor response: 13 FL, 8 CLL, 5 MZL/2 WM, 10 MCL and 15 DLBCL. Median age was 65 years [range 32-85] and 57% were male. At inclusion, median duration of disease was 4 years [range 0-16] and median treatment free interval was 3.9 months [range 1-89]. 43% of patients were refractory to their last treatment. 70% had received more than 3 previous lines of treatment. 60% of FL, MZL, DLBCL and MCL patients were Ann Arbor Stage III/IV, and 25% of CLL patients were Binet Stage C. ORR was 30% (investigator's assessment) with 3 CR and 13 PR. ORR for the 13 FL patients was 46% with 1 CR and 5 PR, 5 patients had SD. In the 8 CLL patients, 1 achieved PR and 7 had SD. Of the 7 MZL/WM patients, 2 achieved PR and 5 a SD. Of the 10 MCL patients, 1 had a CR, 1 a PR and 5 a SD. Of the 15 DLBCL patients, 1 had a CR, 4 a PR and 5 a SD. OS was not mature (68% of patients alive). Only the median of PFS for MCL and DLBCL were mature: 8 weeks [95% CI 4.8-25] for MCL and 12.2 weeks [95% CI 6.2-16.2] for DLBCL. On the 11 FL patients still alive, 6 were on-going without record of PD including 1 CR [5.5 months of follow-up (FU)], 2 PR [3.4 and 9.2 months of FU] and 3 SD [2.8, 4.4 and 7.1 months of FU] and for MZL/WM patients, 1 was still on-going without record of PD with SD [5.7 months of FU]. Grade 3 and 4 drug-related emergent adverse events (EAEs) were experienced by 82% of patients. The most common were grade 3 and 4 thrombocytopenia (78%), neutropenia (29%) and anemia (9%). Three patients experienced grade 4 febrile neutropenia. The most common non-hematological drug-related EAEs were grade 1 and 2 asthenia, diarrhea and nausea. Only few patients experienced grade 3 asthenia (5.5%), diarrhea (1.8%) and nausea (3.6%). No grade 4 was reported for these for the latter.
Summary and Conclusions: The ORR of 30% is encouraging in this patient population, especially in FL (ORR: 46%). Safety profile of abexinostat is manageable with a safety profile expected in this class of drug. Recruitment is still on-going.

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HIGH-DOSE CLARITHROMYCIN IS A FEASIBLE AND ACTIVE MONOTHERAPY FOR PATIENTS WITH RELAPSED/REFRACTORY EXTRANODAL MARGINAL ZONE LYMPHOMA [EMZL]: RESULTS FROM THE "HD-K" PHASE II TRIAL

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Background: The antibiotic clarithromycin displays immunomodulatory properties, partially mediated by the suppression of inflammatory cytokines. This macrolide can directly induce TNF-mediated apoptosis in mouse lymphoma cells, with a DNA fragmentation degree similar to that seen in cells treated with chemotherapeutic agents. The combination clarithromycin-lenalidomide-dexamethasone has been associated with high tumour regression rates in patients (pts) with failed multiple myeloma and Waldenstrom's macroglobulinemia. As single agent, conventional-dose clarithromycin has been associated with an overall response rate (ORR) of 38% and a 3-year PFS of 58% in pts with relapsed/refractory EMZL [Govi *et al.* BJH 2010]. A dose-dependent antineoplastic effect was hypothesized, and a phase II trial addressing high-dose clarithromycin (HD-K) in patients with relapsed/refractory EMZL was conducted (clinicaltrials.gov NCT01516606). Herein, we report tolerability and activity results.
Aims: to address tolerability and activity of high dose clarithromycin in pts with repapsed/refractory extranodal marginal zone lymphoma.
Methods: HIV-negative adults (age ≥18 ys; ECOG-PS ≤3) with relapsed/refractory EMZL and at least one measurable/parametrable lesion were registered and treated with four courses of oral clarithromycin 2 g/day, days 1-14, every 21 days. Activity, in terms of ORR (complete and partial responses), was the primary endpoint; following a two-step Simon minimax, with an ORR of 40% (P0) [Govi *et al.* BJH 2010] and an alternative hypothesis of ORR ≥70% (P1), 21 pts were needed (type-I error 5%, power 80%, two-sided). HD-K would be considered active if ≥12 objective response were obtained.
Results: Twenty-three pts were registered (median age 70 ys, range 47-88; M:F ratio: 0.3). HD-K was the salvage therapy at first relapse in 13 pts, and the 3rd-5th line of treatment in the others. Seventeen pts had local disease at the time of treatment, six pts had multiorgan disease. Ocular adnexae (13), stomach (n=4) and lung were the most commonly involved extranodal organs. No pt had increased LDH serum levels; only one pt had B symptoms. Four pts had concomitant HBV/HCV infections. *H. pylori* and *C. psittaci* infections were successfully eradicated with specific antibiotics as part of first-line treatment, with a median time from bacterial eradication to clarithomycin treatment of 24 (10-90) months. This excludes that a potential clarithomycin antitumor activity could be due to antimicrobial effect. The treatment was completed in 19 pts; it was interrupted in 4 pts due to nausea (1) or progressive disease (PD; 3). Eighty (87%) of 92 planned courses were actually delivered. Tolerability was excellent-good; grade 1-2 nausea was the commonest side effect, but it was manageable and did not require dose reduction, only two pts had G3 nausea. No biochemical abnormalities were detected during or after HD-K. Six pts achieved a complete remission and six pts achieved a partial response (ORR=52%; 95%CI=32-72%); 4 pts had stable disease (4+, 7+, 15+, 18+), 7 pts experienced PD (within 6 months). The number of previous lines of treatment did not influence response. After a median follow-up of 12 months, no pt with responsive or stable disease experienced relapse, with a one-year PFS of 68%. All pts are alive, with a median OS of 45 months (8-214).
Summary and Conclusions: HD-K is a safe and active salvage treatment in EMZL pts; it appears more active than conventional-dose clarithromycin. This antibiotic deserves to be further investigated in EMZL and other lymphoma categories, perhaps in combination with immunomodulators.

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EPIDEMIOLOGY OF NON-HODGKIN LYMPHOMA IN THE US: DISTRIBUTION BY SUBTYPES, DEMOGRAPHICS, AND TRENDS FROM 1998-2011

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Background: Over 15 years have passed since the publication of the International Lymphoma Study Group's classification of non-Hodgkin lymphoma (NHL; *Blood* 1997).
Aims: The objectives of our study are to provide a more contemporary description of the epidemiology of NHL in the US derived from population-based data and to determine whether there is disparity in NHL subtype distribution according to racial groups.
Methods: Patients with all types of NHL diagnosed from 1998 to 2011 were identified from the US National Cancer Data Base (NCDB). We obtained patient level data pertaining to demographics and histology. The NCDB is a nationwide oncology outcomes database for more than 1,500 cancer programs and represents 70% of all newly diagnosed cases of cancer in the US.
Results: There were 515,206 patients diagnosed with NHL. The median age at diagnosis was 67.0 years and 52.5% were males. In 2011, the NHL distribution by cell type were B (82.9%), T/NK (4.7%), and not otherwise specified (NOS; 12.4%). The top 10 distinct histologic subtypes were diffuse large cell (39.2%), follicular (19.9%), marginal zone (10.0%), mantle cell (4.8%), small lymphocytic (5.3%), peripheral T-cell NOS (2.0%), Burkitt (1.9%), lymphoplasmacytic (1.4%), anaplastic large cell (1.1%), and angioimmunoblastic T-cell (0.8%). Over the 14-year study period, significant increasing trends were noted in the male predominance (from 51.6% in 1998 to 53.8% in 2011) and proportion of patients with several subtypes of T-cell NHL (angioimmunoblastic, enteropathy-type, hepatosplenic, peripheral NOS) as well as marginal zone lymphoma. Significant disparities exist based on proportions of histologic subtypes seen according to race. In particular, compared to Blacks and Asians, Whites and Native Americans were nearly twice as likely to develop follicular (12.3%/14.1% *vs* 21.5%/20.2%) and mantle cell (2.3%/2.6% *vs* 4.4%/4.0%) lymphomas. Hepatosplenic T-cell lymphoma was least common among Whites (0.03% *vs* 0.10-0.14%) while enteropathy-type T-cell (0.19% *vs* 0.0-0.09%) and small lymphocytic (7.6% *vs* 2.5-6.7%) lymphomas were more common among Asians. Primary effusion lymphoma was more common among Blacks and Native Americans (0.10% *vs* 0.01-0.03%).
Summary and Conclusions: We provide a contemporary epidemiologic description of NHL in the US population. Certain histologic subtypes are more common among racial subgroups and some appear to be increasing in proportion over time relative to others.

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UBLITUXIMAB (TG-1101), A NOVEL ANTI-CD20 MONOCLONAL FOR RITUXIMAB RELAPSED/REFRACTORY B-CELL MALIGNANCIES

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Background: The introduction of anti-CD20 therapy has resulted in significant improvement in clinical outcomes for patients with B-cell malignancies. Despite these advances, patients continue to relapse from, or become refractory to, rituximab (RTX) based regimens. Ublituximab (UTX) a novel mAb targeting a unique epitope on the CD20 antigen, has been glycoengineered to enhance affinity for all variants of FcγRIIIa receptors and demonstrates significantly greater ADCC than RTX (Le Garff-Tavernier, 2011). A Phase I trial with single-agent UTX in patients with relapsed/refractory CLL reported an ORR of 45% (EHA 2013).
Aims: Herein we report on two Phase I dose-escalation studies of UTX in patients with RTX relapsed/refractory B-cell malignancies: TG-1101-101, a single-agent dose-escalation study of UTX; and TG-1101-102, a dose-escalation study of UTX in combination with lenalidomide (LEN), an immunomodulatory agent that has displayed single-agent activity in NHL and CLL, and has been shown to synergize with anti-CD20 mAbs.
Methods: Eligible patients for both studies were relapsed/refractory to a prior anti-CD20 regimen; had evaluable disease with confirmed CLL/SLL or NHL diagnosis, no active Hep B/C, and provided informed consent to participate. For TG-1101-101 (single agent UTX), the Phase I dose-escalation used a sequential 3+3 design in dose cohorts of 450, 600, 900, and 1200mg. Expansion cohorts were opened at select doses. UTX was administered weeklyx4 doses in Cycle 1 for NHL and on days 1, 8, 15 in Cycles 1 & 2 for CLL patients (cycle=28 days), with maintenance (monthly cycles 3 - 6 followed by quarterly infusions after cycle 6) for all patients. In TG-1101-102 (UTX+LEN), the dose escalation was a sequential 3+3 design with 4 cohorts of escalating UTX, with LEN started at a fixed dose of 10mg and titrated up to 15mg for CLL and up to 20mg for NHL patients per tolerability. UTX was administered days 1, 8, 15 of Cycles 1 & 2 (Cycle=28 days) with maintenance while LEN was started Cycle 1/Day 9 and continued daily (NHL patients, up to a 7 day rest period (days 21-28) was permitted in any cycle). In both studies, safety and efficacy were primary and secondary endpoints, respectively. PK and correlative PD data were collected in both studies.
Results: In TG-1101-101, 32 patients have been enrolled. Median age 68; 17/15 (M/F); median prior therapies=3 (range 1-9). RTX refractory=41%. UTX was well tolerated with no DLT's observed. The most frequent AE for single agent UTX has been Cycle 1/Day 1 infusion related reactions in CLL patients. 29/32 patients (11 FL, 6 MZL, 6 CLL, 5 MCL, and 1 DLBCL) were evaluable for efficacy: 3 CRs (1 FL and 2 MZL); 9 PR's (4 CLL, 2 MZL, 2 FL, and 1 DLBCL); 13 SD (6 FL, 3 MCL, 2 MZL and 2 CLL) and 4 PD (2 FL, 2 MCL) for an ORR of 41%. 86% of patients remained in SD or better for >12 weeks and median PFS for all patients was 34 weeks (95% CI: 19, NA) with no observed progression in 16/29 patients. In TG-1101-102, 10 patients have been enrolled (5 CLL/SLL, 3 MCL, 1 FL and 1 Burkitt's). Median age 66; 7/3 (M/F); median prior therapies=3 (range 3 – 6). Patients were heavily pretreated with 90% refractory to prior treatment and 70% RTX refractory, including two patients previously refractory to a BTK inhibitor and one patient refractory to a PI3Kδ inhibitor. Adverse events with the combination at a LEN dose of 10mg with dose reductions as needed, were manageable, with no DLTs observed. Gr 3/4 AE's included: neutropenia, thrombocytopenia, leukopenia and dyspnea. Lymphocyte depletion in CLL patients has been rapid and profound. 2 patients (1 MCL patient who had previously progressed on idelalisib, and 1 FL patient) achieved a PR.
Summary and Conclusions: UTX has been well tolerated in both Phase I studies with promising clinical activity for single-agent UTX in patients with RTX-relapsed or refractory disease. The safety profile of UTX supports combination therapy, and the combination of UTX and LEN warrants further exploration. Additional combination studies are ongoing with UTX in combination with novel targeted agents (BTK and PI3Kδ inhibitors).

P445

PATIENT-REPORTED OUTCOMES DATA FROM A PHASE 2 STUDY OF IDELALISIB IN PATIENTS WITH REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMA (INHL)

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Background: Idelalisib, a selective oral inhibitor of PI3Kδ, demonstrated considerable anti-tumor activity in patients with relapsed/refractory iNHL in a phase 2 trial (Gopal, 2014).
Aims: The purpose of this analysis was to evaluate patient-reported outcomes (PRO) data to determine whether drug treatment was associated with a change in health-related quality of life (HRQL).
Methods: Eligible iNHL patients (pts) were refractory to both rituximab and an alkylating agent. Idelalisib 150 mg PO BID was administered continuously until disease progression. HRQL was measured by the 42-item Functional Assessment of Cancer Therapy-Lymphoma (FACT-Lym), comprising FACT-G subscales: Physical Well-being (PWB), Social/Family Well-being (SWB), Emotional Well-being (EWB), Functional Well-being (FWB), and the Lymphoma Subscale (LymS). Trial Outcome Index (TOI) is PWB+FWB+LymS. Higher scores reflect better HRQL. Minimally important differences (MID) on the FACT-G ranged 3-7 points (2-5 points for subscales). The FACT-Lym was administered every 4 weeks (0-24), then every 6 weeks (30-48), and at week 60. Change from baseline in FACT-Lym was analyzed.
Results: Enrolled pts (N=125) had a median age of 64 years [range 33-87] and were 64% male. With a median follow up 9.4 months, overall response rate (ORR) is 57% (95% CI=47.6, 65.6) and median DOR is 12.5 months. Median PFS for all pts is 11.0 months. Improvements were noted in the FACT-G, FACT-Lym, and TOI scores during the study, progressively increasing with time. In FACT-G subscales, improvements were noted by 4 weeks for EWB scores. The median best changes from baseline for the FACT-G, FACT-Lym, and TOI total scores were 5.0, 8.3, and 6.0, respectively. LymS change scores exceeded the MID for ≥90% of pts indicating a clinically significant improvement in lymphoma-related concerns at some point in the study. The median best change from baseline for the LymS was 5.0 and median time to improvement was 1.9 months.
Summary and Conclusions: PRO data indicate that clinically significant improvements in HRQL, including lymphoma-related concerns, were noted for most patients.

P446

DROPLET PCR: A NEW SENSITIVE METHOD FOR DETECTING BRAFV600E MUTATION IN HAIRY CELL LEUKEMIA

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Background: Hairy cell leukemia (HCL), that represents approximately 2% of all leukemias, is an indolent lymphoproliferative disease with complete response (CR) rates higher than 80% and long-term survival accounting more than 70% of patients receving rituximab plus 2CdA. In the last recent years, the application of the deep sequencing thechniques allowed to detect the BRAFV600E mutation in up to 100% of the HCL patients, indicating a constitutive activation of the RAF-MEK-ERK pathway. This mutation is also useful for the differentiating HCL cases from other indolent lymphomas, including the splenic marginal-zone or unclassifiable splenic lymphomas or leukemias, that are BRAF unmutated. Different authors described several available molecular techniques for detection of the BRAF mutation: a) the Sanger sequencing, with a sensitivity of 1-5%% (Arcaini L); b) the pyrosequencing, which has a sensitivity of 5% (Laurini JA, Verma S); c) a specific quantitative real-time PCR, with a sensitivity of $2x10^{-4}$ (Schnittger S, Tiacci E).
Aims: The aim of the study was to compare different molecular techniques (real-time PCR and droplet PCR) and their sensitivity/specificity in a series of 40 patients affected by splenic merginal lymphoma and HCL.
Methods: In this study, we assessed 20 untreated cases with splenic marginal lymphoma and further 20 HCL patients by a specific real-time PCR for the BRAFV600E mutation (qBiomarker Somatic Mutation PCR assays BRAF_476, SABiosciences), and by a newer PCR technique: the droplet PCR (PrimePCR™ ddPCR™ Mutation Assay: JAK2 WT for p.V617F, Human- Biorad).
Results: Our HCL patients received R-2CdA, achieved 75% of CR, being the CR the best response for 83% of patients. The 5-year overall survival was 87%, and PFS 66%. All patients, except one HCL case, showed the IgH clonality; as reported even by our group, the sensitivity of this method ranges from $1x10^{-3}$ to $1x10^{-4}$, and this rearrangement was useful for confirming the infiltration of the assessed bone marrow samples. When the same samples were tested for the BRAF mutation by the real-time PCR, all marginal cases resulted wild-type, whereas the mutation was detected in 15 cases (75%) of the HCL patients. The negative case by the IgH resulted mutated by the droplet PCR. Sensitivity tests have been performed for both techniques, diluting a mutated DNA with a pool of wild-type DNAs, from 1x10 to $5x10^{-4}$. The sensitivity of the real-time PCR was $1x10^{-4}$, whereas that of the droplet PCR was $5x10^{-5}$, thus proving that the droplet PCR has got a sensitivity higher than half log. Moreover, the real advantage of the droplet PCR was the possibility of the absolute quantitation of the mutated alleles without necessity of a reference curve, that, at the contrary, is fundamental in the real-time PCR. Twelve HCL cases have been then monitored during the follow-up. At the end of therapy, the droplet PCR showed that the molecular tumor burden reduced of about 2 logs in the whole series; in all cas-

es achieving the CR, the mutation burden reduced, both by real-time and by droplet PCR; in 2 relapsed patients, the RAF mutated allele burden increased; nevertheless, we were not able to predict the relapses, because samples were harvested in concomitance of relapse.
Summary and Conclusions: In conclusion, we demonstrated that the droplet PCR could represent a valid tool either for differential diagnosis of HCL, or for monitoring HCL patients after treatment, including vemurafenib. It is also to consider that costs of this technique are comparable to those of the real-time PCR and that both methods are rapid and exportable in the routinary diagnostic activity.

P447

MINIMAL RESIDUAL DISEASE ANALYSIS BY NEXT-GENERATION SEQUENCING IN BLOOD SERUM FOLLOWING IDIOTYPIC VACCINATION IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA

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Background: A clinical trial was conducted to assess the safety of a plant-based idiotype vaccine in patients with relapsed follicular lymphoma (FL).
Aims: In a correlative analysis, serum samples from patients vaccinated in this study were subjected to minimal residual disease (MRD) assessment using a next-generation sequencing method, termed the LymphoSIGHT™ platform, to evaluate correlations with disease progression.
Methods: The 11 patient cohort was comprised of pretreated, relapsed patients who had not received rituximab in the 4 months prior to enrollment. Enrolled patients underwent lymph node biopsy to manufacture vaccine and received 4-6 months bendamustine-based salvage therapy without antiCD20 antibody followed by 4 months of observation off all therapy to allow for immune recovery. In patients remaining in CR, vaccinations were given monthlyx8 then bimonthlyx4. Serum samples were obtained at the end of observation prior to start of vaccine and collected prior to each vaccination through vaccine 6. MRD assessment was performed using Sequenta's LymphoSIGHT™ platform. Briefly, using universal primer sets, we amplified immunoglobulin heavy chain (IGH) and light chain (IGK) variable, diversity, and joining gene segments from genomic DNA. Amplified products were sequenced and analyzed using standardized algorithms for clonotype determination. Tumor-specific clonotypes were identified for each patient based on their high-frequency within the B-cell repertoire in the lymph node biopsy sample. The presence of the tumor-specific clonotype was then quantitated in serum samples obtained at specified post-vaccination time points. A quantitative and standardized measure of clone level per million leukocytes in each follow-up sample was determined using internal reference DNA.

Table 1. Summary of MRD assessment.

Key: +=positive response; -=negative response; ND=not determined.

Results: Of 11 lymph node biopsy samples screened, we detected a high-frequency clonal rearrangement in 10/11 (91%). Of these patients, the clonotype was also detected in the serum compartment in 4 of 10 (40%) patients at any post-vaccination time point (Table 1). Three patients progressed (T008, T010, T021) prior to completion of all 12 vaccinations; two of whom had positive MRD in the serum at 7 (T008) and 8 (T021) months prior to progression. Patients U001, T003, T006, U011, U016, U017 and T022 completed all 12 vaccinations and were clinically in remission for the 16 month duration of vaccination, suggesting that MRD should have remained negative for all of these patients. However MRD was detected in patients U001 and T006. Further follow-up will determine whether MRD positivity in these patients is indicative of early detection of relapse.
Summary and Conclusions: These proof of concept results demonstrate the application of sequence-based MRD analysis using cell-free samples in FL patients. MRD detection by sequencing is a promising method for assessment of disease status in FL patients, and ultimately, this method may be used as a substitute for repetitive imaging modalities. Future studies will define the optimal use of MRD analysis in prognosis and therapy of FL, as well as the potential role of vaccination strategies in disease control.

P448

CLINICAL COMPARABILITY OF BCD-020 TO INNOVATOR RITUXIMAB IN PATIENTS WITH INDOLENT NON-HODGKIN'S LYMPHOMA

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Background: Approval of the first monoclonal antibody against CD20-antigen, rituximab, heralded the new era of targeted therapy in hematology. Today there are many companies all over the world which have rituximab biosimilar at different stages of development. BIORIX is among the first clinical studies to demonstrate clinical comparability of rituximab biosimilar to innovator rituximab in patients with indolent non-Hodgkin's lymphoma.
Aims: Evaluation of pharmacokinetics, pharmacodynamics, safety and efficacy of BCD-020 (rituximab biosimilar, BIOCAD, Russia) used as monotherapy in patients with indolent B-cell non-Hodgkin's lymphoma in comparison with the parameters of innovator rituximab (RTX).
Methods: 92 patients (aged 18 years and older with diagnosed CD20-positive follicular non-Hodgkin's lymphoma, stage II-IV by Ann Arbor, 1-2 histologic grade, or marginal zone lymphoma) were enrolled into the study. Patients were randomised in 1:1 ratio to receive 375 mg/sq.m of BCD-020 or RTX on days 1, 8, 15 and 22. The primary efficacy endpoint was overall response rate (ORR) at day 50±5. The secondary endpoints included proportion of patients with CR, PR, SD or PD, serum concentration of rituximab, absolute counts of CD20 and CD19-positive cells, as well as safety and immunogenicity.
Results: 72 patients with fNHL were included into the study (35 patients in BCD-020 arm, 37 – in RTX arm) as well as 20 patients with SMZL (11 patients and 9 patients, respectively). ORR on day 50±5 in both arms was equivalent: 39.52% in BCD-020 arm and 36.57% of patients in RTX arm (p=0.8250). The lower limit of 95% CI for difference in proportion of ORR between arms was -17.81% which exceeded predefined non-inferiority margin (-20%). CR was registered in 11.62% of the patients treated with BCD-020 and in none of patients from the reference arm (p=0.0555), CRu – in 2.32% and 2.43% (p=1.00), PR – 25.58% and 34.14% (p=0.4763), SD – in 51.16% and 48.78% (p=1.00), PD – 9.30% and 14.60% of patients respectively (p=0.5151). Within the first week after a single infusion of BCD-020 or RTX, the level of CD19 and CD20-positive cells rapidly decreased to almost undetectable values without any obvious recovery by day 50±5 (upon intergroup comparison p>0.05 at all specified time points). 90% CI for the geometric mean of a BCD-020/RTX AUC_{0-t} ratio fell within standard bioequivalence range 80-125% (80.1-118.2% for the ratio of AUC_{0-168} after a single dose and 81.2-124.8% for the ratio of AUC_{0-1176} after 4 doses). There were equal number of AEs in two arms; 78 AEs in BCD-020 arm and 73 AEs in RTX arm. Adverse events of any grade (by CTCAE v.4.03), which were associated with use of BCD-020 or RTX, were reported in 11 (23.91%) patients in BCD-020 arm and in 8 (17.39%) patients in RTX arm (p=0.6073). There were 4 cases of grade 3-4 AEs in each arm. Throughout the clinical study there were 2 SAEs in total, both in RTX arm (4.35%). In BCD-020 arm, there was one case of premature withdrawal due to AE (grade 4 neutropenia); in RTX arm there were two such cases, one of which was due to a SAE (death). Immunogenicity assessment did not detect the appearance of neutralizing antibodies to rituximab in BCD-020 arm as well as in RTX arm.

Summary and Conclusions: BCD-020 is non-inferior to RTX in terms of efficacy on day 50±5, with equivalent PK and PD profile and immunogenicity. BCD-020 was well tolerated, with the safety profile comparable with RTX parameters. CLINICALTRIALS.GOV IDENTIFIER: NCT01701232.

P449

OCCURRENCE OF CHRONIC THERAPY-RELATED HYPOGAMMAGLOBULINAEMIA IN INDOLENT NON HODGKIN LYMPHOMA: SINGLE CENTRE EXPERIENCE.

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Background: The occurrence of Secondary Hypogammaglobulinaemia (SH) after immune and chemotherapic treatments represents a risk factor for infections in patients affected by indolent Non Hodgkin Lymphomas (NHLs). Although concerns have been expressed about the role of Rituximab, few data are available in literature about the insurgence of SH in patients who receive polichemiotherapy and immunotherapic regimens.
Aims: to investigate risk factors for SH development in patients with NHLs and normal Serum Immunoglobulin (SIg) level at baseline treated with chemotherapies with or without the addition of Rituximab.
Methods: We analyzed 266 patients (126 males, 140 female, median age 66.5 years) with diagnosis of indolent Non Hodgkin lymphomas (138 Follicular lymphoma, 36 Marginal Zone Lymphoma, 56 Small Lymphocytes lymphoma, 4 Mucosa Associated Marginal Lymphoma, 7 Mantle Cell Lymphoma with indolent course, 25 Non Hodgkin Lymphoma non other specified) at first diagnosis or with refractory-relapsed disease, treated with different chemotherapic schedules in our institute from 1993 to 2011. Stage of disease at diagnosis was III-IV in 243 (91.3%) and I-II in 23 (8.7%). One hundred and ten patients received CHOP (Cyclophosphamide, Doxorubicin, Vincristine, Prednisone) +\-Rituximab (R), 34 with CVP (Cyclophosphamide, Vincristine, Prednisone) +\- R, 105 with Fludarabine Based Schedules (Fbs) (Fludarabine-Cyclophosphamide, Fludarabine-Mitoxantrone, Fludarabine-Idarubicine-Cyclophosphamide)+\- R; 16 patients were treated with single agent therapies (Chlorambucil, oral Cyclophosphamide). R was added to CHOP or CVP in 75/144 cases and to Fbs in 64/105 cases. Seventy nine patients received both Fbs+/-R and CHOP/CVP +/-R during their clinical history for relapsed or refractory disease; Sixteen patients with early relapsed or refractory disease underwent to high dose chemotherapy followed by Autologous stem cells transplantation (ASCT).
Results: During a median follow up of 84 months (6-219), 45/266 (16.9%) patients developed a SH after a median time of 12 months (1-86) from treatment administration. Exposure to Fbs+\-R is associated with an Odds Ratio (OR) for SH development of 7.55 (CI:3.39-17-95,p <0.001); exposure to CHOP+\-R is not associated with SSH development, with OR of 0.83 (CI 0.40-1.69,p 0.59,). Similar value has been found for CVP+\-R, with a OR of 0.27 (CI0.3-1.15,p 0.066,). We found a significant higher risk of SH insurgence for ASCT procedure, with a OR of 4.33 (CI 1.28-13.9,p:0.09). Rituximab effect was evaluated comparing patients treated with CHOP-CVP without Rituximab *versus* R-CHOP/CVP and with Fbs alone *versus* R-Fbs, respectively: the addition of Rituximab was not associated with an increased risk of SH development both in CHOP/CVP *versus* R-CHOP/R-CVP (OR 0.43 [CI:0.12-1.43], p:0.12,) and Fbs *versus* R-Fbs group (OR 1.86 [CI 0.41- 11.54], p: 0.37). Median reduction of SIg concentration was of 52% (range 11-83); in 33/45 (73%) sIg dosage shows a mild impairment (SIg between 600 mg/dL and 400 mg/dL), while in 12/45 cases (27%) sIg value was inferior to 400 mg/dL. Recurrent non neutropenic infections were observed in 21/45 cases (46%).
Summary and Conclusions: Our experience underlines that therapy-related SH should be a potential side effect not yet well described; although risk-benefit ratio for Fbs is favourable in indolent NHL, we recommend an intensive SIg monitoring in patients exposed in order to early identify SH and reduce the incidence of infection episodes.

P450

PERIPHERAL BLOOD LYMPHOCYTE/MONOCYTE RATIO (LMR) >2 IS ASSOCIATED WITH BETTER OUTCOME IN FOLLICULAR LYMPHOMA (FL): A SINGLE CENTRE EXPERIENCE

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Background: Whatch & wait (W&W) strategy is still an option even in the rituximab era for follicular lymphoma (FL). FLIPI2 risk criteria and GELF criteria are useful tools to identify candidate patients with low tumor burden for this approach. From a pathogenetic perspective the number and type of lymphocytes and monocytes/macrophages detectable in the peripheral blood and in the lymph nodes of patients with Hodgkin's and non-Hodgkin's lymphomas have been extensively studied and interesting results indicate they may possibly affect the pathogenesis and prognosis of these diseases. Moreover, peripheral blood lymphocyte to monocyte ratio (LMR) have been recently investigated as a new prognostic parameter in diffuse large B cell Lymphoma (DLBCL); the role of this ratio in FL in the rituximab era is unknown.
Aims: We wanted to investigate the prognostic impact of LMR in FL patients at diagnosis.
Methods: We retrospectively evaluated 132 FL patients (70 females, 62 males, median age 63 years) referred to our division from 2001 to February 2014. Ann-Arbor stages I/II and III/IV were, respectively, 31 patients and 101 patients. FLIPI2 risk classes were: low 50 patients (37%), intermediate 28 patients (6.8%), high 54 patients (41%). At diagnosis, W&W approach was performed on 42 patients (17 patients treated with radiotherapy to symptomatic nodes were included in this group, according to previous studies). Patients requiring chemotherapy were treated with rituximab-containing therapy (RCHOP, RCVP, RBM). Wilcoxon test applied to Kaplan-Meyer method was employed to estimate time to treatment start (TTTS), progression free survival (PFS) and overall survival (OS) of patients with different LMR at diagnosis.
Results: we analyzed different LMR cut-off values and we found that the most discriminative LMR was 2. LMR>2 was found in 97 patients, LMR<2 in 35 patients. Patients with LMR>2 had a longer TTTS, respect to patients with LMR<2 (medians 32 months *vs* 2 months, p=0.0096; see Figure 1). 92 patients were treated with Rituximab-chemotherapy (4 pts still on treatment), with an ORR of 97% (CR 75%, PR 22%). Outcome was superior in the LMR>2 group, compared to the LMR<2 group (2 years PFS 85% and 63%, respectively). No difference in OS were found between the two LMR groups.

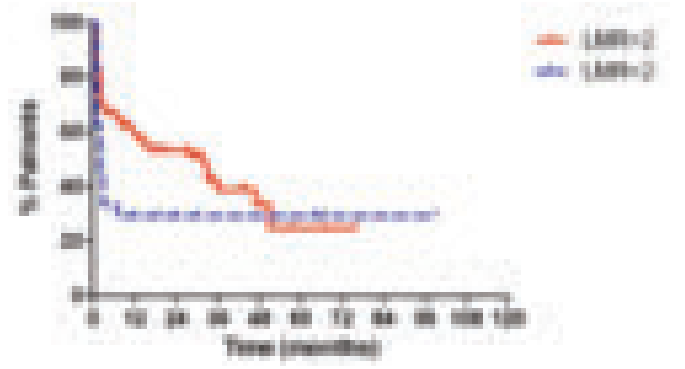

Figure 1. TTTS: time to treatment start.

Summary and Conclusions: LMR at diagnosis can be clinically relevant in patients with follicular lymphoma. In our experience, the majority of patients with LMR<2 required systemic Rituximab-chemotherapy in the first year from diagnosis, with a lower duration of response respect to patients presented with LMR>2 at diagnosis. As recently described in DLBC, this kind of surrogate biomarker of the immune microenvironment may have a prognostic role also in FL and data from perspective studies may help to better explore the exact clinical relevance of LMR. This ratio, easily derivable from WBC count may contribute at diagnosis in the definition of an appropriate follow-up timing for asymptomatic FL patients with low tumor burden. Considering the longer TTTS for patients with LMR>2, the use of this tool may better define selection of ideal candidates for W&W strategy, helping the clinician in transmitting notions to patients about W&W approach.

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THERAPEUTIC CONCEPTS AND OUTCOME OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA OF THE OCULAR ADNEXA: A SINGLE-CENTRE EXPERIENCE

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Background: Although several histological subtypes may occur, orbital marginal zone B-cell lymphoma (OAML) constitutes for the most frequent diagnosis in orbital lymphoma. Relatively little data, however, have been reported in larger cohorts of patients staged in a uniform way, and no randomised trials exist to put the currently data on local and systemic therapy into perspective.
Aims: In view of this, we have retrospectively analysed patients with OAML diagnosed and treated at our tertiary referral centre to assess clinical characteristics and treatment outcome of this relatively large cohort of Austrian patients.

Methods: We report our experience of 60 patients (33 female, 27 male) with OAML managed 1999-2012. Median age at diagnosis was 64 years (IQR 51–75) and follow-up time 43 months (IQR 16–92). All patients had undergone uniform extensive staging and histological diagnosis was made by a reference pathologist according to the WHO classification. This analysis had been approved by the Ethical Board of the Medical University Vienna.
Results: The majority presented with local disease *i.e.* stage IE according to Ann Arbor (n=40/60, 67%), one patient had stage IIE (2%), two patients stage IIIE (3%) and the remaining 17 (28%) stage IVE disease. Seven patients with stage IVE presented with bilateral orbital disease whereas the others showed involvement of further organs. Only one patient had bone marrow involvement. Treatment data were available in 58 patients. Local treatment with either radiotherapy (24%, 14/58) or surgical resection (5%, 3/58) resulted in complete or partial response (CR or PR) in 82% of patients (CR n=13, PR n=1). One patient had stable disease (SD), one progressive disease (PD) and one was lost to follow-up. However, a total of 8 patients needed consecutive further therapy. Median time to progression (mTTP) in this group was 38 months (IQR 24-53). The majority of patients (45%, 26/58) received first line systemic treatment with immuno-/chemotherapy regimens or therapy with CD20+ antibody rituximab and response rate was identical at 85% (CR n=16, PR n=6, SD n=2, PD n=1, no data n=1). Ten patients warranted further therapy after a mTTP of 16 months (IQR 9-31). Nine patients received antibiotics (doxycycline or clarithromycin) as initial therapy. Two patients achieved CR now ongoing for 6 and 83 months; response rate was 38% (PR n=1, SD n=3, PD n=2, no data n=1). Five patients needed further therapy after 1, 2, 4, 15 and 18 months respectively. Watchful waiting was the initial approach in 6/58 patients (10%), and 4 received systemic therapy after 17, 31, 22 and 86 months. The remaining two were lost to follow up. In total 28/58 patients (48%) progressed and were given at least one further line of therapy. Median TTP in this cohort was 20 months (IQR 9-39). No patient died during first line therapy. Kaplan Meier curve for progression free survival according to first line therapy was estimated but no significant difference in TTP after first line therapy was found (p=0.14, see Figure 1). Elevated β-2-microglobulin, plasmacytic differentiation, presence of autoimmune disorder and site of lymphoma were not associated with a higher risk for progress.

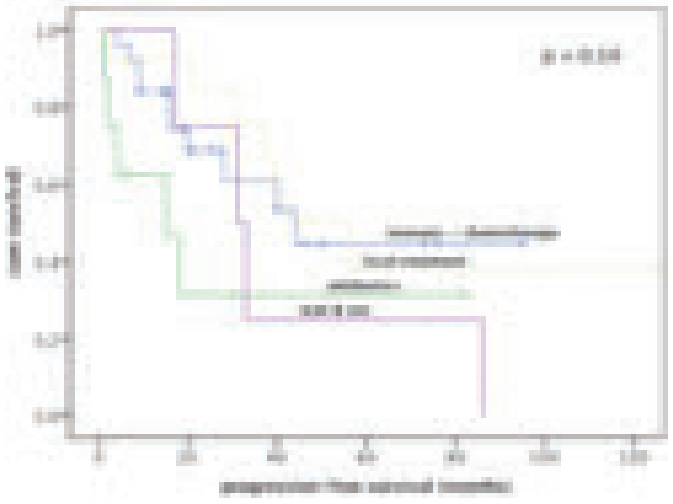

Figure 1.

Summary and Conclusions: Our data underscore the excellent prognosis of OAML irrespective of initial therapy, as there was no significant difference in time to progression and response between local or systemic therapy. In the absence of randomised trials, the least toxic individual approach should be chosen in patients with OAML.

P452

MANAGEMENT OF RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA PATIENTS IN DAILY PRACTICE: FINAL RESULTS OF THE OLYMPE FRENCH NON INTERVENTIONAL STUDY (ML20248)

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Background: There are several unanswered questions on the management of relapsed or refractory (R/R) follicular lymphoma (FL) in the rituximab era. Optimal use of treatments (ttt) such as immunochemotherapy, stem cell transplantation, radioimmunotherapy or rituximab (R) maintenance remains to be clarified.
Aims: The Olympe study was set up to describe, in routine clinical practice, treatment modalities and response rates of R/R FL pt in a French cohort.
Methods: This prospective, multicenter, longitudal, non-interventional study was conducted in patients (pt) with histologically confirmed CD20+ relapsed FL (Grade 1 to 3a) eligible for ttt, regardless of what they had previously received. The primary endpoint was to describe ttt modalities in real life setting in pt treated for R/R FL. Main secondary endpoints included efficacy and safety. Analysis was performed on the total population and on subgroups according to the initiation or not of R maintenance therapy. Data on demographics were collected at inclusion visit while new ttt, efficacy and side effects were prospectively collected.
Results: From January 2007 to April 2013, 260 relapsed FL pt were registered and 241 were analyzed. Nineteen pt were excluded due to either inclusion criteria violation or missing data. At study entry, the median age was 61 years [30-87] and 54% of the pt were men, with Ann Arbor stage I/II (16%) or stage III/IV (84%). Most pt were in 1st relapse (n=143, 59%), and 24.5% of pt were R naive (33.6% in first relapse and 12.2% in 2nd and further relapse). Ttt at time of relapse consisted mainly in R with or without chemotherapy (respectively 74% and 21%), 5% of pt received a ttt without R (chemotherapy or radiotherapy). The median duration of this line of therapy was 3.4 months (mo) [0 – 19] and overall response rate was 52.4% for the whole cohort *(EHA 2010 – Feugier et al.).* Pt were followed-up for a median time of 4.1 years [0.2 – 6.0].
Forty pt did not enter the follow-up phase (31 for disease progression and 9 for premature withdrawal during induction period). Among the remaining 201 pt, 63% (127) received a maintenance ttt with R monotherapy for a median duration of 20 mo [0 – 29]. Disease progression occurred in 106 (44%) pt. Median PFS was 44 mo; it was 64 mo in pt in 1st relapse and 30 mo in pt in ≥2nd relapse (p<0.0001), it was 38 mo in pt without R maintenance and 63 mo in pt with R maintenance. Median OS was not reached and survival estimate at 6 years was 75% (80% in pt in 1st relapse and 67% in pt in ≥2nd relapse). Histologic transformation of FL was reported in only 5% (n=13) of the pt and 2% (n=4) died due to this transformation. A total of 58 (23%) pt died including 30 after the 1st progression: 38 deaths were not related to ttt (disease progression (26 pt), another neoplasy (11 pt), both disease progression and another neoplasy (1 pt), 6 were related to the ttt (all were infections: 2 septic shocks, 1 progressive multifocal leukoencephalopathy, 2 aspergillosis and 1 lung infection), and 14 pt died of causes that did not appear related to ttt or disease. Safety data showed that 166 (68%) pt had an adverse event, the most frequent were infections 47% (n=115) mainly: bronchitis (19%), lung infection (10%), herpes zoster (7%) and pyrexia (22%).
Summary and Conclusions: In France, R/R FL pt receive mainly induction ttt with immunochemotherapy. In the study population, 63% of responder pt received maintenance ttt with R monotherapy. In this difficult-to-treat population, median PFS was 44 mo. These results confirm EORTC 20981 study data in terms of PFS benefit. Safety data are consistent with the known safety profile of immunochemotherapy and R monotherapy, no new safety signal was discovered in this non interventional study.

P453

THE SECOND PRIMARY CANCER RISK AFTER NON HODGKIN LYMPHOMA IS NOT INFLUENCED BY THE SUBTYPE: DATA FROM THE FRENCH CANCER REGISTRIES NETWORK (FRANCIM)

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Background: Non Hodgkin lymphoma (NHL) are known to be a greater risk of second primary cancer (SPC). However, little attention has been focused on SPC risk assessment according to NHL subtypes.
Aims: To determine the risk of second cancer in different NHL subtypes.
Methods: Data from 10 french population-based cancer registries were used to establish a cohort of patients with a first diagnosis of NHL between 1989 and 2004. Seven subtypes of NHL were analysed of NHL were analysed: diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), lymphomaplasmocytic lymphoma (LPL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), hairy cell leukemia (HCL), T-cell lymphoma. Standardized incidences ratios (SIRs) of metachronous SPC were estimated.
Results: Among the 7,546 patients diagnosed with a NHL, the overall SPC risk was 25% higher compared to the reference population (SIR=1.25; 95% CI 1.15-1.36). The SPC risk differed substantially by lymphoma subtype and was spread from SIR at 1.60 (95%CI 1.20-2.10) for MZL to a non-increased risk for HCL (SIR=0.92; 95% CI 0.61-1.34). Among 580 reported cases of SPC, 89%

were solid tumors and 11% were hematological malignancies. Interestingly, multivariate analysis showed that SPC risk did not differ significantly across NHL subtypes after adjustement on the other covariates (p=0.786).
Summary and Conclusions: NHL patients have an increased risk of SPC that is not influenced by the histological NHL subtype. The differences observed in univariate analysis across NHL subtypes may be due to differences in patients'characteristics.

P454

HEAVY/LIGHT CHAIN CHARACTERISTICS OF PATIENTS WITH INDOLENT AND AGGRESSIVE LYMPHOMAS

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Background: Novel nephelometric immunoassays specific for heavy/light chain (HLC) have become available which allow accurate measurement of HLC subsets (IgGk, IgGl, IgAk, IgAl, IgMk and IgMl). The assays improve detection of serum monoclonal immunoglobulins (M-Ig) in plasma cell proliferative disorders. There are limited data on serum HLC in lymphoma patients.
Aims: To assess the use of HLC immunoassays to detect serum monoclonal component in patients with indolent and aggressive lymphomas.
Methods: The study included 145 patients assessed at Heartlands Hospital, Birmingham, UK. 91 patients had indolent lymphoma (41 follicular, 36 marginal, 10 Waldenstrom's macroglobulinemia (WM), 4 hairy) and 54 had aggressive lymphoma (45 diffuse large B-cell (DLBC), 9 mantle). HLC measurements using polyclonal antibody based immunoassays (The Binding Site, Birmingham, UK) were compared to published normal ranges (IgGk:4.03-9.78g/L; IgGl:1.97-5.71g/L; IgGk/IgGl:0.98-2.75; IgAk:0.48-2.82g/L; IgAl:0.36-1.98g/L; IgAk/IgAl:0.80-2.04; IgMk:0.29-1.82g/L; IgMl:0.17-0.94g/L; IgMk/IgMl:0.96-2.30) and to historic serum immunofixation (IFE), protein electrophoresis (SPEP) and free light chain (FLC) results.
Results: Overall 64/145(44%) patients had an abnormal HLC ratio (24 IgGk, 1 IgGl, 3 IgAk, 11 IgAl, 25 IgMk, 16 IgMl; 16 patients had more than one Ig class abnormal and in 2 patients all three Ig classes had an abnormal HLC ratio). By contrast, 45/145 (31%) patients were IFE positive and 38/145 (26%) were SPEP positive. 29/145 (20%) patients had an abnormal FLC ratio (28 FLCk, 1FLCl). Among the indolent lymphoma types, abnormal HLC ratios were observed in 17/41 follicular lymphoma patients (42%: 12 IgGk, 1 IgGl, 1 IgAl, 6 IgMk and 2 IgMl; 4(10%) patients had more than 1 Ig class abnormal); 16/36 marginal (44%: 3 IgGk, 4 IgAl, 4 IgMk, 9 IgMl; 5(14%) patients had more than 1 Ig class abnormal); 10/10 WM (100%: 4IgGk, 1 IgAk, 1IgAl, 6 IgMk, 4 IgMl; 5(50%) patients had more than 1 Ig class abnormal); and 0/4 patients with hairy lymphoma. By contrast IFE identified a M-Ig in 10/41 (24%) follicular, 12/36 (36%) marginal, 10/10 (100%) WM and 0/4 hairy lymphoma patients. Among the aggressive lymphoma types, abnormal HLC ratios were observed in 20/45 DLBC lymphoma patients (44%: 6 IgGk, 2 IgAk, 5 IgAl, 9 IgMk, 3 IgMl; 4(9%) patients has more than 1 Ig class abnormal) and in 1/9 patients with mantle cell lymphoma (11%: IgMl). IFE was positive in 11/45 (24%) DLBC and 2/9 (22%) mantle cell lymphoma patients. Indolent lymphoma patients with abnormal IgMk/l ratios displayed more frequently adverse disease characteristics including low haemoglobin (p=0.009), elevated B2-microglobulin (p=0.08) and bone marrow involvement (p=0.05). No correlation was found between age, LDH levels, stage of disease and HLC ratios.
Summary and Conclusions: HLC ratios are abnormal in a significant percentage of lymphoma patients, particularly those with indolent types; the M-Ig by HLC is predominantly IgM kappa. HLC may provide a useful means of detecting and monitoring M-Ig in lymphoma patients.

P455

R-CHOP THERAPY ALONE FOR LIMITED-STAGE FOLLICULAR LYMPHOMA: A RETROSPECTIVE STUDY

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Background: Limited-stage follicular lymphoma (FL) accounts for 10-30% of all FL cases. Irradiation therapy alone is a standard strategy for limited-stage FL, leading to a 10-year progression-free survival (PFS) rate of 30-50%. However, since 2001, as a primary policy, we have been administering irradiation therapy alone to patients with clinical stage (CS) 1 FL and R-CHOP therapy alone to patients with CS 2 FL.
Aims: To evaluate the treatment efficacy of R-CHOP therapy alone in limited-stage FL.
Methods: A total of 35 patients with newly diagnosed FL received R-CHOP therapy with curative intent between 2002 and 2009 in Yokohama City University Hematology Group. Where possible, patients who achieved partial response (PR) after the completion of the R-CHOP therapy received additional irradiation for any residual lesions. Maintenance therapy was not administered.
Results: The median age of the 35 patients (male, 14: female, 21) was 61 years (range, 25-89 years); 7 patients had in CS 1 FL, and 28 patients, CS 2 FL. The pathological grading of FL among the patients was as follows: FL grade 1 in 11 patients, grade 2 in 12, and grade 3 in 12. FL international prognostic indices were low in 30 patients, intermediate in 4, and high in 1. The median number of R-CHOP cycles was 6. On completion of the R-CHOP therapy, 33 patients achieved complete response (CR) and 1 showed PR. The patient showing PR after the completion of R-CHOP was administered additional irradiation. The remaining 1 patient was not evaluated because of discontinuation of hospital visit. The median follow-up period in the survival cases was 61 months. Four patients died: 2 died from lymphoma and the other 2 from pneumonia in the status of CR of lymphoma. In all the 35 patients, the 5-year PFS rate was 70%, and the 5-year overall survival rate was 92%. In the 15 patients with a PFS >5 years, only 1 patient showed disease progression. The 5-year PFS rates in patients with grade 1 or grade 2 FL and grade 3 FL were 84% and 47%, respectively (*P*=0.03). The 5-year PFS rate in patients with CS 1 and CS 2 FL were 80% and 67%, respectively (*P*=0.32) (Figure 1).

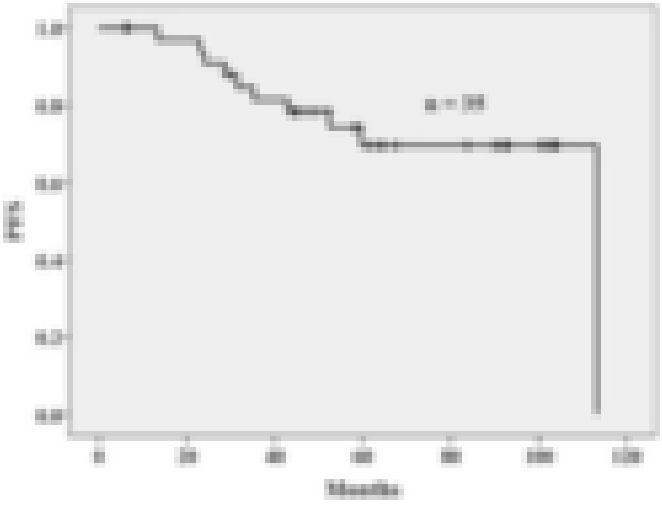

Figure 1.

Summary and Conclusions: The outcome of R-CHOP therapy alone in patients with limited-stage FL was at least equivalent to the reported outcome of irradiation therapy alone. The PFS curve almost plateaued after 5 years. Therefore, R-CHOP therapy could be an alternative to irradiation therapy in patients with limited-stage FL.

P456

PROGRESSION-FREE SURVIVAL AS A SURROGATE ENDPOINT FOR OVERALL SURVIVAL IN RELAPSED-REFRACTORY MANTLE CELL LYMPHOMA

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Background: Overall survival (OS) is becoming increasingly challenging to establish in clinical trials due to advancements in treatment regimens. As a result the use of other endpoints is becoming more important in making regulatory and funding decisions, specifically for oncology products.
Aims: The purpose of this research is to evaluate the potential for median progression-free survival (PFS) as a surrogate endpoint predicting median OS in relapsed and/or refractory (R/R) mantle cell lymphoma (MCL).
Methods: A systematic literature review was undertaken using MEDLINE and EMBASE, evaluating all clinical trials investigating treatments in adult R/R MCL.

Trials included in the analysis had to report median OS and median PFS. To minimise the risk of attrition bias, studies with substantial loss to follow up (*i.e.* >30%) were excluded. The level of surrogacy was quantified using Pearson and Spearman rank correlation statistics, and weighted least squares (WLS) regression using sample size weights. Correlation statistics were also evaluated within different patient subgroups.
Results: A total of 9 studies were identified in R/R MCL corresponding to 12 independent trial arms. Correlation statistics found a strong association between median PFS and median OS (Spearman=0.873; Pearson=0.660; 95% CI 0.100 to 0.903). WLS regression found a statistically significant relationship between median OS and median PFS (coef.=1.413; 95% CI: 0.271 to 2.555), however the low R^2, value (R^2=0.466) is likely due to a small sample size. Correlations within patient subgroups were tested, however, given the sample size no discernable conclusion can be drawn from statistics.
Summary and Conclusions: Median PFS seems to be an acceptable surrogate endpoint at the individual level, exhibiting a strong correlation with OS in this population of patients. Additional data may help to establish the statistical significance of these findings and further research is needed to assess the validity of PFS at the trial level as well, *i.e.*, to evaluate if the treatment effect on PFS can predict the effect of the treatment on OS.

Aggressive Non-Hodgkin lymphoma - Clinical 1

P457

FREQUENCY OF SURVEILLANCE COMPUTED TOMOGRAPHY IN NON-HODGKIN LYMPHOMA AND THE RISK OF SECONDARY PRIMARY MALIGNANCIES

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Background: There is a worldwide growing concern about cancer risk of radiation exposure in medical imaging. With increasing usage of computed tomography (CT) for lymphoma patients with curative-intent treatment, development of secondary primary malignancy related to radiation from CT scans becomes an emerging issue in these long-term survivors.
Aims: To explore the risk of secondary primary malignancy (SPM) related to radiation exposure from CT scans in non-Hodgkin's lymphoma (NHL) patients under curative therapy.
Methods: We conducted a nationwide population-based study analyzing NHL receiving curative-intent treatment between January, 1997 and December, 2010. Dividing patient population into receiving more and fewer CT scans surveillance group according to the medium number of CT scans performed within one year after lymphoma diagnosis, we used the Kaplan-Meier method to compare the cumulative incidence of SPM in these two groups. Propensity score matching was applied to eliminate potential confounders. Group stratification and multivariate analysis under competing risk event was performed to identify independent predictors of SPM.
Results: A total of 4874 patients were enrolled and the medium number of CT scans is eight. Patients receiving **>**8 CT scans had a significantly 2-folds risk of developing SPM (hazard ratio [HR] 2.23, 95% confidence interval [CI] 1.60–3.11; p<0.001) than those with ≤8 and this significant difference was remained even after corrected by propensity scores. Figure 1 revealed the cumulative incidence of patients with more CT scans was higher than those with few significantly (P<0.001). Among 180 SPM identified, those receiving more CT scans had significantly higher SPM incidence in cancers of breast (HR 11.22, 95% CI 1.47–85.64; p=0.02), stomach (HR 5.22, 95% CI 1.17–23.23; p <0.03), and liver and biliary tract (HR 2.18, 95% CI 1.00–4.73; p=0.049) in comparison of those with fewer scans. The risk of SPM would increases 3% per one more CT scans performed.

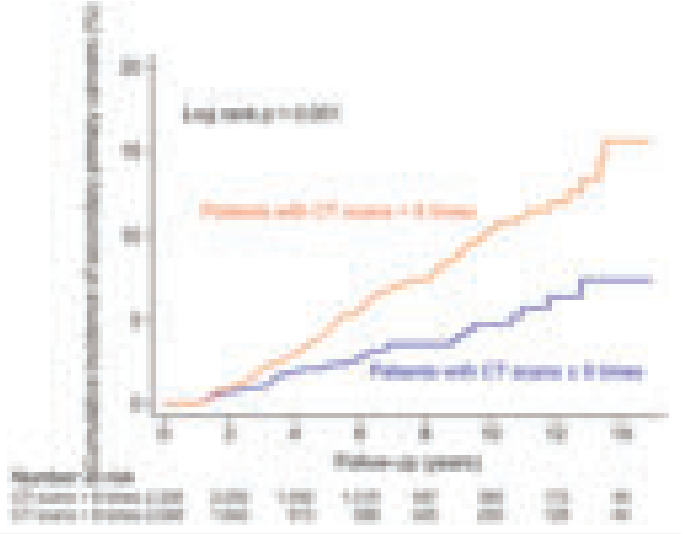

Figure 1.

Summary and Conclusions: Our study demonstrates NHL patients with curative-intent treatment receiving more frequently CT scans surveillance would increase risk of SPM compared to those with few. The incidence of secondary cancer origin from breast, stomach and liver is higher in patients with more CT scans. These sites are usually located at interface or overlapping area and receive double dose of radiation from two CT scans procedures. Physicians should assess the timing of CT scan more carefully and avoid over CT scans, especially in those with complete remission and high curable population.

P458

Abstract withdrawn

P459

MYD88 (L265P) MUTATION IS AN INDEPENDENT PROGNOSTIC FACTOR IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Somatic mutations in the myeloid differentiation primary response gene 88 (*MYD88)* have been described in B cell lymphomas. The leucine to proline exchange at position 265 (L265P) is the most recurrent and biologically potent *MYD88* variation, being found in about 30% of ABC-DLBCL, whereas is uncommon in GCB-DLBCL.
Aims: To study the occurrence of *MYD88* L265P mutation in adult patients with DLBCL and its relation to clinical and biological characteristics and patients' outcome.
Methods: A series of 175 patients with DLBCL diagnosed at our institution between 2000 and 2013 where included. Inclusion criteria where: full clinical data available, treatment with remission intention, enough material for DNA extraction and absence of HIV infection. The presence of *MYD88* L265P mutation was assessed by allele-specific oligonucleotides (ASO)-PCR in DNA samples extracted from FFPE tissue. Overall survival (OS) and progression-free survival (PFS) were calculated since the time from first treatment and according to standard definitions. Multivariate Cox regression was used to determine whether mutation remained independently predictive of PFS and OS after adjusting for clinical variables.
Results: *MYD88* L265P mutation was found in 17 out of 175 cases (10%) and occurred more frequently in males (*P*=.019), cases without B symptoms (*P*=.006) and those with primary extranodal disease (*P*=.02) such as central nervous system (50%), skin (33%) and testes (78%). Univariate analysis showed that *MYD88* L265P mutation (*P*=.001) and intermediate-high or high IPI (*P*=.001) where significantly associated to poor outcome and inferior OS (Figure 1), independently on the primary extranodal origin and the ABC phenotype through Hans' algorithm. For the whole cohort, the median follow-up time was 41 months (range, 2-171 months), with OS at 4 years of 69% and PFS at 4 years of 57%. A total number of 129 patients (74%) received rituximab plus CHOP or CHOP-like schedules, while the remaining cases received other treatments depending on their clinical requirements. We further analysed PFS in those cases treated with rituximab plus CHOP or CHOP-like. The resulting factors associated with high risk of progression were age ≥60 years (*P*=.026), advanced stage (*P*=.001), ECOG 2-4 (*P*=.002), high LDH (*P*=.001), albumin <30 g/L (*P*=.030), IPI intermediate-high or high (*P*<.0001) and *MYD88* L265P mutation (*P*=.011). Only IPI stage and *MYD88* mutation remained significantly associated with a higher risk of progression in the multivariate analysis (*P*<.0001 and *P*=.023, respectively) (Figure 1).

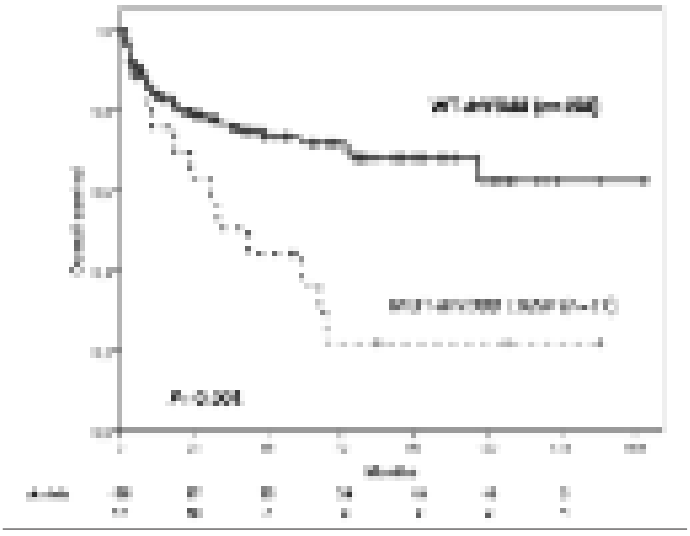

Figure 1.

Summary and Conclusions: Our study shows that *MYD88* L265P is associated with poor prognosis and, although these findings require further validation in prospective studies, suggests that detection of *MYD88* mutation might be useful to identify DLBCL patients at high risk of progression and death with independence of other well-known clinical prognostic factors.

P460

THE ADDITION OF RITUXIMAB TO CODOX-M/IVAC CHEMOTHERAPY IN THE TREATMENT OF BURKITT LYMPHOMA IS SAFE AND IS ASSOCIATED WITH INCREASED EFFICACY IN THE HIV POSITIVE POPULATION

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Background: Burkitt lymphoma (BL) is a rare, aggressive non-Hodgkin lymphoma (NHL), more prevalent in people living with HIV (PLWH) and an AIDS-defining illness. Treatment consists of intensive, short duration chemotherapy regimens such as CODOX-M/IVAC. In the HIV negative (HIV-) population the addition of rituximab improves survival. However, fears of increased toxicity have prevented its routine use in BL in PLWH despite toxicity not being seen in people who do not have HIV infection. Rituximab use in PWLH and other types of NHL results in increased overall survival (OS) but there are few well-populated studies looking specifically at HIV-related BL.
Aims: This retrospective study evaluated the effect of adding rituximab to CODOX-M/IVAC chemotherapy on treatment-related toxicity and efficacy in PLWH.
Methods: 91 patients (74 male) with HIV-related BL, treated in five London treatment centers (Chelsea & Westminster, St Bartholomew's, University College, Royal Free & Kings College Hospitals) between 2003 and 2013, were identified from local databases. Toxicity was assessed according to the EORTC Common Toxicity Criteria version 2.0.

Table 1. Frequency of toxic events during each cycle of chemotherapy.

Figure 1. Kaplan-Meier estimator for overall survival when comparing treatment.

Results: 49 patients received CODOX-M/IVAC and 42 R-CODOX-M/R-IVAC with a median of six doses of rituximab. There was no significant difference in baseline characteristics between groups. All patients received opportunistic infection prophylaxis and combination antiretroviral therapy (cART) during lymphoma treatment. The median follow up is 41 months (3-109). At BL diagnosis the median CD4 count was 244 cells/µl (0-864), 35% were established on cART and 2% had undetectable plasma HIV viral loads. Toxicity Whilst infections were common with all but three patients receiving IV antibiotics at some stage, opportunistic infections were infrequent. There were two confirmed and one suspected fungal chest infections, all in patients receiving R-CODOX-M/R-IVAC. There were five cases of CMV reactivation requiring treatment in three patients receiving R-CODOX-M/R-IVAC and 2 receiving CODOX-M/IVAC. The addition

of rituximab did not confer any significant increase in grade 3/4 toxicity such as infections, mucositis, diarrhea, renal impairment and tumor lysis syndrome. There was also no significant increase in bone marrow suppression with a similar length of neutropenia and GCSF use in both groups. Comparative measurements of HIV viral load, CD4 count, hemoglobin, platelet and white cell counts during and after treatment were not significantly different. There was no significant increase in inpatient hospital stay (94 *vs.* 103 days, p=0.58). Survival Sixty patients were alive at last follow-up. The two-year overall survival is 68%. The overall survival is greater for patients receiving rituximab (2 year OS 72% (95%CI: 0.22-0.92, hazard ratio 0.46) *vs.* 55% (95%CI: 1.1-4.5, hazard ratio 2.2) (logrank p=0.04). Of the 31 deaths, six were due to sepsis during chemotherapy (x4 CODOX-M/IVAC, x2 R-CODOX-M/R-IVAC), 15 from progressive disease, four from disease relapse and two were HIV-related. Four deaths were unrelated to treatment of which two occurred while the patients were in complete remission. There was no significant difference in toxic deaths or disease relapse between groups (p =0.137) (Table 1 and Figure 1).
Summary and Conclusions: This study suggests that HIV-related BL should be treated with the same chemotherapeutic approach as people who do not have HIV infection. The addition of Rituximab is safe, does not increase toxicity and confers a significant survival benefit when compared to CODOX-M/IVAC chemotherapy alone.

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PHASE 2 STUDY OF IBRUTINIB IN RELAPSED OR REFRACTORY MANTLE CELL LYMPHOMA: UPDATED SAFETY ANALYSIS ON PREVALENCE OF INFECTION, DIARRHEA, AND BLEEDING OVER TIME

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Background: Bruton's tyrosine kinase (BTK) is a central mediator of B-cell receptor (BCR) signaling essential for B-cell development and function. Ibrutinib, a first-in-class oral BTK inhibitor, induces apoptosis and inhibits migration and adhesion of malignant B-cells. The phase 2 PCYC-1104 trial showed that ibrutinib was active in relapsed/refractory mantle cell lymphoma (MCL) with an overall response rate of 68% (21% complete response [CR]), durable responses, and a favorable safety profile with a low (8%) rate of discontinuation due to adverse events (Wang *et al.*, *N Engl J Med.* 2013). Since responding patients may receive continuous ibrutinib treatment for prolonged periods, further safety analysis was performed to characterize the pattern of adverse events (AEs) with longer term therapy.
Aims: To evaluate changes in prevalence and severity of infection, diarrhea, and bleeding events over time in the PCYC-1104 study population.
Methods: Patients had relapsed or refractory MCL with measurable disease, and provided written informed consent. Ibrutinib 560 mg PO QD was administered continuously until disease progression or unacceptable toxicity. Safety assessments included monitoring and recording of AEs including serious AEs (SAEs), and measurements of protocol-specified laboratory parameters. AEs were characterized by preferred terms using MedDRA version 16.1, and were evaluated over 6-month time intervals (1–6, 7–12, 13–18, 19–24, >24 months). Prevalence was based on the number of patients with an AE occurring during a given interval (either a new episode or an ongoing episode from the prior 6-month period continuing into the current interval).
Results: Safety data are reported for 111 treated patients. Median time on study was 27 months and median treatment duration was 8 months; 46% of patients were treated for >12 months and 26% continue on treatment. Prevalence rates for all grade infection were 69% for the first 6 months and gradually declined thereafter. One SAE of infection occurred beyond 24 months in the current analysis. Overall, patients achieving CR had a lower incidence rate of grade ≥3 infection than non-CR cases. No substantial changes in serum immunoglobulin (Ig) levels (IgA, IgG, IgM) were observed over time. Median times to onset and resolution of diarrhea, one of the more commonly reported AEs with ibrutinib, were 8 and 5 days, respectively. Prevalence rates for diarrhea were highest in the first 6 months (44% all grade; 4.5% grade ≥3; 1% SAE) and decreased thereafter. No SAE of diarrhea occurred after 6 months of therapy. There were no reported cases of colitis. Overall, bleeding events including bruising of any grade occurred in 51% of patients. Prevalence rates for all-grade bleeding were highest (41%) during the first 6-month time interval, decreasing thereafter (14% at more than 24 months). Major bleeding (protocol specified) occurred with a prevalence of less than or equal to 5% during each interval.
Summary and Conclusions: Analysis of longer term safety data related to infection, diarrhea, and bleeding demonstrates that the prevalence rates of these AEs did not increase with continuous ibrutinib therapy. Prevalence of infection and diarrhea generally decreased over time, and prevalence of bleeding events remained stable throughout the course of the study, with the major bleeding rate ≤5%. These results further elucidate the safety profile of ibrutinib in patients with relapsed/refractory MCL.

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THE BRUTON'S TYROSINE KINASE (BTK) INHIBITOR ONO-4059: PROMISING SINGLE AGENT ACTIVITY IN PATIENTS WITH RELAPSED AND REFRACTORY NHL

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Background: Bruton's tyrosine kinase (BTK) is a critical kinase involved in B-cell receptor signal transduction. ONO-4059, a highly potent and selective oral BTK inhibitor has demonstrated anti-tumour activity in pre-clinical models (Yasuhiro *et al.*, AACR2013) and in the clinic in both NHL and CLL patients (Salles *et al.*, ASH 2013; Rule *et al.*, ASH 2013). Here, we present data from NHL patients enrolled in the ongoing Phase I study ONO-4059POE001.
Aims: Thirty three (33) patients with relapsed (n=16) or refractory (n=17) NHL were administered ONO-4059 as monotherapy, given once daily (QD) at doses ranging from 20-480mg (6 Cohorts), to determine safety, pharmacokinetics and pharmacodynamics, as well as any preliminary efficacy. Patients can receive ONO-4059 for up to a maximum of 2 years and upon completion of the first 6 months of treatment, intra-patient dose escalation is permitted. Patients had a median age of 63 yrs [range 28-88], median of 4 prior therapies [2-10], a median baseline tumour burden of 3,236mm^2 [619-19,509mm^2], with 31/33 patients (94%) having prior exposure to a rituximab-containing regimen and 11/33 patients (33%) who had prior ASCT.
Results: Thirty three (33) patients were evaluable for safety and 31 patients for efficacy. ONO-4059 was found to be well tolerated with a total of sixty eight (68) ONO-4059-related G1-2 adverse events (CTCAE-V4.0) reported in 19 patients. Eight ONO-4059-related G3 events were reported in 6 patients (thrombocytopenia and anaemia in 1 patient [20 mg], proteinuria and hematuria in 1 patient [320 mg], lymphopenia [160 mg], neutropenia and anaemia [320 mg], and adverse drug reaction (maculopapular rash etc.) [480 mg]. One ONO-4059-related G4 event was reported (allergic reaction [320 mg]). The events of allergic reaction (320mg) and maculopapular rash (480mg) were reported as SAEs. Responses occurred from doses of 40mg upwards. Thirty one (31) eligible patients show a best overall response rate of 42% (13/31) with 13 PR (median reduction of lymph nodes was 84.4% [52.4 - 96.1%]), which include 4 refractory patients (3 ABC-DLBCL and 1 MCL), 7 SD and 11 PD. Of note, in a sub-set of 12 ABC-DLBCL patients (all WT for CD79b), 7 have achieved PR (of which 3 were refractory) resulting in a best ORR of 58% (median reduction of lymph nodes was 80.2% [52.4%-96.1%]) Of the 8 evaluable MCL patients, 4 have achieved PR (of which 1 was refractory) resulting in a best ORR of 50% (median reduction of lymph nodes was 86.1% [84.4%-92.4%]). The pharmacokinetics of ONO-4059 reflects a half-life of ~5-7 hours with sustained inhibition of BTK observed in PBMCs up to 24 hours from first dose.
Summary and Conclusions: ONO-4059 showed a very favorable safety profile along with promising preliminary efficacy in this difficult-to-treat patient population, most notably encouraging responses observed in ABC-DLBCL and MCL patients. The study is ongoing with additional dose escalation cohorts underway.

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APOPTOTIC POLYMORPHISMS ARE INVOLVED IN AGGRESSIVENESS AND PROGNOSIS OF DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Apoptosis plays a key role in non-Hodgkin lymphoma (NHL)

development. BCL-2 family members are crucial regulators of apoptosis, and include the anti-apoptotic *BCL-2* gene and the pro-apoptotic *BAX* gene. The ability to induce apoptosis of damage cells is variable in humans, since several proteins enrolled in process are encode by genes with single nucleotide polymorphisms (SNPs). The variant A allele of the *BCL2* C(-717)A SNP was related to higher expression of the encoded protein compared to the C wild allele. The G wild allele of the *BAX* G(-248)A SNP was associated with higher protein level in some studies but with lower transcriptional activity in others, when compared with the variant A allele. However, their roles in diffuse large B cell lymphoma (DLBCL) are still not clear.

Aims: This study aimed to evaluate whether the SNPs *BCL2* C(-717)A and *BAX* G(-238)A influence the risk, clinical manifestation and outcome of DLBCL patients.

Methods: Our prospective analysis included 135 consecutive DLBCL patients at diagnosis seen at the Haematology and Haemotherapy Centre, from December 2007 to February 2014 and 240 healthy subjects matched to patients by age, gender and race. Median age of patients was 57 years old (range: 17-89); 95 patients (70.4%) had B symptoms, 26 patients (19.2%) had bone marrow involvement (BMI), 61 patients had higher lactate dehydrogenase (LDH) values (45.2%), 15 (11.1%) patients had high risk disease (IPI 4 and 5), and 50 (37.0%) patients had stage IV tumors. Patients received 6 cycles of R-CHOP as treatment. Genomic DNA from peripheral blood of all patients and controls was analyzed by polymerase chain reaction followed by enzymatic digestion for discrimination of distinct SNPs genotypes. Multivariate analysis using the logistic regression model served to obtain age, gender and ethnic origin adjusted odds ratios (ORs) with 95% confidence intervals (95% CIs), and to assess the associations between genotypes and DLBCL. Overall survival (OS) was calculated using the Kaplan-Meier estimate probabilities, and differences between survival curves were analysed by the log-rank test.

Results: The frequencies of *BAX* GG and *BCL-2* CA+AA genotypes were higher in patients than controls (83.7% *versus* 77.9% and 77.8% *versus* 70.0%), respectively. However, only a tendency for statistical significance of differences could be found in study (P=0.06 and P=0.05), reflecting a trend increased risk of 1.81 and 1.63 for DLBCL in individuals with the respective genotypes. When only patients were analyzed, an excess of the *BAX* GG genotype was seen in patients with stage IV tumors compared with those with tumors of I+II+III stages (94% *versus* 80%, P=0.04). The median time of observation of patients enrolled in the study was 20 months (range: 1-68). On univariate analysis, presence of B symptoms (68% *versus* 86%, P=0.04) and BMI (52% *versus* 78%, P=0.004), high levels of LDH (69% *versus* 80%, P=0.02), disease of high risk (52% *versus* 87%, P=0.003), and stage IV (64% *versus* 83% P=0.01) were predictive of worse outcome of DLBCL patients at 24 months of follow up. In addition, patients with *BCL2* -717 CA+AA genotype (68% *versus* 88%, P=0.04) had worse outcome at 24 months of follow up.

Summary and Conclusions: The data present, for the first time, preliminary evidence that inherited abnormality in intrinsic pathway of apoptosis, related to the *BCL2* C(-717)A and *BAX* G(-238)A SNPs, influence the aggressiveness and outcome of DLBCL patients treated with conventional chemotherapy regimen.

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OUTCOME OF AGGRESSIVE LYMPHOMA PATIENTS WITH A NEGATIVE PRETREATMENT POSITRON EMISSION TOMOGRAPHY SCAN

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Background: Virtually all aggressive lymphomas are 2-deoxy-2-(^{18}F)fluoro-D-glucose positron emission tomography (^{18}FDG-PET) positive. It has been firmly established that negative midtreatment and end-of-treatment ^{18}FDG-PET scans after chemotherapy with or without Rituximab confer an excellent long term prognosis. A favourable prognostic value of a negative pretreatment ^{18}FDG-PET scan is generally assumed, but not substantiated by a large background of data. The Positron Emission Tomography Guided Therapy of Aggressive Non-Hodgkin's Lymphomas (PETAL) trial was initiated to resolve the question whether a change in treatment protocol may improve the outcome of patients with a positive midtreatment ^{18}FDG-PET scan (EudraCT-Nr.: 2006-001641-33). In the PETAL trial, patients with a negative pretreatment ^{18}FDG-PET scan were registered but treated off-protocol according to the physician's discretion.

Aims: This companion study to the PETAL trial aimed at establishing the frequency of negative pretreatment ^{18}FDG-PET scans within a large aggressive lymphoma cohort, encompassing the base line patient's characteristics in pretreatment ^{18}FDG-PET negative and positive patients, recording treatment practice in pretreatment ^{18}FDG-PET negative individuals and gathering information about the long term outcome of these patients.

Methods: Patients with a negative pretreatment ^{18}FDG-PET scan were identified in the PETAL database. The protocol for this study was approved by the German Federal Institute for Drugs and Medical Devices and institutional review boards. Participants provided signed informed consent before enrollment. Information about patient's baseline characteristics, treatment provided and survival status were retrieved and analysed with the SAS software data suite. This analysis of the pretreatment ^{18}FDG-PET negative patients is an explorative additional analysis.

Results: 1075 patients were registered in the PETAL-trial, 80 of whom had a negative pretreatment ^{18}FDG-PET scan (7,4%). More males than females were found in the pretreatment ^{18}FDG-PET negative group (56 *vs.* 24, p=0.03, Fisher's exact test). 11 (13,8%) were diagnosed with aggressive T- cell lymphomas, 69 (86,2%) with diffuse large B-cell-lymphoma or follicular lymphoma grade 3 a/b (^{18}FDG-PET positive patients: 99 (9,7%) aggressive T-cell lymphomas, 928 (90,3%) diffuse large B-cell-lymphomas or follicular lymphomas grade 3 a/b). For pretreatment ^{18}FDG-PET negative and positive patients median age was 60,7 and 57,8 years, respectively (p=0,45, Wilcoxon-test). A favourable International Prognostic Index (IPI) category of 0, 1 or 2 was observed in the majority of pretreatment ^{18}FDG-PET negative patients (69), two patients had an unfavorable IPI of 3 (data pending for nine patients). Eight patients received less than six cycles of Cyclophosphamide, Doxorubicin, Vincristin, Prednisone (CHOP) or Rituximab (R)-CHOP. One Patient received three cycles of R-Bendamustine and, at relapse, six cycles of R-CHOP. At a median follow up of 530 days, overall survival was 93.8% (75/80). Three patients died from lymphoma relapse. Two patients died following a cardiovascular or cerebrovascular event. The three relapsed patients received less than six cycles of R-CHOP as first line therapy.

Summary and Conclusions: Patients with aggressive lymphomas and a negative pretreatment ^{18}FDG-PET scan appear to have an excellent prognosis and a low rate of disease recurrence. Relapse and subsequent death from lymphoma occurred only in patients treated with less than six cycles of R-CHOP. Thus, treatment de-escalation in pretreatment ^{18}FDG-PET scan negative patients is not recommended outside a clinical trial.

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BRENTUXIMAB VEDOTIN IN REFRACTORY OR RELAPSED PERIPHERAL T-CELL LYMPHOMA: THE FRENCH NAME PATIENT PROGRAM EXPERIENCE IN 65 PATIENTS

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Background: Outcome of patients with peripheral T-cell Lymphoma (PTCL) in first relapse or refractory is poor with an overall survival of 6.7 months (Mak V, JCO 2013). Brentuximab Vedotin (BV) is an antibody drug conjugate directed against CD30. Its efficacy was demonstrated in a phase 2 study in patients with relapsed or refractory systemic anaplastic large cell lymphoma (ALCL), with an

overall response rate of 86% and a complete response rate of 57% (Pro B, JCO 2012). ALCL is characterized by a strong and uniform CD30 expression, but little is known about the efficacy of BV in other T-cell lymphoma subtypes with different CD30 expression levels.

Aims: The aim of the study was to investigate the efficacy of BV in patients with relapsed or refractory PTCL.

Methods: Before drug approval, from March 2011 to January 2014, 74 patients were treated in France in a compassionate patient program for recurrent T-cell lymphoma. Diagnosis was confirmed for 65 patients after histopathologic review, according to the 2008 WHO classification. Central review and CD30 immunostaining (with monoclonal mouse anti-Human CD30, Clone Ber-H2, Dako) were performed.

Results: Characteristics of the 65 patients with histological review, before BV treatment, are summarized in Table 1. The median follow-up was 13.4 months [range 0.4-28.9]. Patients were treated for: ALCL (n=24): ALCL ALK- (n=14) and ALCL ALK+ (n=10); systemic PTCL (n=22): PTCL NOS (n=12), adult T-cell leukemia/lymphoma (n=4), enteropathy-associated T-cell lymphoma (n=4), and angioimmunoblastic T-cell lymphoma (n=2); and primary cutaneous lymphoma (n=19): Mycosis Fungoides/Sézary syndrome (n=11) and cutaneous CD30+ T-cell lymphoproliferative disorders (n=8). CD30 was scored as >75%, 10-70% and undetectable (<5%) in respectively 48.8%, 30.2% and 21% of the tested cases. At the time of initiation of BV, 36 patients were refractory to their last line of treatment and 25 were in relapse. Patients underwent a median number of 5 cycles of BV [range 1-16]. Eleven patients received chemotherapy in association with BV. Forty-two patients achieved response during treatment with 32 patients (49.2%) achieving complete response (CR) and 10 (15.4%) partial response (PR) with a median of 4 cycles before best response [range 1-12]. BV was discontinued in 58 patients (89.2%) because of progression (n=18), high-dose consolidative therapy followed by transplantation (n=18), optimal response (n=9), death (n=8), and toxicity (n=5). At the end of the treatment, 29 patients (45.3%) were in CR, and 7 (10.9%) in PR. The median duration of overall response was 8 months [95% CI 4.4; NR]. Median disease-free survival and progression-free survival were 15.5 months [95% CI 5.0; NR] and 6.8 months [95% CI 5.1; 9.4], respectively. The most common adverse events of any grade, regardless of relationship to BV were: neuropathy (n=23), hematological toxicities (anemia (n=22), neutropenia (n=19), thrombocytopenia (n=17)) and infection (n=13), for which 8 patients required a dose reduction. Nineteen patients received after BV a consolidative autologous (n=9) or allogeneic (n=9) stem cell transplantation or radiotherapy (n=1). Median survival was 25.9 months [95% CI 11.9; NR].

Table 1.

Patients characteristics before BV	N= 65
Age at diagnosis (Years) ; Median [Range]	51.5 [18;81]
ECOG: <2/>2/Not evaluated	40/18/7
Ann Arbor stage : I-II/ III-IV/Not evaluated	8/52/5
Number of prior chemotherapy regimens; Median [Range]	3 [1;8]
Prior autologous/ allogenic Steam Cell Transplantation	9/4

Summary and Conclusions: BV has already been proved effective in ALCL and seems also promising in other PTCL. Further analysis will attempt to determine if the response to BV is correlated with histological subgroups or CD30 expression level.

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THE ORAL SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) SELINEXOR (KPT-330) ACTIVITY IN DOUBLE HIT DIFFUSE LARGE B CELL LYMPHOMAS (DLBCL) IN PRECLINICAL MODELS & CLINICAL ACTIVITY IN PATIENTS WITH DLBCL

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Background: Amongst the ~60,000 cases of DLBCL diagnosed each year in EU/US, ~5% are "double-hit lymphomas" (DH-DLBCL) which overexpress MYC and BCL2/BCL6. DH-DLBCL is relatively resistant to available therapeutic agents including combination chemo-immunotherapy. Selinexor (KPT-330) is a SINE that forms a slowly reversible covalent bond with exportin 1 (XPO1). Inhibition of XPO1 forces the nuclear retention and activation of >10 tumor suppressor proteins (TSP) and is also associated with reduction in protein levels of c-myc and BCL family members. Selinexor showed anti-lymphoma activity in murine models of DLBCL and in spontaneous canine aggressive lymphomas that are similar to human DLBCL.

Aims: Here we investigate the effect of selinexor on XPO1 protein levels *in-vitro* as well as present updated results of selinexor in patients (pts) with DLBCL.

Methods: XPO1 protein levels were assessed by IHC in 62 human DLBCL specimens and compared with levels in normal human tonsils. Cytotoxicity at 48h was evaluated in 9 DLBCL cell lines, including several DH-DLBCL lines. MYC, BLC2 and BLC6 mRNA levels were determined in the nucleus and cytoplasm of the cells. Oral selinexor was given at 8-10 doses / 28-day cycle. XPO1 inhibition leads to rapid elevations in XPO1 mRNA, representing a pharmacodynamic (PDn) marker for selinexor. Tumor biopsies were performed. Response evaluation was done in cycles 1, 2, and every 2 cycles. All pts had heavily pretreated DLBCL with objectively progressive disease (PD) on study entry.

Results: XPO1 levels were overexpressed relative to normal tonsils in 49 of 62 (79%) human DLBCL samples. Selinexor showed potent cytotoxicity against all DLBCL cell lines (IC_{50s} 9.5 - >1000 nM) independent of MYC or BCL2/6 protein levels. Selinexor (10nM) lead to significant reductions in cytoplasmic to nuclear ratios of MYC, BCL2 and BCL6 mRNA levels in in OCI-Ly7 DLBCL cells. Nineteen pts (10 M, 9 F; median age 60 yrs; ECOG PS 0/1: 6/13; median prior regimens: 3.8 range 1-8) received selinexor across 7 dose levels (3 to 45 mg/m^2). Dosing at 60 mg/m^2 twice weekly (BIW) is ongoing and MTD has not been reached. Cycle 1 (DLT period) Grade 3/4 events include neutropenia (2pts) and thrombocytopenia (3pts). The most common Grade 1/2 AEs in Cycle 1: nausea (58%), vomiting (47%), anorexia (37%), & fatigue (32%). Supportive care with appetite stimulants and anti-emetics improved constitutional symptoms. Eighteen pts with progressive disease on study entry were evaluable for efficacy. Best responses included 4 Partial Responses (22%), 8 Stable Disease (44%), and 6 Progressive Disease (33%). Reductions in target lesions of 4 - 93% were observed in 9 pts (Figure 1). 4/19 pts have remained on therapy for >4 months (>4-18) months without clinically significant toxicities. Two pts with DH-DLBLC achieved 20% and 74% reductions in tumors in cycle 1 and continue on study (30+ and 180+ days).

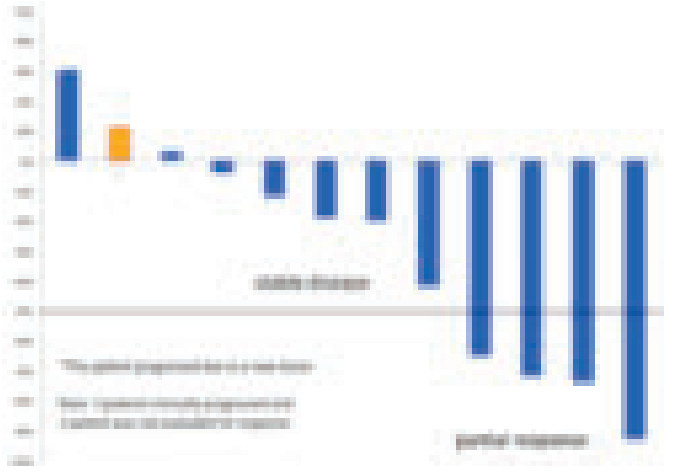

Figure 1. Waterfall Plot: Target Lesions Maximal Percent Change DLBCL Patients

Summary and Conclusions: Most human DLBCL overexpress XPO1 and are highly sensitive to XPO1 inhibition. In addition to forcing the nuclear localization of TSPs, selinexor reduces the relative levels of MYC, BLC2 and BCL6 cytoplasmic mRNAs leading to reduced translation to protein. Oral selinexor is generally well tolerated and can be administered over prolonged periods. Durable single agent activity was observed in heavily pretreated DLBCL pts, including those with DH-DLBCL. Phase 2 studies in DLBCL are planned.

P467

PATIENT PREFERENCE FOR SUBCUTANEOUS OR INTRAVENOUS ADMINISTRATION OF RITUXIMAB IN PREVIOUSLY UNTREATED CD20+ NON-HODGKIN LYMPHOMA: INTERIM DATA FROM THE PREFMAB STUDY

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Background: Rituximab is a mainstay of treatment for follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). A subcutaneous (SC) rituximab formulation has been developed to improve patient convenience and reduce healthcare resource burden without sacrificing clinical activity for patients receiving rituximab for B-cell hematologic malignancies.
Aims: PrefMab (NCT01724021) is an ongoing, randomised, open-label multicentre study to evaluate preference for rituximab intrvenous (IV) or rituximab SC during immunochemotherapy for non-Hodgkin lymphoma.
Methods: Patients with untreated CD20+ Grade 1–3a DLBCL or FL received 8 cycles of rituximab in combination with 6–8 cycles of CHOP, CVP or bendamustine, per standard local practice. Patients randomised to Arm A received 1 cycle of rituximab IV (375 mg/m^2) then 3 cycles of rituximab SC (1400 mg) followed by 4 cycles of rituximab IV (375 mg/m^2) after interim staging. Those randomised to Arm B received 4 cycles of rituximab IV (375 mg/m^2), then 4 cycles of rituximab SC (1400 mg) after interim staging. Dosing route preference was assessed via a Patient Preference Questionnaire (PPQ; preference recorded as SC, IV or 'no preference') post-therapy in cycles 6 and 8. Chemotherapy Treatment Satisfaction Questionnaires (CTSQ) and Rituximab Administration Satisfaction Questionnaires (RASQ) were completed at cycles 4 and 8. Questionnaire responses were compared using descriptive statistics. Adverse events (AEs) were monitored throughout the study. All patients provided written informed consent.
Results: At November 8th 2013, 433 patients had received treatment (Arm A: n=215; Arm B: n=218); baseline characteristics were well balanced with a mean age of 59 years. Most patients had DLBCL (62%) and 38% had FL. At the data cut-off, 69 patients (16%) had completed treatment; 34 (8%) discontinued study treatment prior to cycle 8, mainly due to AEs (n=13; 11 after IV doses [8 in cycle 1] and 2 after SC doses), disease progression (n=5), stable disease at interim staging (n=4) and withdrawn consent (n=4). The intent-to-treat (ITT) population included 445 patients (Arm A: n=221; Arm B: n=224). In 190 completed PPQs at cycle 6 (see Table 1), rituximab SC was preferred by 157 patients (83%; 95% confidence interval [CI]: 76–88; Arm A: n=76 [82%]; Arm B: n=81 [84%]), and rituximab IV by 15 patients (8%; 95% CI: 4–13; Arm A: n=10 [11%]; Arm B: n=5 [5%]). A similar preference was apparent in 125 completed PPQs for cycle 8: 108 patients (86%; 95% CI: 79–92; Arm A: n=50; Arm B: n=58) preferred rituximab SC, 45% with a 'very strong' and 35% a 'fairly strong' preference. Eleven patients (9%; 95% CI: 4–15) preferred rituximab IV (Arm A: n=7; Arm B: n=4). Reasons for administration route preference were the same as those given at cycle 6 (see Table 1). Data from the RASQ and CTSQ supported patients' preference for SC over IV administration. The occurrence of AEs was similar in both arms, and no new safety issues were detected. There were 7 deaths on-study (4 in Arm A, 3 in Arm B), with one (febrile neutropenia) considered probably rituximab-related.

Table 1. Responses to PPQ in cycle 6.

Summary and Conclusions: These data demonstrate patients' preference for SC *vs* IV rituximab administration during immunochemotherapy for FL and DLBCL. 'Less time in the clinic' was cited by patients as the primary reason for preferring SC administration, followed by 'less emotional distress', and 'more comfortable administration'.

P468

CLINICAL IMPACT OF INCLUSION AND TIMING OF RADIOTHERAPY IN THE TREATMENT OF LIMITED-STAGE NK/T-CELL LYMPHOMA

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Background: For the treatment of limited-stage (stage I or II stage) natural killer (NK)/T-cell lymphoma, combined treatment modality including radiotherapy (RT) is widely recommend. However, the best standard has not been clearly determined.
Aims: In this study, we analyzed the role and the optimal timing of RT, and the difference of clinical outcome among patients with limited-stage NK/T-cell lymphoma.
Methods: We retrospectively analyzed patients with limited-stage NK/T-cell lymphoma, diagnosed between January, 2004 and April, 2013 from six Korean institutes.These patients were categorized into 3 groups, *i.e.*, 1) anthracycline or non-anthracycline-based chemotherapy followed by radiotherapy (CTx/RT), 2) concurrent chemoradiotherapy (CCRT) followed by non-anthracycline-based chemotherapy (CCRT/CTx), and 3) CTx alone group according to therapeutic modalities.
Results: In total 104 patients with limited-stage NK/T cell lymphoma, the median age was 52 years (range 28~85) and 70 (67.3%) patients were male. Seventy-three (70.2%) patients had RT as initial treatment protocol. According to therapeutic modalities, 29 (27.9%) and 44 (42.3%) patients were treated by CTx/RT and CCRT/CTx, respectively, and the remaining 31 (29.8%) patients were managed by CTx alone as initial therapy. Overall response (OR) rate was 80.8% including complete response (CR) rate 70.2% after initial therapy. Patients who had CTx/RT or CCRT/CTx achieved markedly high CR rate (76.7%) comparing to those had CTx only group with 54.8% (p=0.009). With median follow up of 47.6 months, the 5-year progression free survival (PFS) was 44.2% and overall survival was 75.1%. The 5-year PFS of patients with RT containing protocol and those with CTx only group were 52.1% and 25.8%, respectively (p=0.003). Among patients treated with RT containing protocol, the 5-year PFS was superior in CTx/RT group as 60.6% than CCRT/CTx group as 48.8% (p=0.001). Note that, among CCRT/CTx group, there were 7 patients who could not proceed to the planned following chemotherapy. The causes were disease progression just after CCRT (n=4), and decreased performance status during CCRT (n=3).However, all patients treated with CTx/RT could finish the planned protocol. The site of relapse within RT containing protocol did not have different pattern (Figure 1).

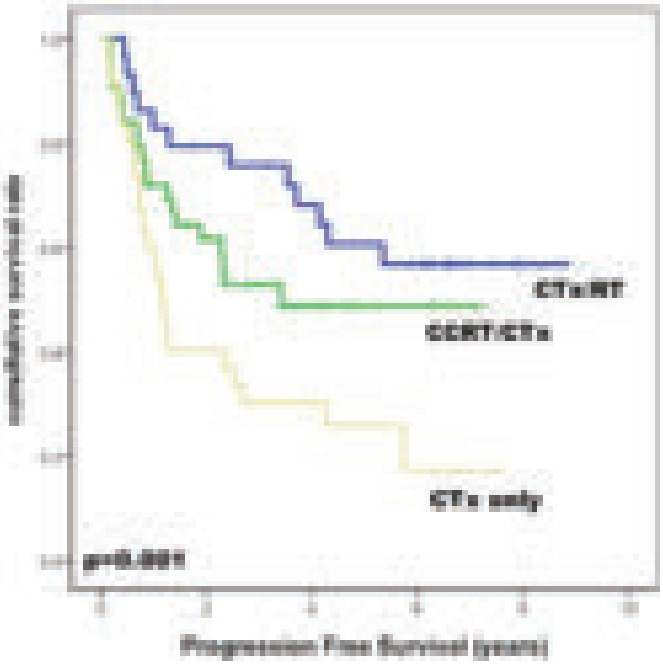

Figure 1.

Summary and Conclusions: Based on this analysis, we were able to emphasize the role of RT for treating limited-stage NK/T cell lymphoma, again. However, the optimal timing of RT in the disease course should be determined carefully in order to complete of planned treatment and prevent disease progression during localized RT. Moreover, RT at upper aerodigestive tract could decrease the therapeutic compliance of next following chemotherapy.

P469

EFFICALY OF M-NHL-BFM-90 TREATMENT PROTOCOL FOR PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) is an aggressive diffuse large B-cell lymphoma entity. It represents less than 3% of all non-Hodgkin lymphoma cases,, but is over-represented in the young adult population.
Aims: To evaluate the efficacy of modified NHL-BFM-90 (m-NHL-BFM-90) protocol in the treatment for adult PMBCL patients.
Methods: 71 patients (26 men, 45 women) were eligible for our study; median age was 30 years (18 - 70). PMBCL staging criteria developed by Ann Arbor were used to stage the patients. All patients were diagnosed with II stage, in 8 (11%) cases had breast and/or soft tissues involvement. Bulky mediastinal disease was found in 65 (91.5%) patients with PMBCL. Serum lactate dehydrogenase level was increased in 64 (90%) patients. aa-IPI 1 was established in 14 patient (20%), 2 – in 57 (80%), 0 and 3 – in 0. 71 newly diagnosed, previously untreated patients participated in the study performed in the National Research Center for Hematology between November 2004 and February 2014. The treatment is based on the modified NHL-BFM protocol (the dose of methotrexate was reduced to 1500mg/m^2 (12 h) in course A, B and C, doxorubicin (50mg/m^2) was included in the course A). A number of courses (4 or 6) were determined depending on achieving the remission. The patients with residual tumor in mediastinum underwent radiotherapy as consolidating treatment in total dose of 36 Gray.
Results: The 5-year disease-free survival (DFS) and overall survival (OS) were 83% and 89% with PMBCL, during an average follow-up of 38 months. The 5-year DFS and OS were 29% and 57% of patients with soft tissues and/or muscles tumor involving. Serum lactate dehydrogenase level and bulky mediastinal disease didn't impact on DFS and OS. One of the patients has died from chemotherapy complications. Eight patients turned out to be primary-resistant.
Summary and Conclusions: The modified NHL-BFM-90 is a highly effective protocol. The 5-year DFS and OS were 83% and 89% with PMBCL. Soft tissues and/or breast involvement were adverse prognostic factor in patients with PMBCL.

P470

BISPECIFIC T-CELL ENGAGER (BITE®) ANTIBODY BLINATUMOMAB IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): AN OPEN-LABEL PHASE 2 STUDY

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Background: The bispecific T-cell engager (BiTE®) antibody, blinatumomab, redirects cytotoxic T cells to CD19$^+$ B-lineage cells, resulting in serial lysis of the target cells. In a phase 1 study in pts with relapsed/refractory non-Hodgkin lymphoma, blinatumomab exhibited anticancer activity and acceptable toxicity. In a subset of pts with DLBCL, stepwise dose escalation with blinatumomab resulted in an overall response rate (ORR) of 55% in evaluable patients (Goebeler *et al. Hematol Oncol* 2013;31[suppl 1]:197).
Aims: The objective of this open-label phase 2 study is to investigate the efficacy and tolerability of blinatumomab in pts with relapsed/refractory DLBCL, comparing stepwise dose escalation with constant target dosing.
Methods: Eligible pts were ≥18 years of age, had relapsed/refractory DLBCL and an ECOG performance status ≤2. Pts received blinatumomab by continuous intravenous infusion for up to 8 weeks. In part 1, a target dose of 112 µg/d was achieved using a double-step escalation dosing regimen (cohort I: 9, 28, and 112 µg/d during weeks 1, 2, and thereafter, respectively) or a flat dose regimen (cohort II: 112 µg/d throughout). Based on the overall benefit/risk assessment for part 1, the dose administered in cohort I was selected for further evaluation in part 2 (cohort III). Pts achieving an objective response could receive a 4-week consolidation cycle, following a 4 week treatment-free period. All pts received prophylactic dexamethasone. The primary endpoint was ORR by Cheson (2007) revised response criteria for malignant lymphomas per independent radiologic assessment. Secondary endpoints included rates of complete response (CR) and partial response (PR), and incidence of adverse events (AE).
Results: 19 pts have been enrolled and treated: 9, 2, and 8 in cohorts I, II, and III, respectively. All pts provided informed consent. Median age was 66 years (range, 39–85); 47% of pts were women. Blinatumomab was given as third-line (median) systemic treatment (range of prior treatments, 1–7). Eight (42%) pts had received previous radiotherapy; 6 (32%) had received autologous hematopoietic stem cell transplantation. At the time of this analysis, 16 pts were evaluable for response (cohort I, n=8; cohort II, n=1; cohort III, n=7). Per independent radiologic assessment, ORR was 44% (cohort I: CR=2, PR=2; cohort II: PR=1; cohort III: CR=1; PR=1). Three pts had stable disease (1 in cohort I and 2 in cohort II). Six pts had progressive disease (3 each in cohorts I and III). Three pts were not evaluable for ORR per protocol definition (1 each in cohorts I, II and III). Further enrollment in cohort II was terminated due to AEs. The most common AEs in cohorts I and III, regardless of causality, were pyrexia (47%), tremor (41%), fatigue (29%), and edema (24%). Fifteen of 17 pts in cohorts I and III had ≥1 grade 3 AE, regardless of causality. Thirteen of the 17 pts in cohorts I and III had CNS AEs, mostly tremor (41%) and speech disorders (18%). Four pts from cohorts I and III had grade 3 CNS AEs; there were no grade 4 or 5 CNS AEs.
Summary and Conclusions: In this ongoing phase 2 study in heavily pretreated adult pts with relapsed/refractory DLBCL, blinatumomab showed antitumor activity. Part 1 of the study established stepwise dosing (9, 28, 112 µg/d) as the recommended blinatumomab dose for this pt population. Evaluation of pts in cohort III is ongoing.

P471

NON PEGILATED LIPOSOMAL DOXORUBICIN IS SAFE AND EFFECTIVE AS PART OF R-COMP IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AND MODERATE/SEVERE HEART DISEASE. RESULTS OF HEART01 TRIAL BY THE FONDAZIONE

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Background: R-CHOP is the standard treatment for Diffuse Large B-Cell Lymphoma (DLBCL) but is usually contraindicated in patients with concomitant heart disease due to the unsafe cardiac profile of doxorubicin. The encapsulation of doxorubicin into liposomes modifies its pharmacokinetic reducing its concentration in the heart.
Aims: We conducted a multicenter phase II trial investigating activity and safety of R-COMP regimen where conventional doxorubicin was substituted with non-pegilated liposomal doxorubicin (TLC-D99; Myocet ™) in patients with DLBCL presenting with moderate/severe concomitant heart disease.
Methods: Main inclusion criteria were: age >18 years, diagnosis of DLBCL, cardiac disorder defined by at least one of the following: left ventricular ejection fraction (LVEF) <50%, left ventricular hypertrophy, uncontrolled moderate/severe arterial hypertension, history of ischemic cardiopathy, clinically significant ventricular arrhythmia, atrial fibrillation (AF), pulmonary hypertension, moderate/severe mitral valvular disorder, moderate aortic valvular disorder. Enrolled patients received 6 courses of 3-weekly R-COMP (rituximab 375 mg/m^2, cyclophosphamide 750, mg/m^2, vincristine 1.4 mg/m^2, TLC-D99 50 mg/m^2, prednisone 40 mg/m^2/day day 1-5).
Results: Between 2009 and 2011, 51 patients were enrolled. Median age was 77 years (range 53 - 90 years), 34 (68%), were male and 32 (64%) had stage III-IV disease. Sixty-two cardiac disorders were identified at baseline; the most frequent was ischemic cardiopathy (n=20), followed by AF (n=9), baseline LVEF <50% and left ventricular hypertrophy (n=8); 2 and 3 concomitant cardiac disorders were described in 3 e 4 patients respectively. Treatment was completed in 39 (78%) of patients with a median dose intensity for TLC-D99 of 99%. R-COMP was interrupted prematurely due to lack of response (n=1), CEs (n=5), severe infection (n=2), deep venous thrombosis (n=1), renal failure (n=1), other toxicity (n=1), and patient's decision (n=1). As requested by the sequential Bayesian model, the CR rate and the rate of CE never fell outside activity and safety boundaries. In fact, 27 patients achieved a CR (54%; 95%CI=42-72%) and 6 CE were observed, which included significant LVEF reduction (2 patients), clinically significant s-troponine increase (2), hearth failure (1), and cardiac arrest (1). Median LVEF after cycle 3 and at the end of treatment was 59% (range 25-72%) and 57% (30-69%), without significant modifications from baseline values. With a median follow-up of 30 months (range 1-44) 11 patients had lymphoma progression, 8 experienced relapse and 8 died without signs of lymphoma relapse/progression. Overall, 19 patients died due to lymphoma (9), severe infection (2), second cancer (1), hemorrhage (1), myocardial infarction (2), renal insufficiency (1), respiratory insufficiency (1), and unknown cause (2). The 3-year OS and PFS were 54% (95%CI: 34-70%) and 40% (95%CI: 25-54%), respectively. Median LVEF was 55%(n=16, range 30-69%), 56% (n=15, range 30-65%), 58% (n=12%, range 52-66%) and 60% (n=8, range 53-76) at 6, 12, 18 and 24 months respectively. In 8 patients a decrease in LVEF ≥20%

compared with baseline value has occurred, 3 cases after 3 cycles, 1 at the end of the treatment, 1 at 6, 2 at 12 and 1 at 18 months after the end of treatment.
Summary and Conclusions: The substitution of conventional doxorubicin with non pegilated liposomal doxorubicin (TLCD99-Myocet™) in the R-CHOP regimen is a safe and active option for patients with DLBCL presenting with concomitant moderate/severe cardiac disorders.

P472

HIGH DOSES OF ANTIMETABOLITES FOLLOWED BY HIGH-DOSE SEQUENTIAL CHEMOIMMUNOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT IN SYSTEMIC B-CELL LYMPHOMAS WITH CNS INVOLVEMENT: A MULTICENTER PHASE II TRIAL

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Background: We report results of a multicentre phase II trial (ClinicalTrials.gov NCT00801216) addressing feasibility and efficacy of a new chemoimmunotherapy combination in patients (pts) with B-cell lymphomas (BCL) and CNS involvement. Experimental treatment is based on the encouraging experiences with high doses of antimetabolites in pts with primary CNS lymphoma (Ferreri *et al.* Lancet 2009) and with high-dose sequential chemoimmunotherapy (R-HDS) supported by autologous stem cell transplant (ASCT) in pts with relapsed BCL (Tarella *et al.* JCO 2008).
Aims: to address tolerability and efficacy of a new intensified chemoimmunotherapy regimen supported by ASCT in pts with secondary CNS lymphoma.
Methods: HIV-negative pts (18-70 ys; ECOG PS ≤3) with systemic BCL and CNS disease at diagnosis or relapse were enrolled. Experimental treatment consisted of 2 c. of methotrexate 3.5 g/m^2 d1 and cytarabine 2 g/m^2 x2/d d2-3, followed by R-HDS with cyclophosphamide 7 g/m^2 d1, cytarabine 2 g/m^2 x2/d d22-25 and etoposide 2 g/m^2, d43, and BCNU-thiotepa conditioning and ASCT. Treatment included 8 doses of rituximab and 4 doses of intrathecal liposomal cytarabine 50 mg. Two-year PFS was the primary endpoint; the planned accrual was 38 pts.
Results: 40 pts were registered (median age 59ys, range 32-70 ys; M/F ratio 1.5): 34 had DLBCL, 3 had blastoid variant of MCL and 3 had FL. CNS disease was detected at presentation in 17 pts (all pts had concomitant extra-CNS disease) and at relapse in 23 (8 had extra-CNS disease), and consisted of brain parenchyma (n=23), meninges (6) or spinal cord (2) lesions, or involvement of multiple CNS organs (9). Response was complete in 23 pts (58%) and partial in 1 (ORR=60%; 95%CI=45-75%), 1 pt had SD, 11 experienced PD (CNS in all pts; extra-CNS in 4), and 4 died of toxicity (sepsis 2, stroke). G4 neutropenia, thrombocytopenia and anemia were recorded in 90%, 76% and 8% of courses. Febrile neutropenia was recorded in 26% of courses; opportunistic infections (CMV in 6 pts, aspergillosis in 1) were successfully managed. Transient increase of transaminases levels (2) and intestinal perforation (1) were the only G4 non-hematological toxicities. ASC collection was successful in 23 (92%) of the 25 referred pts (median 9.5x10^6/kg; range 5.8-18.9); 20 pts eventually received ASCT. At a median follow-up of 3 ys, 16 pts remain relapse-free, with a 2-yr PFS of 40±8%. Seventeen pts are alive and NED, with a 2-yr OS of 41±8%; 2-yr OS of transplanted pts was 64±11%. Age, extra-CNS disease and/or meningeal dissemination did not affect survival.
Summary and Conclusions: This intensified treatment is feasible and effective in pts ≤70 ys with systemic BCL and CNS involvement. Toxicity is almost exclusively haematological and manageable. Survival benefit is also attained in pts with extra-CNS and/or meningeal disease. Since randomized trials are unrealistic in this context, authors recommend this combination as first choice in routine practice.

P473

R-ESHAP-LENALIDOMIDE AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA CANDIDATES TO STEM-CELL TRANSPLANTATION: UPDATED RESULTS OF A PHASE IB GELTAMO STUDY

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Background: Diffuse large B-cell lymphoma (DLBCL) patients with relapsed or refractory disease after rituximab-containing first-line therapy have a poor outcome with the current salvage regimens. Consequently, new drugs designed to increase the response rate of salvage regimens should be explored.
Aims: We conducted a phase Ib trial to establish safety, and to determine the maximum-tolerated dose (MTD) of lenalidomide in combination with R-ESHAP (LR-ESHAP) in patients with relapsed or refractory DLBCL. Efficacy data were also collected as secondary objective. EUDRACT number: 2010-018463-41.
Methods: Eligible patients must have refractory or relapsed disease following first-line treatment with rituximab and anthracycline-containing regimens, and be eligible for autologous stem-cell transplantation (ASCT). Subjects received three cycles of lenalidomide (5, 10 or 15 mg) given on days 1 to 14 of every 21-day cycle, in combination with R-ESHAP salvage chemotherapy at standard doses (rituximab 375 mg/m^2 day 1, etoposide 40 mg/m^2 days 1-4, cisplatine 25 mg/m^2 days 1-4; citarabine 2000 mg/m^2 day 5, and methilprednisolone 500 mg days 1-5). Responding patients received BEAM followed by ASCT.
Results: During the escalating phase, 3 patients had dose-limiting toxicity in the 15 mg cohort: 1 grade 3 angioedema, and 2 mobilization failures. The MTD was therefore established at 10 mg of lenalidomide and this cohort was expanded to further explore the safety and efficacy of LR-ESHAP. A total of 20 patients (3, 13, and 4 in the 5, 10, and 15 mg cohorts) were enrolled. 1 patient (10 mg cohort) was excluded due to a major deviation protocol in exclusion criteria (severe hepatic disease not due to lymphoma), so 19 patients were evaluable (17 with DLBCL-NOS, and 2 with Burkitt-like lymphoma). Median age was 58 (23–70) years (60% male). First-line treatment consisted of R-CHOP or similar in 17 patients and Burkitt's lymphoma protocols in 2. Disease status at LR-ESHAP was: primary refractory disease in 13 patients, and relapsed disease in 6 (3 early and 3 late relapses). IPI was 0-1 in 37%, 2-3 in 40%, and 4-5 in 25%. To date, 17 serious adverse events have been reported: 6 episodes of febrile neutropenia, 3 pneumonia, 2 sepsis, 1 ionic imbalance, 1 renal toxicity, 1 facial angioedema, 2 thrombosis (associated to central venous catheter), and 1 graft failure after ASCT, all of them recovered except the graft failure. There were no treatment-related deaths. 17 patients (90%) completed the planned three cycles (3 without lenalidomide in the third cycle due to significant toxicity during the second) and 1 patient two cycles (due to persistent neutropenia and thrombocytopenia after the second cycle). One patient discontinued treatment during the first cycle due to grade 3 facial angioedema. The overall response rate to LR-ESHAP was 74% (42% complete remission). All 18 patients who received at least 2 cycles were successfully mobilized after one (13 patients) or two (5 patients) mobilization procedures, and 14 patients (74% of the overall series) underwent ASCT according to protocol. Reasons for not performing the ASCT were: early progression (n=4), and clinical trial discontinuation due to toxicity (n=1). At the time of this analysis, 9 patients had disease progression and 7 of them have died. With a median follow-up of 11.9 (5.9 to 30) months, the estimated 1-year progression-free survival and overall survival were 61% and 63%, respectively.
Summary and Conclusions: The MTD of lenalidomide in combination with R-ESHAP is 10 mg. LR-ESHAP shows an acceptable safety profile and encouraging activity in rituximab-pretreated relapsed or refractory DLBCL patients. Further investigation with this regimen is warranted.

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NCCN-INTERNATIONAL PROGNOSTIC INDEX (NCCN-IPI) IS A POTENTIAL PROGNOSTIC MODEL IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMA

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Background: Peripheral T-cell lymphomas (PTCLs) represent the most prevalent subtype among T-cell neoplasms and constitute a heterogeneous group of disorders. Prognostic characterization of patients is an essential prerequisite for risk-adapted therapies in PTCL. Initially designed for aggressive B-cell lymphoma, the International Prognostic Index (IPI) is the most commonly used prognostic scoring system in PTCL. The National Comprehensive Cancer Network (NCCN)-IPI has recently been proposed and demonstrated better discrimination of risk subgroups than IPI in diffuse large B-cell lymphoma. Thus prognostic significance of NCCN-IPI in PTCL remains great interest.
Aims: To validate the prognostic significance of NCCN-IPI in PTCL patients.
Methods: We conducted a retrospective analysis in 252 PTCL patients treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone)-based chemotherapy (eight cases received subsequently high-dose chemotherapy and autologous stem cell transplantation). Informed consent was obtained from all patients, in accordance with the regulations of the Shanghai Rui Jin Hospital and Shanghai Hua Shan Hospital Review Boards.
NCCN-IPI used a maximum of 8 scoring points for categorized age >40-60 (1 pt.), >60-75 (2 pts.) and >75 years (3 pts.), LDH ratio >1-3 (1 pt.) and ≥3 (2 pts.) upper limit of normal in addition to extranodal disease in major organs (either bone marrow, CNS, liver/GI tract or lung), Ann Arbor stage III/IV and ECOG

PS≥2, each having a score of 1. Four risk groups were formed: low (0-1), low-intermediate (2-3), high-intermediate (4-5) and high (6-8).
Results: The estimated 5-year progression free survival (PFS) and overall survival (OS) rate were 21.1% and 36.5%, with median PFS and OS at 12.6 and 30.8 months, respectively. The prognosis of the patients varied from histological subtypes, but in agreement with previous cohort (Figure 1A). NCCN-IPI owned lower Akaike information criterion value (PFS: 1496.267 *vs* 1502.117, OS: 1161.130 *vs* 1172.765) and higher concordance probability index (PFS: 0.72 *vs* 0.69, OS: 0.78 *vs* 0.75) than IPI, which indicated better model fitting and discrimination. Meanwhile, when controlling for NCCN-IPI, histological subtype was no longer a significant predictor of survival (PFS: P=0.232, OS: P=0.132). In the multivariate analysis including the five factors of NCCN-IPI, all the enhancements in NCCN-IPI as the categorization of age, LDH and specific extranodal sites were independent prognostic factors of PFS (P=0.022; P=0.044; P=0.013) and OS (P=0.006; P=0.001; P=0.011), further supporting the predictive efficacy of NCCN-IPI. According to risk subgroups, the survival rate of low-risk patients was remarkably higher in NCCN-IPI than in IPI (5-yr OS 88.9% *vs* 67.6%, 5-yr PFS 48.7% *vs* 38.3%, Figure 1B-E). The identification of these 'ultra' low-risk patients by NCCN-IPI is of paramount importance, since they may benefit from conservative chemotherapy like CHOP regimen to achieve optimal prognosis. Regarding high-risk patients, NCCN-IPI and IPI are both efficient for predicting poor disease outcome (2-yr OS 14.8% *vs* 19.1%, 2-yr PFS 10.0% *vs* 16.3%). These patients should thus receive intensive treatment with stem cell transplant and/or novel targeted agents.

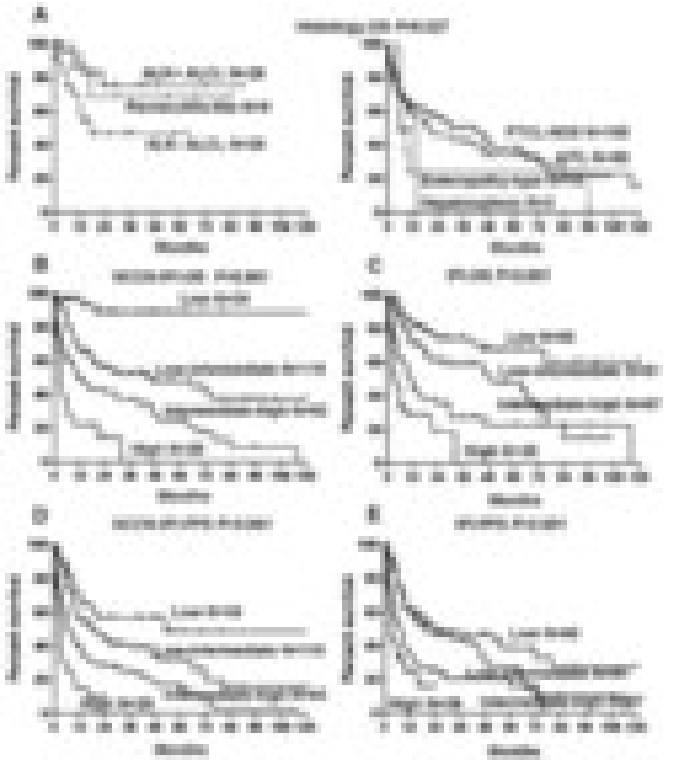

Figure 1. Survival curves of patients with peripheral T-cell lymphoma. (A) Overall survival (OS) according to histological subtypes of PTCL. (B-E) OS (B and C) and progression-free survival (PFS, D and E) according to NCCN-IPI and IPI (Xu *et al.*).

Summary and Conclusions: Similar to B-cell lymphoma, NCCN-IPI is also more powerful than IPI for risk prognostication in PTCL.
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RITUXIMAB-CHOP PLUS INTRATHECAL METHOTREXATE AND CONTRALATERAL TESTIS IRRADIATION IN UNTREATED PRIMARY TESTICULAR DIFFUSE LARGE B-CELL LYMPHOMA: LONG-TERM RESULTS OF THE IELSG-10 TRIAL

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Background: Primary testicular lymphoma (PTL) has poor prognosis with an high risk of failures in central nervous system (CNS) and contralateral testis. The IELSG-10 international trial showed the efficacy of conventional Rituximab-CHOP21 (R-CHOP21) associated with CNS intrathecal prophylaxis and contralateral testis irradiation (Vitolo, J Clin Oncol 2011).
Aims: Aim of the current analysis is to evaluate long term outcome and incidence of CNS and contralateral testis relapses.
Methods: From 2001 to 2006, 53 patients were enrolled. Inclusion criteria were: untreated stage I or II diffuse large B-cell lymphoma PTL. After diagnostic orchiectomy, patients were planned to receive 6 or 8 courses of R-CHOP21 followed by prophylactic irradiation (IF-RT) to the contralateral testis at 25 to 30 Gy for all patients. During chemotherapy, intrathecal methotrexate (IT-MTX) was administered as CNS prophylaxis (12 mg, 4 times).
Results: Fifty-three patients were enrolled. Clinical characteristics were: median age 64 years (range 22-79); stage I in 40 patients (75%) and stage II in 13 (25%); bilateral testis involvement in 4 (8%). No patients have CNS involvement at diagnosis. With a median follow-up of 65 months, we previously reported 5-years progression free survival (PFS), overall survival (OS), cumulative incidence of lymphoma progression or death as a result of lymphoma (TTP) and cumulative incidence of CNS relapse were: 74%, 85%, 18% and 6%, respectively. With an extension of follow-up time to a median of 9 years, PFS is 67% (95% CI: 52-78%): one patient failed during treatment and died; 6 died due to causes not related to lymphoma and 10 patients relapsed. Relapses involved lymph node alone in 3 patients, extranodal organs (pleura, skin, CNS) with or without lymph nodes in 7 patients. Four patients experienced CNS relapses: two of them had isolated relapses (one meningeal and one brain parenchymal), one had a concurrent meningeal and lymph nodal relapse and the fourth one had a parenchymal CNS relapse while in second remission after nodal relapse. At 9 years the cumulative incidence of CNS relapse, taking into account the competitive risk of death, remains 6% (95% CI, 0-12%). Contralateral testis relapses continue to be absent. 9- year TTP is 27% (95% CI: 15-39%) and 9-year OS is 75% (95% CI: 60-84%); 14 patients died, 8 of progressive disease, one of gastric cancer after 9 months off therapy, one of heart failure after 17 months, two of acute myelogenous leukemia after 21 and 60 months, one of hepatocarcinoma after 63 months and one of metastatic melanoma after 84 months. (Figure 1).

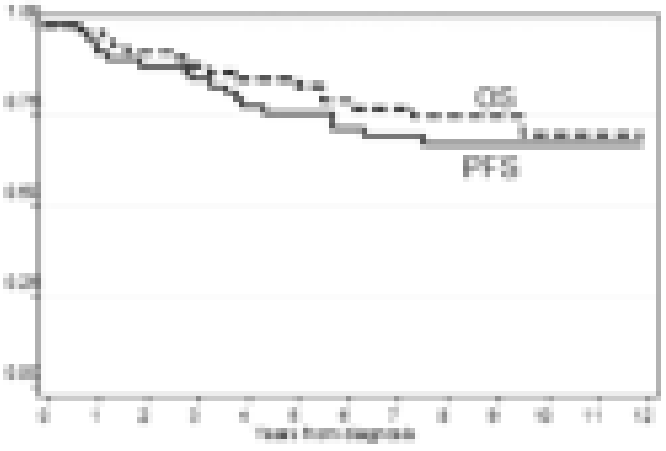

Figure 1. Kaplan-Meier estimates of progression free and overall survival.

Summary and Conclusions: This update of IELG10 study, with a prolonged follow-up, shows a long-term benefit of the R-CHOP21 treatment associated with intrathecal methotrexate and testicular radiotherapy in PTL. To further improve the outcome and reduce CNS relapse, FIL and IESLG planned the ongoing international trial, IELSG30, to test the use of an intensified CNS prophylaxis such as intrathecal liposomal citarabine and systemic intermediate-dose methotrexate.

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THE NOVEL ROLE OF BONE MARROW INVOLVEMENT DETECTED BY BOTH STAGING AND INTERIM FDG PET-CT IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Background: Bone marrow involvement (BMI) detected by either bone marrow biopsy (BMB) or PET-CT at the initial staging (PET(0)-CT) may have an important value in newly diagnosed diffuse large B-cell lymphoma (DLBCL).

Aims: The aim of our study was to confirm the diagnostic and prognostic value of BMI assessed by PET(0)-CT (PET(0)-BMI), evaluate the impact of PET(0)-BMI on the classical International Prognostic Index (IPI) and the significance of BMI detected by PET(inter)-CT (2-4 cycles of standard R-chemotherapy) (PET(inter)-BMI).
Methods: One hundred thirty-five patients with DLBCL were retrospectively enrolled in our study, for whom underwent both a PET(0)-CT and staging BMB. The presence of BMI in patients was determined by BMB and PET(0)-CT results.
Results: BMI was detected by PET(0)-CT in 35 (25.9%) and by BMB in 18 (13.3%) cases with the total number of BMI in 38 (28.2%) according to our criteria. By multivariate analysis, only IPI□2 (P=0.007) and PET(0)-BMI(+) (P=0.005) remained independent predictive factors of progression-free survival (PFS). Patients with PET(0)-BMI(+) had worse PFS than patients with PET-BMI(−), P<0.0001. The same pattern was observed for BMB (P=0.032). Among the 60 patients with IPI of inter-risk, the patients with PET(0)-BMI(+) had significantly inferior PFS than patients with PET(0)-BMI(−) (P=0.005). Among the 35 patients with PET(0)-BMI(+), patients with PET(inter)-BMI(−) had superior PFS than patients with PET(inter)-BMI(+) (P=0.009), the same pattern was observed for ΔSUVmax (P=0.052).
Summary and Conclusions: PET(0)-BMI demonstrates a better diagnostic and prognostic performance. The patients with IPI of inter-risk meanwhile with PET(0)-BMI(+) should be treated as IPI score of high-risk. Furthermore, PET(inter)-BMI(−) appears vital in particular in patients with PET(0)-BMI(+).

Stem cell transplantation - Experimental

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MIR-155 IS ASSOCIATED WITH THE LEUKEMOGENIC POTENTIAL OF CLASS IV G-CSFR IN CD34+ PROGENITOR CELLS

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Background: Granulocyte-colony stimulating factor (G-CSF) is a major regulator of granulopoiesis via the G-CSF Receptor (G-CSFR). The class IV G-CSFR (G-CSFRIV) lacks 3 tyrosine residues and the dileucine internalization motif present in the class I G-CSFR (G-CSFRI), and demonstrated to be differentiation defective. Recently, an oncogenic role of G-CSFRIV in human myeloid leukemia has been suggested. MicroRNAs (miRNAs) have been implicated in various pathologies including hematologic malignancies. Whether miRNAs especially miR-155 are related to the leukemogenic potential of G-CSFRIV was not evaluated yet.
Aims: To investigate the leukemogenic mechanism associated with the high expression levels of G-CSFRIV in human CD34+ progenitor cells.
Methods: G-CSFRI or IV overexpressing cells were stimulated with 10-400 ng/ml G-CSF for 1-3 days. Proliferative potential of G-CSFRI and G-CSFRIV overexpressing (G-CSFRI+ and G-CSFRIV+) progenitor cells were analyzed by BrdU incorporation and colony forming unit (CFU) assays. Ki67 and PI cell staining was used to analyse the cell cycle. Annexin V/PI staining was used to detect apoptosis. Signaling pathways were analyzed using phosphflow technology. Levels of miR-155 and its candidate target genes were quantified by real time-PCR. Inhibition assays were performed by using a STAT5 inhibitor and by RNA interference.
Results: Higher proliferation rates were observed in G-CSFRIV+ progenitor cells in comparison to G-CSFRI+ progenitor cells as indicated by enhanced BrdU incorporation (15.8%, p=0.0142 at 100ng/ml and 14.1%, p=0.0022 at 400ng/ml) upon G-CSF stimulation. G-CSFRIV+ progenitor cells generated higher colony numbers (52.0±20.2 *vs.*102.3±6.7, p=0.0087) in the CFU assay, but the cells expressed significantly lower levels of maturity markers. Cell cycle analysis demonstrated an increase in the number of G-CSFRIV+ CD34+ cells in S phase and G2-phase. Accordingly, an increase of Ki67 staining (169.0±47.44 *vs.* 231.7±31.79, p=0.0316) in G-CSFRIV+ progenitor cells was observed. Significantly less apoptosis was observed in G-CSFRIV+ progenitor cells cultured with G-CSF. Furthermore, G-CSF induced sustained Stat5 activity in G-CSFRIV+ progenitor cells. Interestingly, an induction of miR-155 was observed in G-CSFRIV+ progenitor cells in presence of G-CSF (RQ: 0.70±0.11 *vs.* 1.41±0.36, p=0.0040). Moreover, significantly reduced levels of miR-155 target gene transcripts including PU.1, Gfi-1 and TP53INP1 were observed in G-CSFRIV+ progenitor cells. Importantly, a Stat5 inhibitor prevented G-CSF-induced upregulation of miR-155 levels in G-CSFRIV+ progenitor cells (RQ: 1.76±0.36 *vs.* 1.05±0.08 for with and without Stat5 inhibitor respectively, p=0.0112) and the subsequent inhibition of its target genes. Furthermore, the hyperproliferation of G-CSFRIV+ progenitor cells was significantly decreased by the Stat5 inhibitor (p=0.0157) or the shRNA targeting miR-155.
Summary and Conclusions: This study shows that G-CSFRIV mediates a prolonged activation of Stat5 which causes an increase in miR-155 levels and consequently a decrease in the expression of its targets. Moreover, the Stat5/miR-155/miR-155 targets pathway promotes proliferation and survival of progenitor cells indicating of leukemic transformation. We identify that the leukemogenic potential of G-CSFRIV is associated with a Stat5-dependent dysregulation of miR-155 and its target genes. In consideration of the wide applications of G-CSF in the clinic, it is highly recommended to prescreen for G-CSFRIV expression levels in patients and stem cell donors before G-CSF administration. Moreover, our data suggests that Stat5 or miRNA-155 inhibitors strongly contribute to the abrogation of the leukemogenic potential of G-CSFRIV. This may represent a novel therapeutic approach to decrease the risk of leukemia or relapse in patients showing an elevated G-CSFRIV/G-CSFRI ratio.

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THE ROLE OF EPITHELIAL TO MESENCHYMAL TRANSITION IN THE REMODELING OF LUNG INJURY INDUCED BY ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Noninfectious lung injury caused by graft-*versus*-graft disease (GVHD) is a life-threatening complication after allogeneic hematopoietic stem cell transplant (allo-HSCT). Epithelial injury is a central event in the pathogen-

esis of noninfectious lung injury. Recent studies have shown that alveolar epithelial cells are able to self-renew and reestablish a functional alveolar epithelium. In the inflammatory and fibrotic lung diseases, differentiated epithelia also can acquire a myofibroblast phenotype in the process termed epithelial to mesenchymal transition (EMT), which contributes to aberrant healing and fibrosis. Many factors such as inflammatory cytokine TGF-β have been suggested to induce EMT. However, the role of EMT in the remodeling of acute GVHD (aGVHD) induced lung injury is unclear.
Aims: To clarify whether EMT is involved in airway remodeling in an animal model of aGVHD induced lung injury.
Methods: BALB/c mice were lethally irradiated and transplanted T cell-deleted (TCD) bone marrow plus whole spleen cells from C57BL/6 mice as aGVHD group, and only transplanted TCD bone marrow cells as control group. Alveolar epithelial cells were isolated from mice of two groups and Ep-CAM expression was measured by flow cytometry. The mRNA expression of cytokines including IFN-γ and TGF-β was detected by RT-PCR. The mRNA and protein expressions of specific markers, including E-cadherin, vimentin, Snail and surfactant proteins (SP)-C in lung tissue, were detected by RT-PCR and western blot.
Results: All mice in the aGVHD group showed diffuse periluminal infiltrates and parenchymal pneumonitis by histopathology, while the mice in the control group did not show any lung injury evidence. TGF-β mRNA expressions were markedly up-regulated but IFN-γ expressions were down-regulated in the lung injury group as compared to the control group (*P*=0.025 and *P*=0.045). Alveolar epithelial cells of injured lung expressed higher Ep-CAM (*P*=0.017) and lower SP-C (*P*=0.023). RT-PCR and western blot analyses revealed a significant decrease in epithelial marker E-cadherin (*P*=0.029) and increase in mesenchymal marker vimentin (*P*=0.026) in the GVHD damaged lung. Snail, a key EMT related transcription factor, was significantly elevated at mRNA and protein level in comparison to control group (*P*=0.015).
Summary and Conclusions: EMT is involving in the remodeling of lung injury induced by aGVHD. The effects and mechanisms of IFN-γ and TGF-β imbalance in aGVHD induced lung injury are deserved to be further explored.

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COMPARISON OF UMBILICAL CORD BLOOD AND HAPLOIDENTICAL DONOR GRAFTS IN ADULTS WITH HIGH RISK HEMATOLOGIC DISEASES AFTER FLUDARABINE CYCLOPHOSPHAMIDE AND TBI 2 GY BASED REDUCED-INTENSITY CONDITIONING RE

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Background: For patients without a suitable matched related donor, Alternative donors such as mismatched unrelated, cord blood and mismatched family donors could be searched. The aim of this retrospective study was to compare the results of graft source on outcome of patients after haploidentical related donor (Haplo), and the unrelated umbilical cord blood (UCB) transplantation in the setting of Non myeloabalative conditioning regimen (NMA). We retrospectively analyzed outcomes in 150 adult patients with high risk hematologic diseases who received Allo-SCT from alternative donors from two centers (Institut Paoli-Calmettes at Marseille France and Humanitas cancer center at Rozzano, Italy): These two centres have been applying common transplant approaches and procedures during the study period. Sixty nine patients received Haplo and 81 patients received UCB. The NMA regimen consisted of fludarabine (Flu), cyclophosphamide (Cy) and low dose TBI (2 Gy) combination in the two groups. The GVHD prophylaxis consisted of Cyclosporine A (CsA) and MMF in all patients in the two groups. In the Haplo group all patients received also 50 mg/kg Cy at day 3 and 4 post transplant. Of note, supportive care was the same during the whole study period. All supportive care measures included red blood cell, and platelet transfusions were significantly increased in cord blood transplantation group. The cumulative incidence of transplant related mortality (TRM) at one year was 23% in the UCB group *versus* 17% in the Haplo group (P=0.39). Grade 2-4 acute graft-*vs.*-host disease (GVHD) and extensive chronic GVHD incidences were 52% *versus* 29% (P=0.05), and 12% *versus* 6% (P<0.0001), in the UCB group *versus* the Haplo group, respectively. The overall survival at 2 years was 45% (95%CI, 34-56%) in the UCB group *versus* 69% (95%CI, 58-80%) in the Haplo group, (P=0.10). The estimate of progression-free survival at 2 years was 36% (95%CI, 25-47%) in the UCB group *versus* 65% (95% CI, 53-77%) in the Haplo group (P=0.01).
Aims: The main difference between the 2 groups was the significantly higher incidence of acute and chronic GVHD in the UCB group. In this study, relapse and PFS was lower in Haplo group than in UCB transplants.
Results: Our results suggest that haploidentical transplants are a good and promising alternative option for patients with high risk hematological diseases who lack an HLA-matched donor (sibling or unrelated donor). This should be now investigated in prospective comparative studies.
Summary and Conclusions: The main difference between the 2 groups was the significantly higher incidence of acute and chronic GVHD in the UCB group. In this study, relapse and PFS was lower in Haplo group than in UCB transplants. Our results suggest that haploidentical transplants are a good and promising alternative option for patients with high risk hematological diseases who lack an HLA-matched donor (sibling or unrelated donor). This should be now investigated in prospective comparative studies.

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IMMUNOREGULATORY MECHANISMS OF BLOCKING IL-21 SIGNALING IN ACUTE GVHD MODEL: DUAL REGULATION OF T AND B CELL HOMEOSTASIS

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Background: Interleukin (IL)-21 is a pro-inflammatory cytokine produced by T helper cell (Th) 17 that affects a broad range of cells and is a critical regulator of Th17 development. Several autoimmune diseases accompanied by high levels of IL-21 suggest that blocking the IL-21 signal might ameliorate autoimmune symptoms. Increased IL-21 leads to progression of B cell-mediated autoimmune diseases with rapidly induced cell proliferation, plasma cell differentiation and immunoglobulin production during the response to antigens and cognate helper T cells. Thus, IL-21 plays a key role as a cytokine modulator between T- and B cell for immune homeostasis. Recent reports have also showed that inhibiting IL-21 also decreases disease severity in murine models of acute graft-*versus*-host disease (GVHD). Specifically, disruption of IL-21 signaling, either genetically or via neutralizing mAbs, reduces transplant-related weight loss, tissue pathology, and mortality. Although murine models have indicated that IL-21 blockade is an attractive strategy to reduce GVHD-associated injuries, these studies have not elucidated the molecular mechanisms affecting both T and B cell responses.
Aims: Interleukin (IL)-21 plays a key role in the development of acute graft-*versus*-host diseases (GVHD) after allogeneic bone marrow transplantation (allo-BMT). Therapeutic manipulation of IL-21 activity may improve acute GVHD in early-post-transplant period. We investigated the mechanisms regulating T- and B cells during IL-21 blockade in acute GVHD.
Methods: Recipient mice (BALB/C, H-2K^d) were irradiated with 800 cGy and injected i.v 5x10^6 BM and 5x10^6 spleen cells from donor IL-21$^{-/-}$ and WT mice (C57BL/6, H-2K^b). Survival after BMT was monitored daily, and the degree of clinical GVHD was assessed weekly using a scoring system. Post-transplant immunologic changes were evaluated by serum level of proinflammatory cytokines, real-time PCR of transcriptional factors, immunophenotyping using FACS and confocal examination and Western Blotting.
Results: IL-21 blockade enhanced Treg and Th2 cell differentiation, while inhibiting Th1- and Th17-derived transcription factors and cytokines as a modulator of activated T cells. IL-21$^{-/-}$ cell recipients had increased mature B, marginal zone B and decreased memory B, germinal center formation and plasma cells that did not lead to immunoglobulin production. B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) are involved in the induction and maintenance of T- and B-cell responses. We observed decreased levels of only BAFF during acute GVHD and confirmed that this regulation was through BAFF-receptor (BAFF-R) as mammalian target of rapamycin complex 1 (mTORC1) in IL-21 blockade.
Summary and Conclusions: Therefore, this study suggested that IL-21 blockade effects modulate activated T cell as well as B cell homeostasis via BAFF pathway-mediated inhibition in acute GVHD following murine allo-BMT.

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IDENTIFYING NOVEL REGULATORS HEMATOPOIETIC STEM CELL EXPANSION *EX VIVO*

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Background: Maintenance of hematopoietic stem cell (HSC) self-renewal *ex vivo* remains a major challenge in order to exploit sources with limited numbers of HSCs, such as umbilical cord blood, clinically. Self-renewal is a unique attribute of stem cells to maintain the pool of stem cells during cell division. During asymmetrical divisions, one cell retains multi(pluri)potency, while expansion of the stem cell pool is the result of symmetrical divisions. Both intrinsic and extrinsic factors can influence the self-renewal capability of HSC. During embryogenesis, in particular in the fetal liver, HSCs not only give rise to mature progeny but also symmetrically expand (self-renewal) their number in order to create an adequate pool of HSC be adequate for post-natal life. Fetal liver is the organ where expansion of definitive hematopoietic stem cell pool occurs.

Aims: Considering the importance of hematopoietic stem cells from a clinical standpoint, understanding the biology of HSCs and their microenvironment, during early developmental stages would enable us to find out novel intrinsic and extrinsic regulators in the process of migration, homing and expansion (self-renewal).
Results: Here, we performed genome-wide transciptome analysis of murine fetal liver and postnatal bone marrow derived HSC and the murine fetal liver niche (at e12.5-16.5) to identify novel factors involved in symmetrical divisions and hence expansion of HSC using RNA-Seq. Transcriptional regulators were shortlisted after comparing HSC isolated from e14 fetal liver and adult bone marrow. The role of these intrinsic regulators in hematopoiesis is being screened by morpholino knockdown studies, and subsequent RT-PCR using Gata-1(dsred)/Flk(GFP) transgenic Zebrafish lines. Cell extrinsic factors identified by RNAseq from laser-captured microdissection cells that surround Lin-/CD11b+/Sca1+ cells (expressed in e14 fetal liver but not either in e12.5 or e16.5 fetal liver) for which receptors were found on HSC were also shortlisted. The extrinsic regulators are being screened for their role in maintenance of HSC self-renewal using an *ex vivo* culture system wherein the identified secreted molecules are added.
Summary and Conclusions: The ongoing functional validation of transcriptional regulators in HSC and fetal liver HSC niche extrinsic factors will provide novel insights in mechanisms that support symmetrical selfrenewal of HSCs, which may be of great interest for clinical HSC transplantation, gene therapy, or creation of mature blood cells from stem cells.

P482

RECIPIENT BATF3 DEPENDENT DENDRITIC CELLS SUPPRESS ACUTE GRAFT-*VERSUS*-HOST DISEASE AFTER EXPERIMENTAL ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background: Graft-*versus*-host disease (GVHD) is a frequent life-threatening complication following allogeneic haematopoietic stem cell transplantation (HSCT) and induced by donor derived T cells that become activated by host antigen presenting cells.
Aims: The aim of our study was to assess the relevance of host dendritic cell (DC) populations in the initiation of acute GVHD.
Methods: We used three transgenic mouse strains deficient of $CD11c^+$ DC populations in a mouse model of acute GVHD where bone marrow and T cells from BALB/c donors are transplanted into C57BL/6 or CD11c-DTA, CD11c-iDTR or *Batf3* deficient (*Batf3*$^{-/-}$) hosts. DC phenotypes post HSCT were characterized by flow cytometry and *in vivo* cytokine production by quantitative real-time PCR. ^{3}H-thymidin uptake was used to assess T-cell proliferation in allogeneic MLR.
Results: Surprisingly a strong increase in GVHD related mortality was observed in the absence of $CD11c^+$ cells. Likewise *Batf3*$^{-/-}$ mice that lack $CD8\alpha^+$ DCs also displayed a strongly increased GVHD related mortality. In the absence of $CD8\alpha^+$ DCs, we detected an increased activation of the remaining DCs post HSCT, leading to an enhanced priming of allogeneic T cells. Importantly, this was associated with reduced numbers of regulatory T (Treg) cells and TGF-β levels indicating a failure of peripheral tolerance mechanisms after HSCT in the absence of $CD8\alpha^+$ DCs.
Summary and Conclusions: Our results indicate for a critical role of $CD8\alpha^+$ DCs as important inducers of Treg mediated tolerance to control DC activation and T-cell priming in the initiation phase of GVHD.

P483

DISRUPTED TOLL-LIKE RECEPTOR SIGNALING OF INTESTINAL EPITHELIUM IN INTESTINAL GRAFT-*VERSUS*-HOST DISEASE

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Background: The intestine is preferentially damaged in acute graft-*versus*-graft disease (aGVHD). Patients with intestinal GVHD are usually associated with drug-resistant diarrhea and microflora disturbance. Recent studies suggest that toll-like receptor (TLR) signaling can protect the intestinal epithelial barrier and confer commensal tolerance in health. But less is known about how functional *versus* dysfunctional TLR pathway opposes or favours the intestinal GVHD.
Aims: To identify the role of TLR signaling played in the intestinal GVHD.
Methods: In the current study, BALB/c mice were transplanted whole spleen and T cell deleted (TCD) bone marrow cells from C57BL/6 mice as GVHD group, and transplanted TCD bone marrow cells as control group. The jejunum, ileum, colon and rectum epithelium were harvested and total RNA were extracted in two groups. The mRNA expression of classical TLR pathway TLRx/MYD88/IRAK4 signaling molecules (TLR2, TLR4, MYD88, IRAK4 and Tollip) and cytokines (IFN-γ, TNF-α and TGF-β) were detected by RT-PCR.
Results: The intestine of aGVHD recipients showed severe mucosal edema and erythema with histologic changes of apoptotic epithelial cells and crypt cell dropout, while the intestine of recipients in the control group did not show any intestinal GVHD evidence. TLR2 expression was markedly down-regulated and little TLR4 expression was observed in GVHD intestinal epithelium in comparison to control group. MYD88 and IRAK4 expression were lower in the entire GVHD damaged intestine but only significant in colon and rectum between the two groups. Tollip, a TLR signaling inhibitor by interfering IRAK, was found much higher in the GVHD group. For cytokines, both of IFN-γ and TNF-α expression were markedly up-regulated from proximal to distal intestine in GVHD group as compared to control group. There was no difference in TGF-β expression between the two groups.
Summary and Conclusions: We propose TLR signaling in the intestinal epithelium, especially in colon and rectum, presents disruption in intestinal graft-*versus*-host disease. IFN-γ and TNF-α might contribute to accelerate TLR pathway alteration.

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CYCLOSPORIN A DOES NOT IMPAIR EARLY GRANULOCYTE EFFECTOR FUNCTION

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Background: Severe infections with A*spergillus fumigatus* play a relevant role in immunodeficient patients after myelo-affecting chemotherapy or under long-term immunosuppressive medication, *e.g.* in allogeneic hematopoietic stem cell or solid organ transplantation. In these patients prolonged neutropenia is a major risk factor for fungal infections indicating an essential role of innate immune system in antifungal defense. Data from previous studies suggest that NFAT is a key player in signaling pathways of innate immunity e. g. in polymorphonuclear neutrophils (PMN) and that it can be modulated by commonly used agents like cyclosporin A (CsA).
Aims: The aim of our work is to elucidate the relevance of NFAT-dependent activation signals in PMN whether PMN effector functions are influenced by calcineurin/NFAT inhibitors and if NFAT-dependent signals are essential in immune responses against *Aspergillus fumigatus*.
Methods: To address the above mentioned questions we firstly performed *in vitro* experiments using human (healthy donors) PMN after purification and from whole blood regarding their effector functions in absence or presence of CsA in titrated doses appropriate to therapeutic levels. Phagocytosis and activation-induced shedding of CD62L was measured by flow cytometry using polychromatic microspheres and appropriate surface markers (CD11b-PB, CD62L-APC, CD66b-FITC). Generation of reactive oxygen species was analyzed by dichlorofluorescein assay (DCF) and activation-induced synthesis of IL-8 by enzyme-linked immunosorbent assay (ELISA) and intracellular flow cytometry. Secondly, we applied a murine *Aspergillus fumigatus* pneumonia model to study the antifungal innate immune response *in vivo* with or without intraperitoneal administration of CsA (18mg/kg, appropriate to a serum level of 600ng/ml). Here, PMN recruitment to the lungs, pulmonary clearance of *Aspergillus fumigatus* and survival of infected mice was examined. PMN-depleted mice using an murine Gr1-antibody served as controls.
Results: Administration of CsA had no significant influence on human granulocyte expression of activation markers and shedding of CD62L. Moreover, additional functional testing indicated also no substantial influence on generation of reactive oxygen species (5245 RFU +/- 354 *vs.* 5763 +/- 520 (control) after stimulation with LPS, mean +/- SEM) compared to controls. Likewise the activation-induced synthesis of IL-8 was not reduced in presence of CsA *in vitro* (519pg/ml +/- 81 *vs.* 463 +/- 131 (control) after stimulation with LPS). In contrast, phagocytosis was rather enhanced in presence of CsA (83.5% +/- 1.7 *vs.* 71.0 +/- 1.5 (control) after stimulation with LPS). Regarding survival of *Aspergillus fumigatus* infected mice, successful granulocyte-dependent clearance of fungal infection was not impaired in relevant degree through CsA medication.
Summary and Conclusions: Early granulocyte-based innate immune function is not inhibited by CsA, suggesting that NFAT may play a more crucial role in granulocyte differentiation under steady state conditions. Therefore, we are planning *in vivo* experiments with long-term CsA-pretreated mice and also *ex vivo* analysis of patient blood samples under continuous CsA medication. Finally, we expect our results to lead to a deeper understanding of innate immune response after fungal infection and will therefore contribute to improved therapeutic options for immunodeficient patients.

P485

RECONSTITUTION OF GPI-ANCHOR-NEGATIVE NK CELL POPULATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING ALEMTUZUMAB-BASED CONDITIONING

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Background: The anti CD52-antibody Alemtuzumab is used for T cell depletion (TCD) in the context of allogeneic hematopoietic stem cell transplantation (HSCT). We already showed long-term persistence of CD52-/Glykosylphosphatidyl-Inositol (GPI)-negative T cells with impaired antiviral function in patients after HSCT. In addition, we observed a correlation between the persistence of GPI^{neg} regulatory T cells with impaired immunosuppressive capacity and severe acute GvHD.
Aims: Since NK cells influence antitumor reactivity, antiviral defense and GvHD, in this study, we analyzed GPI expression on natural killer (NK) cells of patients undergoing HSCT.
Methods: We examined peripheral blood samples of 12 patients after HSCT following reduced intensity conditioning including Alemtuzmab. By using multicolor FACS-analysis, we phenotypically distinguished the NK cell subpopulations $CD56^{bright}CD16^{dim}$, $CD56^{dim}CD16^{bright}$, $CD56^{dim}CD16^{int}$ and $CD56^{dim}CD16^{neg}$ at d+60 and d+365 after HSCT. GPI^{neg} NK cell subsets were characterized regarding their expression of specific surface antigens involved in cytotoxicity (NKp44, NKp46, NKG2A and NKG2D), maturation and activation (CD62L, CD69). CMV-reactivity was investigated by IFNγ ELISPOT-assay in two patients.
Results: Early after HSCT, we found $CD56^{dim}CD16^{int}$, to be the major NK cell population, at d+356 $CD56^{dim}CD16^{bright}$, becomes, the most prominent population. At d+60, $CD56^{dim}CD16^{bright}$, and $CD56^{bright}CD16^{dim}$ showed 70-90% of GPI^{neg} cells, which decreased over time. $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$ NK cells were mostly GPI^{pos}. Independent from GPI expression NKp46 was positive in the $CD56^{dim}CD16^{bright}$, / $CD56^{bright}CD16^{dim}$ populations. In contrast, $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$, NK cells remained, NKp46 negative at d+60. Early after HSCT, all NK cell subsets lacked NKG2D, whereas NKG2A expression remained stable over time. Interestingly, NKp44 was only observed on GPI^{pos} NK cells, and its expression increased late after HSCT, especially in $CD56^{dim}CD16^{int}$, NK cell populations. The activation marker CD69 was mainly expressed on $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$. CD62L was prominent in $CD56^{dim}CD16^{neg}$ and $CD56^{dim}CD16^{int}$ early after HSCT,, but increased in the $CD56^{dim}CD16^{bright}$/$CD56^{bright}CD16^{dim}$ NK cell population at d+365. Remarkably, we observed the highest frequency of CMV-specific IFN-γ production by NK cells early after transplantation in the absence of T cells.
Summary and Conclusions: We previously showed the reconstitution of functionally impaired GPI^{neg} T cells which may be partly responsible for some of the viral complications after Alemtuzumab-based conditioning. In this context, NK cells play a major role especially early after HSCT. Here we provide for the first time data on a relevant percentage of GPI^{neg} NK cells and their specific subset distribution over time. We observed many NK cells with $CD56^{dim}CD16^{int}$ phenotype early after HSCT, whereas later $CD56^{dim}CD16^{bright}$, NK cells were dominant comparable to the natural NK distribution in healthy donors. Depended on the time after HSCT, the expression of GPI-anchors and natural cytotoxicity receptors, maturation- and activation markers varied. These alterations may impact the capacity of NK cell mediated immune responses depending on the GPI-anchors itself. Further investigations on different NK subsets and their specific function will help to identify patients with a need for specific cellular therapies such as NK cell based DLI.

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INDUCTION OF MIXED CHIMERISM USING COMBINATORY CELL-BASED IMMUNE MODULATION WITH MESENCHYMAL STEM CELLS (MSCS) AND REGULATORY T CELLS (TREGS) FOR SOLID ORGAN TRANSPLANTAION

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Background: Establishment of mixed chimerism is an ideal approach to induce donor-specific tolerance expanding its potential in various clinical settings. Despite the developments in partial conditioning regimens, improvements are still needed in reducing toxicity and BMT related complications. Recently, cell-based therapies including mesenchymal stem cells (MSCs) have been incorporated in establishing noncytoreductive mixed chimerism protocols; however, its efficacy is only partial and show reversed immunosuppressive properties.
Aims: We hypothesize that the interaction between MSCs and Tregs may lead to greater inhibition of host immune responses. We investigated the effects of cell-based immune modulation (CCIM) of MSCs and Tregs with a low-intensity conditioning regimen and achieved persistent mixed chimerism and tolerance in an MHC-mismatched transplantation mouse model.
Methods: Recipient mice underwent total-body irradiation (TBI) at a dose of 150 cGy 3 days before BMT, followed by intravenous infusion of 3×10E7 donor BM cells on day -2 and cyclophosphamide (CY, 100 mg/kg) on day -1. On the day of BMT, recipient mice received TCD BM cells or total BM cells. On days +1 and +3 following BMT, they received combinatory cell-based immune modulation of Tregs (2×10E6) and MSCs (2×10E6). To establish GVHD, recipients were lethally irradiated with 750 cGy 1 day before injected 2×10E7 donor BM cells on day 0. On days +1, +10, +16 and +23, recipient mice received MSC alone or combination of MSC and Treg. To assess immune function *in vivo*, full-thickness skin grafts were transplanted from allogeneic mice and syngeneic mice to the dorsa of the recipient mice. All animals were monitored for clinical signs, histopathologic changes, post-transplant immunologic changes, the degree of mixed chimerism, and donor-specific tolerance.
Results: Compared to single cell therapy, CCIM induced a higher engraftment rate and robust donor-specific tolerance to skin allografts across full MHC barriers. These regulatory effects were associated with inhibition of NK cell cytotoxic activity, CD4+IL-17+ cells, memory B cells, plasma cells and immunoglobulin production levels with increased frequencies of CD4+Foxp3+ cells, IL-10-producing mature B cells and myeloid-derived suppressor cells (MDSC). Furthermore, CCIM was able to regulate mortality in a GVHD model through reciprocal regulation of Treg/Th17.
Summary and Conclusions: Taken together, we suggest combinatory cell-based immune modulation (CCIM) of MSCs and regulatory T cells (Tregs) as a clinically applicable strategy for facilitating the induction of mixed chimerism and permanent tolerance for solid organ transplantation.

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ACTIVATED MONOCYTE-PHAGOCYTES PROBABLY INITIATE THE ONSET OF PRE-ENGRAFTMENT SYNDROME (PES) FOLLOWING UMBILICAL CORD BLOOD TRANSPLANTATION

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Background: Cord blood transplantation (CBT) is being increasingly used for treatment of hematological malignancies and showed many advantages. The immaturity of T cell contained in the graft and the T cell reconstitution is later than bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT), but the relapse rate after CBT to be comparable to that BMT or PBSCT. Our clinical analysis revealed the outcome of CBT for high risk acute leukemia relapse after HLA-mismatched CBT was lower than that BMT or PBSCT, but the incidence of graft-*versus* host disease (GVHD) was similar. Pre-engraftment syndrome seems to be a distinct clinical syndrome from engraftment syndrome, which developed usually after cord blood transplantation. PES was defined as unexplained fever >38.3C not associated with infection and unresponsive to antimicrobials, and/or diarrhea, jaundice, and weight gain which occurred before neutrophil recovery. If only the PES patients have simple fever and rash, just the right amount of methyl prednisolone treatment can make the symptoms disappear, but some patients is not sensitive to hormone therapy can accentuate illness. Because when PES occurs the white blood cell number of transplantation patient is extremely low, so they can easy get high fever with the serious complications such as hemorrhage, which may threaten transplant patients life and UCBT success rate. The pathogenesis of PES is not clear. It has been speculated that cytokines induced by the initial immune/inflammation reaction are the primary cause of PES, but no data are available to confirm this supposition. Proteomic patterns have been studied in the fields of transplantation, but no specific marker has been identified also. Our prior studies suggest monocyte-phagocytes from the donor initiate the cause of PES.
Aims: To explore the main effective cells and the pathogenesis of pre-engraftment syndrome (PES) in patients with hematological malignancies after umbilical cord blood transplantation
Methods: We selected 26 patients with PES and 6 patients without PES as control; they were all treated with umbilical cord blood transplantation after myeloablative conditioning regimens. We collected the serum samples before transplantation, PES and after PES been controlled and detected the cytokines related to monocyte-macrophages, T cells and NK cells by multicolor flow cytometry or /and ELISA. The miRNA sequencing was performed on Illumine HiSeq 2000 using TruSeq Rapid SBS Kits. The target genes may be predicted according to the differentially expressed miRNA then analyze GO and pathway by comparing the database of mirbase, miranda, targetscan. Data are summarized as mean±SD. Student t test was used to determine whether there was a statistically significant difference between samples, with two-tailed P values less than 0.05 indicating a significant difference.
Results: 1) The cytokines of IL-1B, IL-6,IL-18 and MCP-1 that related to monocyte-macrophages increased significantly in the 26 CBT patients with PES

compared to before transplantation or the same period of six patients without PES($p<0.01$). Those cytokines declined to the level prior to the occurrence of the PES when the symptom controlled, but neither the cytokines of Th1/Th2/Th17 nor NK had no significant difference. The levels of IL-1b, IL-6, TNF-a and IL-17 in stool of two patients with PES were significantly higher than the peripheral blood. 2) There were no significant difference between PES and the control groups in the levels of serum complement C3a, C4a, C5a ($p>0.05$).3) There were no significant difference between PES and the control groups in the proportion of peripheral blood T or NK cells and the expression of CD11b, CD27, CD57 on NK cells($p>0.05$) on 14 days. 4) We collected nine serum samples for miRNA sequencing in three patients who treated by single umbilical cord blood transplantation at the time of before, occurrence and symptom of PES controlled. The results showed 39 differentially expressed miRNA such as hsa-let-7i-5p, hsa-mir-98-5p, hsa-mir-152-3p, hsa-mir-223-3p and so on. It could be deduced three signaling pathways related to active the monocyte-phagocytes such as TOLL,NOD and RIG-I-like receptor by predicting the target genes +GO+Pathway analysis when development of PES.

Summary and Conclusions: Our preliminary finding confirmed that the monocyte-phagocytes are the effective cells of PES. They are activated by Toll, NOD or RIG-I-like receptor signaling pathway and produce immunological functions. This will provide a reliable experimental basis for the further research of pathogenesis of PES.

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INFLUENCE OF HLA-G POLYMORPHISM ON VIRAL REACTIVATION AND OVERALL SURVIVAL AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Human leukocyte antigen-G (HLA-G) is a non-classical class Ib HLA molecule characterized by tolerogenic role widely studied *in vitro*: inhibition of T CD4+ alloproliferative response, inhibition and apoptosis of NK and CD8+ T cells, inhibition of APC, induction of immunosuppressive cells.

The HLA-G expression is regulated by a deletion(del)/insertion(ins) polymorphism of 14 base pair (bp) at the 3' untranslated region of exon 8. The 14bp-insertion is associated with a decreased mRNA stability and a lower HLA-G protein expression.

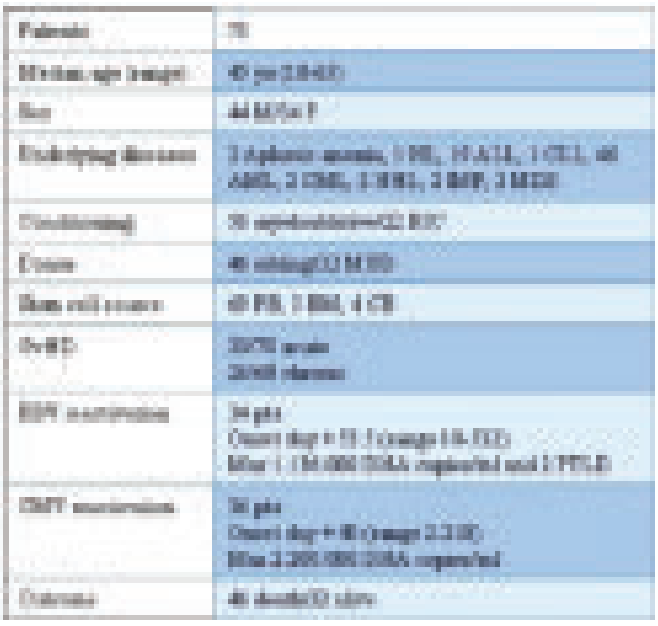
Aims: We analyzed the possible role of HLA-G polymorphism in the setting of stem cell transplantation (SCT), in terms of Graft-*versus*-host disease (GvHD), viral reactivation, OS and DFS.

Methods: We studied retrospectively 78 patients (pts) (Table 1), 44M/34F, with a median age of 45 years (range 10-65) underwent SCT. The underlying diseases were: 2 Aplastic anemia, 1 HL, 19 ALL, 1 CLL, 46 AML, 2 CML, 2 NHL, 3 IMF, 2 MDS. A myeloablative SCT was performed in 56 pts, while 22 pts received a reduced intensity conditioning. GvHD prophylaxis was obtained with Cyclosporine A and short course of methotrexate. Stem cell source was BM in 5 pts, CB in 4 pts and PB in 69 pts, with a median dose of CD34+ of $6.83*10^6$/Kg (range 1.2-19.7). Donor was sibling in 46 cases and MUD in the other 32 cases. HLA-G polymorphism was determined on genomic DNA extracted by donor and recipient samples before transplant, amplified by standard PCR and visualized on agarose gel electrophoresis. Data were analysed using IBM SPSS Statistics 20 Core System.

Results: No correlation was found between HLA-G and GvHD or DFS. Surprisingly, HLA-G polymorphism showed a relationship with viral reactivation after SCT. After SCT, 14 pts showed a CMV reactivation, 11 pts an EBV reactivation and 21 pts revealed both them. In the setting of CMV reactivation, viral replication achieve a maximum load of 2.200.000 copies/ml, while EBV reactivation reached a maximum of 1.136.000 genome copies/ml. Furthermore, two pts developed EBV-related post-transplant lymphoproliferative disease without circulating viremia. In pts receiving SCT from ins/ins donor, EBV reactivation was detected after a median of 41 days (range 10-102) compared to 64 days (range 16-532) in pts transplanted by ins/del or del/del donor ($p=0.034$). Moreover in the setting of CMV reactivation after SCT, the maximum viral copies number reached by pts receiving stem cells from del/del donor was lower than viral copies titer detected in pts transplanted by ins/del and ins/ins donor, 4140 genome copies/ml (range 1040-2200000) and 10.000 copies/ml (range 6000-331180) respectively ($p=0.0366$). Donor and recipient del/del polymorphism was also associated with a prolonged OS both in terms of TRM (toxicity, MOF, GvHD, infections) and relapse mortality. Median OS was 18 months for del/del pts, 10 months for ins/del pts and 6 months for ins/ins pts ($p=0.023$), while it was 22 months for del/del donor, 9 months for ins/del donor and 6 months for ins/ins donor ($p=0.011$).

Summary and Conclusions: These data highlight that tolerogenic power of HLA-G might play a role in viral infection control after SCT. Particularly, ins/ins donor polymorphism associated with a decreased level of soluble HLA-G molecule, allows earlier viral reactivation (EBV) and a broad viral replication (CMV) after SCT.

Table 1. Patients characteristics.



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MYELOID DERIVED SUPPRESSOR CELLS ARE MOBILIZED BY G-CSF IN MICE AND MEN

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Background: In solid cancers, the frequency of Myeloid derived suppressor cells (MDSCs) correlates with tumor stage. Progressive disease has been associated with MDSC function as MDSCs are believed to mediate tumor escape from T-cell–mediated immunosurveillance. The relevance of this population in the context of allogeneic hematopoietic stem cell transplantation (HSCT), Graft-*versus*-Host Disease (GvHD), and Graft-*versus*-Leukemia effect (GvL) is largely unknown.

Aims: Therefore, we determined the frequency of MDSC in bone marrow (BM) and leukapheresis products (LPHs) collected for HSCT.

Methods: MDSCs were defined as monocyte-like cells with a lack or low expression of HLA-DR ($CD14^+HLA\text{-}DR^{low/-}$). We quantified MDSCs in the peripheral blood (PB), LPH and BM of healthy volunteer donors by multi-color flow cytometry. For routine diagnostic purposes, we compared leukocyte isolation by density gradient centrifugation (DGC) and red blood cell lysis (RBC-lysis). PB was drawn before (PB) and after receiving G-CSF at 7.5µg/kg/day for 5 days (PB+G-CSF). LPH and BM samples were obtained at the day of donation. All samples were prepared and stained with a panel of antibodies within 24 hours after donation. In C57BL/6J mice, monocytic (CD11b/Ly6C) MDSCs frequency in PB was analyzed before and after subcutaneous application of GCSF (250µg/kg for 6 consecutive days). Functionality of MDSCs was examined using an in-vitro proliferation suppression assay of autologous T-cells.

Results: The frequency of MDSCs in PB of human donors was lower than in BM, but we observed a significant increase of MDSCs in PB upon G-CSF treatment (PB: 29±16%; BM: 41±16%; PB+G-CSF: 44±18%; LPH: 52±14% of CD14+), while relative CD14+HLA-DR+ and CD14+ cell counts remained unchanged. However, the largest number of MDSCs was found in LPHs (PB *vs.* PB+GCSF $p<0.05$; PB *vs.* LPH $p<0.001$; Figure 1). Consistently, G-CSF stimulation in mice resulted in a significant increase of the MDSC frequency in PB (monocytic MDSC: 3.7±0.9% of total leucocytes). In contrast to MDSCs isolated from PB, PB+G-CSF and LPH, MDSCs from BM did not inhibit the proliferation of stimulated autologous T-cells. RBC-lysis shows comparable MDSC frequencies to DGC in PB, PB+G-CSF, LPH and BM, respectively. As RBC-lysis allows a faster processing of samples, this method might represent a more useful technique in routine diagnostics.

Summary and Conclusions: In healthy volunteers, the frequency of phenotypically defined MDSCs was higher in BM compared to PB, but G-CSF treat-

ment led to a significant increase in MDSCs in PB, which was reproduced in the murine system. Finally, the leukapheresis procedure led to a further enrichment of MDSCs in the grafts. However, phenotype did not necessarily predict functionality, as MDSCs isolated from BM did not show any suppressive capacity in an in-vitro proliferation assay. The consequences of these findings on engraftment, GvHD and GvL after allogeneic HSCT remain to be elucidated.

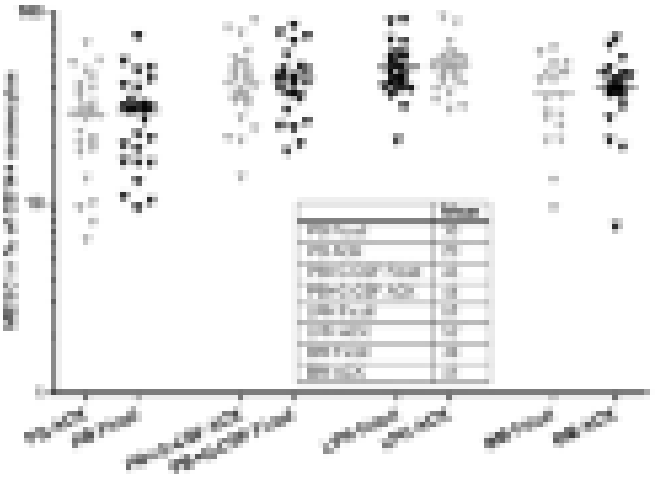

Figure 1.

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GRANULOCYTE COLONY-STIMULATING FACTOR MOBILIZATION AFFECTS THE EXPRESSION OF TH1/TH2 CHEMOKINES AND THEIR RECEPTORS

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Background: Granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cell (PBSC) has been used more frequently than bone marrow as the source of stem cells in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although it contains more mature T cells, neither the incidence nor the severity of acute graft-*versus*-host disease (aGVHD) is higher compared with bone marrow transplantation. This might be due to the immunoregulatory effects of G-CSF on adaptive immunity, including that G-CSF directly modulated via its receptor on T cells or indirectly modulated T cell immune responses via effector cells and cytokines. However, these mechanisms are not fully understood, and whether G-CSF could influence the expression of Th1/Th2 chemokines and their receptors remains unknown.
Aims: To investigate the effect of G-CSF mobilization on the expression of Th1/Th2 chemokines and their receptors.
Methods: The expression levels of Th1 chemokines (CXCL9, CXCL10, and CXCL11), Th2 chemokines (CCL17, CCL22) and their receptors CXCR3 and CCR4 were analyzed in peripheral blood mononuclear cells (PBMCs) from 25 donors before and after G-CSF mobilization, using real-time RT-PCR with SYBR Green-staining. The β_2-microglobulin gene (β_2-MG) was used as an endogenous reference, and the relative mRNA expression level of each gene was evaluated by the $2^{-\Delta C}$, ^{t}×100% method.
Results: The median expression level of CXCR3 was similar before and after G-CSF mobilization (0.1426% and 0.1109%) (P=0.278), while the expression level of CCR4 after G-CSF mobilization (0.0985%) was significantly lower than that before mobilization (0.1415%) (P=0.039). The median expression levels of CXCL9, CXCL10, and CXCL11 genes before mobilization (0.0048%, 0.0576% and 0.0079%) were not significantly different from that after G-CSF mobilization (0.0143%, 0.0666% and 0.0088%)(P=0.086, P=0.535 and P=0.680). The median expression levels of CCL17 and CCL22 were also similar before and after G-CSF mobilization (P=0.155, P=0.476). The expression pattern of three Th1 chemokines before mobilization was CXCL10>CXCL11>CXCL9, whereas it changed to CXCL10>CXCL9>CXCL11 after mobilization. The expression pattern of two Th2 chemokines before mobilization was CCL17>CCL22, whereas it changed to CCL22>CCL17 after mobilization. The relative expression levels of CXCL10 and CXCL11 genes before mobilization both showed a positive correlation to that after mobilization (P<0.001, r=0.760; P=0.024, r=0.470). Before mobilization, significant positive correlation was observed between the expression levels of CXCL9 and CXCL10, CXCL11 and CXCR3, CXCL11 and CCL22, CXCR3 and CCL22 (P<0.001, r=0.902; P=0.003, r=0.584; P=0.022, r=0.473; P<0.001, r=0.674, respectively). After G-CSF mobilization, significant positive correlation was observed between the expression levels of CXCL9 and CCL22, CXCL10 and CXCL11, CXCR3 and CCR4, CXCR3 and CCL22, CXCR3 and CCL17, CCL22 and CCL17 (P=0.001, r=0.653; P=0.001, r=0.665; P=0.002, r=0.602; P=0.028, r=0.458; P<0.001, r=0.738; P=0.044, r=0.424, respectively).
Summary and Conclusions: G-CSF mobilization might mainly influence the expression level of CCR4 genes in Th1/Th2 chemokines and their receptors. The expression patterns of Th1 chemokines and Th2 chemokines might both change after G-CSF mobilization.

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GRANULOCYTE COLONY-STIMULATING FACTOR MOBILIZATION AFFECTS THE EXPRESSION OF REGULATORY GAMMA DELTA T CELLS

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Background: The immune modulatory effect of granulocyte colony-stimulating factor (G-CSF) on T cells resulted in an unexpected low incidence of graft-*versus*-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation. Our previous studies demonstrated that G-CSF mobilization influenced the distribution and clonality of TRGV and TRDV repertoire (T cell receptors of γδ, T cells), and significant positive correlation was observed between the invariable clonality of TRDV1 gene repertoire after G-CSF mobilization and low incidence of GVHD in recipients (P=0.015, OR=0.047) (Li Xuan *et al.* Journal of Translational Medicine 2011). Regulatory γδ T cells (γδ Tregs), which express Foxp3 and primarily belong to $CD27^{+}CD25^{high}$, phenotype, are a novel subset of cells with immunosuppressive function (Xiaoyan Li *et al.* Journal of Immunology 2012). However, whether G-CSF could influence the expression of γδ Tregs remains unknown.
Aims: To investigate the effect of G-CSF mobilization on the expression of γδ Tregs.
Methods: The immunophenotyping of γδ Tregs was analyzed in peripheral blood mononuclear cells (PBMCs) from 20 donors before and after G-CSF mobilization, using flow cytometry.
Results: Compared with that before mobilization, the Vδ1 proportion was significantly increased (*P*=0.001), whereas the proportions of total γδ T cells and Vδ2 subsets were significantly decreased after G-CSF mobilization (*P*=0.011, *P*=0.005). The proportions of $CD25^{+}$ and $CD27^{+}$ subsets were also significantly increased after mobilization (*P*=0.006, *P*=0.015), and the proportion of $Foxp3^{+}$ subset was similar between the two groups (*P*=0.768). In addition, there was a significant increase in the proportions of $Foxp3^{+}$Vδ1, $CD27^{+}$Vδ1, $CD25^{+}Foxp3^{+}$, and $CD25^{+}CD27^{+}$, subsets (*P*=0.008, *P*=0.007, *P*=0.011, *P*=0.014), and a significant decrease in the proportions of $CD27^{+}$Vδ2 and $CD25^{+}$Vδ2 subsets after mobilization (*P*=0.021, *P*=0.007). We then compared the Foxp3, CD27 and CD25 phenotypes in total γδ T cells, Vδ1 and Vδ2 subsets. We observed a significant increase in the proportions of $CD27^{+}Foxp3^{+}$, Vδ1, $CD25^{+}Foxp3^{+}$, Vδ1 and $CD25^{+}CD27^{+}$Vδ1 subsets after G-CSF mobilization (*P*=0.005, *P*=0.024, *P*=0.040, respectively).
Summary and Conclusions: G-CSF mobilization significantly increased the proportions of Vδ1 subsets, including $Foxp3^{+}$Vδ1, $CD27^{+}Foxp3^{+}$, Vδ1 and $CD25^{+}Foxp3^{+}$, Vδ1 subsets, whereas decreased the Vδ2 proportion.

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USE OF UNRELATED DONORS IN ELDERLY PATIENTS (AGE >60 YEARS) UNDERGOING REDUCED-INTENSITY CONDITIONING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

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Background: We retrospectively analyzed outcomes in 62 consecutive hematologic malignancy patients aged >or =60 years (median, 62 years; range: 60-70 years) undergoing reduced intensity conditioning regimens (RIC) from URD. In this study, URD was used only when a MRD was not available.
Then we compared the outcome of 17 elderly patients (age >65 years) with 44 younger patients aged between 60 and 65 years.
Aims: The lower morbidity and mortality of reduced-intensity conditioning (RIC) regimens have allowed allogeneic hematopoietic cell transplantation (HCT) in older patients. However, there are only limited data on the feasibility and outcomes of URD HCT in elderly patients.The aim of the study was to compare the outcome in OS and PFS for patients transplanted using unrelated donor (URD) in patients age 60 or older.
Results: No patients experienced graft rejection. The median HCT comorbidity index score was 2 (range, 0 to 6). With a median follow up of 36 months (range, 5-74), the cumulative incidence of grades II to IV acute GVHD was 28% and of grades III to IV acute GVHD, 13%. At 2 years, the cumulative incidence of chronic GVHD was 27%, progression-free survival (PFS) was 62%, overall

survival (OS) was 63%, and relapse was 14%. Non relapse mortality (NRM) was 24% at 2 years.
The cumulative incidence of grade II–IV Acute GVHD was 43% for the younger group and 17% for the older group (P=0.056). The cumulative incidence of chronic GVHD was not different between the two groups (23% *vs.* 45% (p=0.3), respectively). Two-year OS and PFS was 57% *versus* 86% (P=0.059) and 55% *versus* 86% (P=0.03), in the younger and the older group respectively. The 2-year NRM and relapse was 26% *versus* 14% (P=0.4) and 19% *versus* 0% (P=0.04), in the younger and older group respectively.
Summary and Conclusions: This retrospective study suggest that RIC HCT from URD is a safe and effective option for patients aged >or =60 years or older, and in the absence of suitable related donors, well-matched URD may offer a very reasonable alternative, and that does not appear to be associated with a detrimental outcome. However these results are encouraging showing once again that with an adequate selection, age is not a definitive limitation.

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MICAFUNGIN *VERSUS* FLUCONAZOLE OR ITRACONAZOLE FOR PROPHYLAXIS AGAINST INVASIVE FUNGAL INFECTIONS DURING NEUTROPENIA IN PATIENTS UNDERGOING HAPLO-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Over the past decade, invasive fungal infections (IFI) have remained an important problem in patients undergoing hematopoietic stem cell transplantation (HSCT). The optimal approach for prophylactic antifungal therapy has yet to be determined.
Aims: we conducted a retrospective, bi-institutional comparative clinical study, (Institut Paoli-Calmettes at Marseille France and Humanitas cancer center at Rozzano, Italy), and we compared the efficacy and safety of Micafungin 50mg/day (iv) with those of fluconazole (400mg/day) or itraconazole 200mg/day (iv) as prophylaxis for adult patients with various haematological diseases receiving haplo-identical allogeneic stem cell transplantation (haplo-SCT). Patients received prophylaxis with the beginning of the transplant conditioning regimen until the hospital discharge, or until occurrence of an IFI. We compared the incidence of proven or probable IFI (the primary end point) between the micafungin and fluconazole or itraconazole groups; death from any cause and time to death was secondary end points. Patients were followed for 100 days after haplo-SCT and for 30 days after the last dose of the prophylaxis drug administrated.
Results: From January 2009 to May 2013, a total of 99 patients were identified; 30 patients received micafungin, and 69 patients received fluconazole or itraconazole. 81 patients (82%) received a non myeloabaltive conditioning regimen (NMA), with Fludarabine, Cyclophosphamide and Total body irradiation (TBI) 2 Gy based, or Fludarabine, Busulfan, and Cyclophosphamide based (3%) or other (9%), while five patients (5%) received a thiotepa-based conditioning regimen. The patients and transplant details are shown in the Table 1. Proven or probable invasive fungal infections were reported in 2 patients (7%) in the micafungin group and 8 patients (12%) in the fluconazole or itraconazole group (absolute reduction in the micafungin group, −5%; 95% confidence interval, 0.0565-3.1395, P=0.72). Fewer patients in the micafungin group had invasive aspergillosis (1 [3%] *vs.* 5 [7%], P=0.6). A total of 4 (13%) patients in the micafungin group and 23 (33%) patients in the fluconazole or itraconazole group received empirical antifungal therapy (P=0.14). No serious adverse events related to treatment were reported by patients and there was no treatment discontinuation because of drug related adverse event in both groups. Overall Survival and disease free survival were similar among the two groups (P=0.97). 6 patients (20%) in the micafungin group died within 100 days, as did 10 patients (14%) in the fluconazole or itraconazole group (P=0.57). Interestingly the transplant related mortality (TRM) at 100 days was 0% in the micafungin group *vs* 13% in the second group [CI 95% (0-22)] (p=0,06), whereas the relapse or progression rate at 100 days was 27% *vs.* 8% respectively [CI 95% (14-44)] (p=0,14).
Summary and Conclusions: In patients undergoing to haplo-SCT, antifungal prophylaxis with micafungin is well tolerated and effective to prevent IFI. Furthermore, the incidence of IFI and invasive aspergillosis seems lower even if this did not attend statistical power, probably due to low number of patients.

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EFFECT OF UPREGULATED TLR2 EXPRESSION FROM G-CSF-MOBILIZED DONOR GRAFT ON ACUTE GRAFT-*VERSUS*-HOST DISEASE IN MICE

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Background: Our previous study demonstrated that TLR2 expression was increased on donor graft mainly myeloid cell types by G-CSF treatment, which contributed rapid engraftment after allogeneic hematopoietic stem cell transplantation (HSCT) in mice (Cytokine 54:36-42; Immunol Lett. 143: 177-183).
Aims: In current study, we investigated the effects of upregulated TLR2 expression in G-CSF-mobilized donor graft on acute graft-*versus*-host disease (GVHD).
Methods: B6 donor mice were subcutaneous injected with recombinant human G-CSF (10 mg) daily for 5 days. The expression of TLR2 was analyzed on CD11b+, Gr-1+, CD3+, and CD19+ from splenocytes by flow cytometry**.** To evaluate the effect of TLR2 on acute GVHD, lethally irradiated (950 cGy) B6D2F1 mice were transplanted with splenocytes ($2x10^7$) from G-CSF-injected WT B6 or TLR2 KO B6 mice plus WT B6 TCD-BMs ($5x10^6$). Another experimental setting, using blocking antibody, recipients given splenocytes from G-CSF-injected B6 plus TCD-BMs were administrated with 0.2 mg of anti-TLR2 (T2.5) mAb on day 0, 2, and 5 after transplantation. Survival and clinical GVHD scores were monitored every 2 days.
Results: We found that TLR2 was highly expressed on myeloid cell population (CD11b+Gr-1+) but not T and B cells of splenocytes from G-CSF-treated donor mice. Mortality and severity of recipients were not significantly different between G-CSF-treated WT and TLR2 KO donor graft. Moreover, there was no difference on GVHD between anti-TLR2 mAb and control IgG.
Summary and Conclusions: Our results demonstrate that upregulated TLR2 expression in G-CSF-mobilized donor graft affects little on acute GVHD. Our study suggests that TLR2 is valuable target for increasing allo-HSCT efficiency to enhance engraft without acute GVHD enhancement.

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ALDEHYDE DEHYDROGENASE-BRIGHT CELLS CORRELATED WITH THE COLONY FORMING UNIT-GRANULOCYTE/MACROPHAGE ASSAY OF THAWED CORD BLOOD UNITS

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Background: Together with total nucleated cells (TNC) and CD34+ cells, the colony-forming unit-granulocyte/macrophage (CFU-GM) has been regarded as a marker predicting transplantation outcome in hematopoietic stem cell transplantation, because the CFU-GM represents the clonogeneic and proliferative potentials of hematopoietic stem cells. However, CFU assay takes 14 days; therefore, there are some difficulties in application as routine examination. Recently, the number of cells with high aldehyde dehydrogenase (ALDH) activity, namely, ALDH-bright ($ALDH^{br}$) cells, has been suggested as a viable marker of hematopoietic stem cell function.
Aims: We evaluated the suitability of $ALDH^{br}$ cell analysis in the quality assessment of post-thaw cord blood (CB) units.
Methods: We used the 245 CB units rejected from the Seoul Metropolitan Government Public Cord Blood Bank inventory due to inappropriate test results after conventional processing. After thawing of cryopreserved CB units, the numbers of TNC, CD34+ cells, $ALDH^{br}$ cells among CD34+ cells (CD34+$ALDH^{br}$ cells), $ALDH^{br}$ cells, CD34+ cells among $ALDH^{br}$ cells ($ALDH^{br}$CD34+ cells), CFU-GM, and CFU-granulocyte/erythrocyte/macrophage/megakaryocyte (GEMM) were examined. We could extract the numbers of TNC and CD34+ cells before cryopreservation from inventory database. Simple linear regression analysis was performed to assess the correlation among the parameters: the number of TNC (pre-cryopreservation and post-thaw), the number of CD34+ cells (pre-cryopreservation and post-thaw), the numbers of post-thaw CD34+$ALDH^{br}$ cells, $ALDH^{br}$ cells, $ALDH^{br}$CD34+ cells, CFU-GEMM, and CFU-GM.
Results: CD34+ cells were found to represent 0.14%±0.10% of all TNC, and 74.5%±13.8% of CD34+ cells were found to express high ALDH activity (*i.e.*, CD34+$ALDH^{br}$ cells). $ALDH^{br}$ cells were found to represent 0.16%±0.10% of all TNC, and 69.9%±15.5% of $ALDH^{br}$ cells were found to express CD34 (*i.e.*, $ALDH^{br}$CD34+ cells). CFU-GEMM was significantly correlated with the numbers of TNC and CD34+ cells before and after cryopreservation (TNC, r=0.251 and r=0.250, respectively; CD34+ cells, r=0.391 and r=0.347, respectively) and the numbers of CD34+$ALDH^{br}$ cells, $ALDH^{br}$ cells, and $ALDH^{br}$CD34+ cells (r=0.297, r=0.297, and r=0.252, respectively). Although CFU-GM was not correlated with the numbers of TNC before and after cryopreservation, CFU-GM was significantly correlated with the number of CD34+ cells before and after cryopreservation (r=0.418 and r=0.359, respectively) and the numbers of CD34+$ALDH^{br}$ cells, $ALDH^{br}$ cells, and $ALDH^{br}$CD34+ cells (r=0.426, r=0.455, and r=0.469, respectively). CFU-GM showed higher correlation coefficient with the number of CD34+$ALDH^{br}$ cells, $ALDH^{br}$ cells, and $ALDH^{br}$CD34+ cells than with the number of CD34+ cells before and after cryopreservation.
Summary and Conclusions: The high correlation found between the numbers of CD34+$ALDH^{br}$ cells, $ALDH^{br}$ cells, and $ALDH^{br}$CD34+ cells and CFU-GM number supports the suitability of ALDH analysis in the quality assessment of post-thaw CB units.

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A MACHINE LEARNING BASED MODEL FOR PREDICTION OF NRM 100 DAYS POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT). A EUROPEAN REGISTRY DATA MINING STUDY

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Background: Allo-HSCT has been shown to increase survival and improve cure in acute leukemia (AL). However, this procedure is accompanied by high rates of morbidity and mortality. Several risk scores based on conventional statistical methods may aid HSCT risk assessment, but these methods carry inherent limitations, which might lead to sub-optimal candidate selection. Machine learning (ML) is a field in computer science stemming from artificial intelligence and is part of the data mining approach for data analysis. ML algorithms are commonly applied in technological and commercial settings. They allow for coping with complex data scenarios, thus are potentially suitable for outcome prediction in HSCT. With this background, and using a ML algorithm - the alternating decision tree (ADT), we developed an interpretable prediction model for allo-HSCT outcomes in AL patients.
Aims: The primary endpoint was prediction of NRM 100 days post allo-HSCT. Secondary outcomes were prediction of NRM, leukemia free survival (LFS) and overall survival (OS) at 2 years according to the primary model.
Methods: A cohort of 28,236 adult allo-HSCT recipients from the acute leukemia working party registry of the European Group for Blood and Marrow Transplantation (EBMT) was used to construct the model. Twenty variables were analyzed, including year of transplant (range, 2000-2011), diagnosis (Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia), disease stage (CR1, CR2, advanced/ refractory disease), conditioning regimen (myeloablative or reduced-intensity conditioning), graft type (peripheral blood or bone marrow), donor type (HLA matched related or unrelated), etc. For model development and validation, the dataset was randomly divided into a training set (n=19,765) and a validation set (n=8,707). ADT model includes a graphical tree like structure, detailing variable assigned score, dependence and predictive influence. Patients evaluated are plotted on the tree and gather a cumulative score correlating with the endpoint. For model calibration and risk stratification, the score was transformed into probabilities using logistic regression. Discrimination was assessed using the area under the receiver operating characteristics curve (AUC) and compared for the primary endpoint with the EBMT score.
Results: For prediction of 100 days NRM ten variables were selected by the ADT model, with varying dependence and predictive influence. Patients were stratified into ten risk groups, reflecting ten score intervals with increasing probabilities for the primary outcome (Figure 1). Calibration was excellent. The ADT Model discrimination for day 100 NRM, as assessed by the AUC, was 0.69 and 0.68 on the training and validation sets respectively, performing better than the EBMT score (AUC of 0.62 for both data sets, p-value<0.0001). Two years outcomes (NRM, LFS, OS), correlated with score intervals generated for the primary outcome. For instance, the highest and lowest score intervals corresponded with NRM of 38% and 10% at two years respectively.
Summary and Conclusions: We present a novel prediction model for NRM at day 100 post allo-HSCT, potentially extending to two years. The model enables risk assessment, in a stratified manner, prior to transplantation. Data from approximately 30,000 patients was used for model development, assuring robustness and validity. Incorporation of our model with conventional risk scores, such as the Hematopoietic Cell Transplant-Co-morbidity Index, may further improve predictive performance.

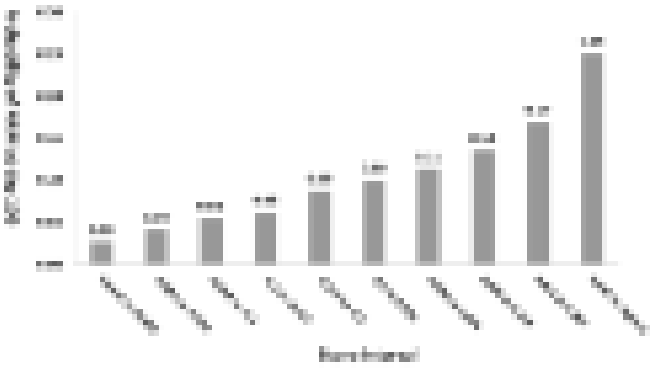

Figure 1.

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CONTRASTING EFFECT OF MISSING SELF MODEL AND ACTIVATING DONOR KIR GENES WITH RELEVANT HLA LIGAND IN RECIPIENTS ON PREVENTING RELAPSE AFTER T-CELL-REPLETE HAPLOIDENTICAL TRANSPLANTATION IN CML

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Background: The effect of NK alloreactivity in HLA haploidentical SCT is still under debate. This might be a result of patient heterogeneity and the involvement of activating KIR genes besides the effect of different haploidentcial transplant protocols.
Aims: We prospectively analyzed the HLA typing of donor-recipients pairs as well as the KIR typing of the donor in the relatively purified CML disease aim to further address the predictive roles of NK cells on relapse undergoing T-cells-replete haploidentical transplantation.
Methods: We studied the HLA genotype of 97 donor-recipient pairs and their donor KIR genotype, who underwent T-cells-replete haploidentical transplantation during 2003-2009 in our center.
Results: Among the 97 pairs of donor-recipients, there were 74 donor-recipients pairs with missing self and 23 without missing self. There was a trend towards increased molecular and hematologic relapse rate in patients with missing self between donor and recipients compared to those without missing self (P=0.003 and P=0.015). We found a significantly reduced risk of molecular relapse (P=0.009) and hematologic relapse (P=0.009) in patients with HLA-C1C2 or C2C2 accepting donors with KIR 2DS1 positive or patients with HLA-Bw4 accepting donors with KIR3DS1 positive ("recipient with relevant KIR ligand for donor activating KIR", n=25) compared with "other" (n=72). Meanwhile, among 74 patients absent KIR ligand for donor inhibitory KIR genes, there were 13 patients in "recipient with relevant KIR ligand for donor activating KIR" group and 61 patients in "others" group. The molecular relapse and hematologic relapse in patients of "recipient with relevant KIR ligand for donor activating KIR" group were significantly lower than patients of "others" group (P=0.04 and P=0.03) among patients with missing self between donor and recipients. Multivariate analysis confirmed that recipient with relevant KIR ligand for donor activating KIR were independent factors for preventing relapse.
Summary and Conclusions: we demonstrated that donor-derived "alloreactive" NK cells could be predicted *in vitro* using donor activating KIR gene (KIR2DS1 or KIR3DS1) with their relevant KIR ligand in recipients. Contrasting roles of missing self between donor and recipients on relapse in this T-cells-repleted haploidentical transplantation suggested NK cells alloreactivity might be altered by the process of NK functional education *in vivo*.

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IMPACT OF SMOKING ON THE NON-RELAPSE MORTALITY, THE OVERALL SURVIVAL, AND THE INCIDENCE OF SECOND CANCERS AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

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Background: The impact of smoking on health has been extensively studied in epidemiology, yet the effect of smoking on the allogeneic stem cell transplantation (alloSCT) outcomes has not been determined yet.
Aims: This retrospective monocentric study aimed at understanding whether

smoking affects the survival outcomes of hematologic patients undergoing reduced intensity conditioning (RIC) alloSCT.
Methods: We searched for information on the smoking history (packs-year and duration of exposure) of 301 patients who received RIC alloSCT between 2001 and 2012 in our division, reviewing the clinical charts and by telephone interviews. We analyzed the impact of smoking on non-relapse mortality (NRM) by Cumulative Incidence method, on overall survival (OS) and progression free survival (PFS) by log-rank method. We ran a Cox multivariate analysis for NRM, OS and PFS using pre-transplant disease status, donor type, and smoking as covariates.
Results: We had complete data of 202 patients. All the patients received RIC alloSCT for lymphoma (60%), multiple myeloma (21%), acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) (17%), or myeloproliferative diseases (MPD, 2%). The median age at alloSCT was 49 years (range, 18-69). Donors were HLA-identical siblings (43%), mismatched siblings (2%), unrelated (43%), or haploidentical (12%). At alloSCT, 50% of patients were in complete response (CR), 36% in partial response (PR), 3% of patients were stable (SD) and 11% were in progression (PD). Forty-nine percent of patients were former smokers, 28% were hard smokers (defined as smokers of >=1 pack per day). Nine percent of patients were still smokers at admission of alloSCT. The exposure to smoking was a median of 1 pack per day (range, 0.1-3) for a median of 15 years (range, 1-40), and smokers had quit smoking by a median interval of 5 years before transplant (range, 0-41). The median follow-up after alloSCT was 4.9 years (range, 0.2-11.9). The NRM at 100 days was 5%, at 1 year 9%, at 3 years 11%. Three-year OS and PFS were 77% and 55%. In univariate analysis, smoking increased NRM (p=0.008), and reduced the OS (p=0.006). The multivariate analysis showed that smoking increased the NRM by a factor of 5.4 (p=0.001). Having a haploidentical donor also impacted NRM (p<0.001). Smoking significantly impacted OS increasing the risk of death by a factor of 2.8 (p=0.001). Overall survival was also impacted by haploidentical donor (p=0.001) and pre-transplant disease in PR (p=0.01) or PD (p<0.001). Smoking did not affect PFS, which was impacted by active disease before alloSCT (p<0.001 for PR, SD and PD). Ten patients had a second cancer after alloSCT (5%). Three cancers occurred in non-smokers (skin carcinoma, breast, and bladder carcinoma), 7 cancers occurred in smokers (bladder carcinoma - 2 patients, skin carcinoma, oropharingeal carcinoma, AML, MDS and MPD). The crude incidence of second cancers was 3% in non-smokers and 7% in smokers. In hard smokers, the incidence of second cancers after alloSCT was 9%. Smokers had a 2.5 risk increase of developing a second cancer compared to non-smokers. Three deaths were caused by a second cancer, and all occurred in smokers.
Summary and Conclusions: Smoking is a significant independent factor that impacts NRM and OS in patients undergoing a RIC alloSCT, and it may increase the incidence of second tumors. Smoking should be considered a comorbid condition before alloSCT. Aggressive anti-smoking campaigns should be undertaken to decrease this avoidable risk.

P499

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT) FOR PRIMARY PLASMA CELL LEUKEMIA (PPCL): A PROSPECTIVE STUDY OF IFM GROUP

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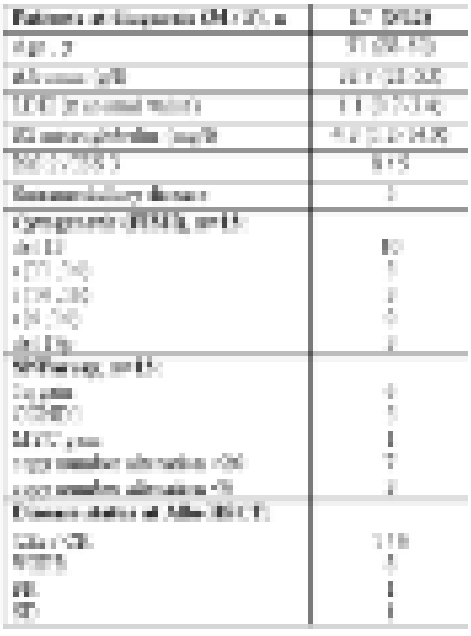
Background: pPCL is a rare plasma cell malignancy with very poor outcome. Novel therapeutic strategies including transplantation (Auto or Allo-HSCT) may improve survival but the prognosis remains poor. Only retrospective studies incorporating up-front Allo-HSCT for pPCL patients have been published.
Aims: We conducted a prospective study incorporating tandem Auto/Allo-HSCT after an induction with doxorubicin-bortezomib-cyclophosphamide and dexamethasone for pPCL patients less than 66-year old.
Methods: After the induction 17 patients (Female 12/Male 5) underwent Allo-HSCT: 1 syngenic, 1 after a reduced intensity conditioning (RIC)-Allo-HSCT and 15 after a tandem Auto/RIC-Allo-HSCT. At diagnosis median age was 51 year [28-60], 8 patients presented an ISS2, 5 ISS3 and 5 an extramedullary disease. The majority of patients had high-risk cytogenetic assessed by FISH or SNParray (Table 1). Patients have been allotransplanted at a median time of 7.4 months [7-18] from diagnosis, in sCR (n=1), CR (n=6), VGPR (n=8), PR (n=1) and in SD (n=1). The conditioning regimen used were: fludarabin-busulfan- antithymoglobulin (n=13), fludarabin-melphalan (n=1), bortezomib-fludarabin-melphalan (n=2) and high-dose melphalan for the syngenic transplantation (n=1). All patients were grafted with peripheral blood stem cells except one who received bone marrow and one cord blood unit, from related (n=9) or HLA-matched unrelated donors (n=8). The median infused CD34 cells number was 6.8 10^6/kg [3-8.5]. All patients received ciclosporine +/- methotrexate as GvHD prophylaxis.
Results: All patients achieved engraftment. The median time for neutrophil (≥1 10^9/l) and platelet (≥25 10^9/l) engraftment was 19 [7-31] and 11 days [10-43] respectively and the median duration of hospitalisation 28 days [1-77]. Six patients developed an acute GvHD which responded to steroid in 5 cases and 1 was steroid-resistant and responded secondary to anti-IL2Rα antibody. At day 100, 16 patients were evaluable: 12 (76%) achieved a VGPR or better (3 in sCR, 4 in CR and 5 in VGPR), 2 (12%) PR and 2 patients (12%) died from EBV reactivation. Three patients received, 3 months after Allo-HSCT as immunomodulation, donor lymphocyte infusions plus Lenalidomide-Cyclophosphamid-Fludarabin (n=1), Bortezomib (n=1) and Lenalidomide (n=1): one patient in PR (transplanted in SD) achieved CR and the two others in VGPR maintained their response. The median follow-up was 22 months [7-41] from diagnosis and 14 months [1-32] from Allo-HSCT: 6/17 (35%) patients relapsed and 5/17 (29%) died. At analysis, median progression-free survival (PFS) and overall survival (OS) were not reached. One year post-diagnosis OS was 87% and post-Allo-HSCT 68%, PFS 86% and 65% respectively. Five patients experienced chronic GvHD: mild (n=4) and extensive (n=1).

Table 1.



Summary and Conclusions: This is the first large prospective trial for pPCL patients including Allo-HSCT. Tandem Auto/RIC-Allo-HSCT or RIC-Allo-HSCT is feasible, inducing a high response rate and allowing a prolong survival. In addition immunomodulation after transplantation may be proposed to patients.

P500

IMPACT OF GRAFT SOURCE ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION IN ADULTS WITH PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Allogeneic hematopoietic cell transplantation (allo-HCT) remains a curative option for adults with Philadelphia chromosome-negative acute lymphoblastic leukemia (Ph-negative ALL). An unrelated bone marrow (uBM) donor or an umbilical cord blood (CB) donor becomes an alternative source when a suitable related donor is not available.
Aims: To assess the impact of donor source on outcomes of allo-HCT in adults with Ph-negative ALL.
Methods: We analyzed the data from 96 patients with Ph-negative ALL who

underwent allo-HCT between 2000 and 2012 at 4 institutes of the Yokohama City University Hematology Group. We assessed the impact of donor sources on outcomes using univariate and multivariate analyses.

Results: The cohort included 53 men and 43 women with a median age of 32 years (range, 15-62 years). The stem cell source was a related bone marrow (rBM) donor in 25 patients, an uBM donor in 43, a related peripheral blood (rPB) donor in 10, and a donor of a single unit of unrelated umbilical CB in 18. At the time of allo-HCT, 19 patients (76%) in the rBM group, 20 (47%) in the uBM group, 4 (40%) in the rPB group, and 14 (77.8%) in the CB group were in complete remission (CR). Eighty-nine patients received a myeloablative conditioning regimen with cyclophosphamide (CY) plus a total body irradiation based-regimen or CY plus busulfan, while only 7 patients received a reduced intensity conditioning regimen with a fludarabine and melphalan based therapy. The median observation period for the surviving patients was 45 months (range, 3-178 months). The 3-year overall survival (OS), 3-year leukemia-free survival (LFS), and relapse rates at 3 years of all the 96 patients were 45%, 40%, and 34%, respectively. The patients with a CB source had better 3-year OS (72%) and 3-year LFS (69%) than those with a rBM source (61%, and 41%, respectively), an uBM source (38%, and 27%, respectively), or a rPB source (50%, and 44%, respectively; *P*=0.013, and *P*=0.028, respectively). The relapse rates at 3 years were lower in the CB group (13%) than in the other groups (rBM, 33%; uBM, 41%; rPB, 50%; *P*=0.049). No statistically significant difference in non-relapse mortality (NRM) at 3 years or in cumulative incidence of acute graft-*versus*-host disease (aGVHD) was observed between the 4 groups. In the multivariate analysis, the following 3 independent factors were significantly associated with inferior OS and LFS: uBM source (HR, 3.29; 95% CI, 1.2-8.8; *P*=0.02, and HR, 3.3; 95% CI, 1.2-8.8; *P*=0.02, respectively), having no CR at the time of allo-HCT (HR, 3.3; 95% CI, 1.6-6.9; *P*=0.001, and HR, 3.1; 95% CI, 1.5-6.3; *P*=0.003, respectively), and performance status >2 at the time of allo-HCT (HR, 29.7; 95% CI, 4.6-191.5; *P* <0.001, and HR, 14.5; 95% CI, 2.5-84.4; *P*=0.003, respectively). The multivariate analysis demonstrated also that use of a rPB source (HR, 13.9; 95% CI, 2.6-75; *P*=0.002) and having no CR at the time of allo-HCT (HR, 4.4; 95% CI, 1.6-11; *P*=0.003) were significantly associated with a higher risk for relapse. For NRM, only grade 2-4 aGVHD was a significant adverse prognostic factor (HR, 3.3; 95% CI, 1.2-9.1; *P*=0.02) (Figure 1).

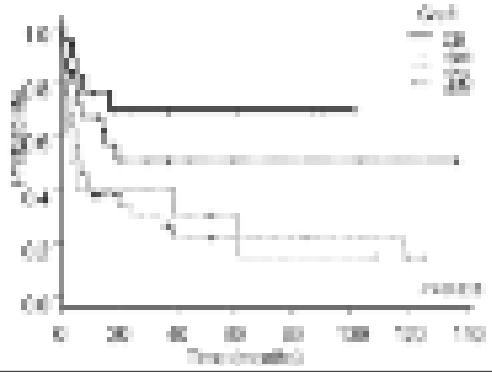

Figure 1. Kaplan-Meier curves for overall survival according to graft sources.

Summary and Conclusions: In our study, allo-HCT using CB resulted in better outcomes with higher OS and LFS rates than that using other sources. With the results of the multivariate analysis, CB source was not associated with poor outcomes, while uBM and rPB were independent adverse prognostic factors. CB could be a good alternative donor source for patients with Ph-negative ALL in CR when a related donor is not available.

P501

A POTENTIAL BENEFIT OF INHIBITORY KIR-MISMATCHED UNMANIPULATED HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOLLOWING TBI-BASED CONDITIONING IN ADULT PATIENTS WITH AML

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Background: Donor natural killer (NK) cells, regulated by killer Ig-like receptors (KIR), are known to recognize specific groups of HLA class I alleles for inducing alloreactivity. Effectiveness of donor NK cell alloreactivity determined by inhibitory KIR (iKIR)-ligand incompatibility model in graft-*versus*-host (GVH) vector had been shown in patients with acute myeloid leukemia (AML) receiving haploidentical stem cell transplantation (haplo-SCT) in T cell-deplete (TCD) setting. However, extensive TCD transplant is relatively discouraged due to infectious complications and conflicting impact of NK alloreactivity on transplantation outcome from different haplo-HSCT settings were reported.

Aims: We aimed to evaluate a potential NK cell alloreactivity on transplant outcome after unmanipulated haplo-SCT with total body irradiation (TBI)-based conditioning regimen in adult Korean population with AML.

Methods: A total of 89 consecutive patients with AML received peripheral blood stem cell haplo-SCT at first complete remission (CR1, n=49), second CR (CR2, n=16), or relapsed/refractory (n=9) status between 2008 and 2013. The median age was 41 years (range, 16-69) and identical conditioning regimen (fractionated 800 cGy TBI, reduced doses of fludarabine and busulfan, and anti-thymocyte globulin) was used in all patients. NK cell alloreactivity in GVH vector was predicted when the donor expresses but the recipient lacks an iKIR-ligand (C1, C2, Bw4 or A3/A11) and that in host-*versus*-graft (HVG) vector was determined when the recipient expresses but the donor lacks the ligand. The frequency of iKIR-ligand assessed by high-resolution HLA typing showed that the majority of donors and recipients were C1/C1 homogygous (71%) and Bw4/Bw6 heterozygous (62%), while only 26% had A3/A11 ligand.

Results: All patients achieved neutrophil recovery (median, 11 days) without delayed engraftment failure. After a median follow-up of 24 months, 25 patients died after relapse (n=14) or due to non-relapse mortality (n=11). Two-year relapse-free survival (RFS) from transplant was 62% in all patients and was significantly different between CR1 (76%) *versus* ≥CR2 (33%, p=<0.0001). Patients were then classified according to iKIR-ligand incompatibility model: 28 donor/patient pairs were iKIR-ligand mismatched in GVH vector only (31%; Group 1), 25 pairs mismatched in HVG vector only (28%; Group 2), 32 pairs ligand-compatible in both vectors (36%; Group 3): 4 pairs with iKIR-ligand incompatible in both vectors were excluded from further analysis due to the small number. Two-year RFS was 72%, 51%, and 50%, respectively (p=0.497). However, in patients with CR1, 2-year RFS was 92%, 67%, and 53%, respectively (p=0.043), and cumulative incidence of relapse (CIR) was 8%, 0%, and 41%, respectively (p=0.003) (Figure 1), while significant difference was not found in patients transplanted at ≥CR2. Significantly more number of group 1 patients (n=10, 36%) had advanced status at transplant compared to group 3 (n=3, 12%; p=0.051). By adjusting disease status and donor age (n=85), patients with iKIR-ligand mismatched in HVG vector only (Group 3) showed significantly worse posttransplantation outcome compared to patients in group 1: 2-year RFS (HR 4.03, p=0.013) and CIR (HR 9.40, p=0.007).

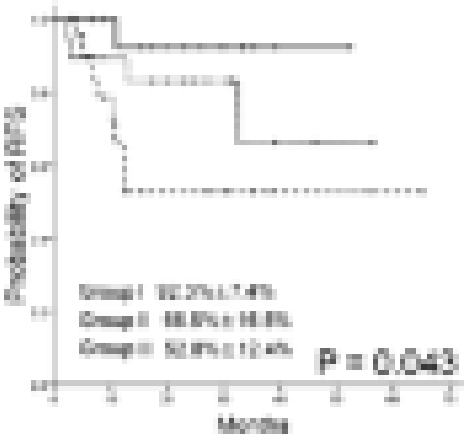

Figure 1.

Summary and Conclusions: Although limited by small number of patients and short duration of follow-up, our study results of posttransplantation outcome after T-cell replete haplo-SCT in adult patients with AML may suggest the beneficial effect of iKIR-ligand incompatible in GVH vector and compatible in HVG vector, showing low CIR leading to better RFS, while transplantation of iKIR-ligand compatible in GVH vector and incompatible in HVG vector may be expected with worse outcome. Further studies using KIR genotyping should be pursued. Meanwhile, donor/patient iKIR-ligand compatibility assessed by HLA genotyping seems to be useful in choosing adequate donor among multiple haploidentical donors.

P502

HIGH BURDEN OF CD34+/CD38-CELLS PREDICTS WORSE OUTCOME IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (HCT) WITH REDUCED INTENSITY CONDITIONING (RIC)

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Background: Most patients (pts) with acute myeloid leukemia (AML) achieve complete remission (CR), but the majority subsequently relapses and dies of the disease. In AML, leukemia initiating cells (LICs) are enriched within the CD34+/CD38- cell compartment. LICs are assumed to be less chemotherapy sensitive, enriched in minimal residual disease cell populations and responsible for relapse. Furthermore, they are described to be less immunogenic. The therapeutic approach of RIC-HCT is mainly based on an immunological graft-*versus*-leukemia (GvL) effect. The prognostic impact of the CD34+/CD38-burden at diagnosis remains unknown.
Aims: To analyze the prognostic impact of the CD34+/CD38-burden at diagnosis in AML pts receiving RIC-HCT.
Methods: We analyzed 120 AML pts (median age 65 years [y]; range 47-75y) who received RIC (Fludarabin 30mg/m^2 at day-4 to -2 & 2Gy total body irradiation day 0)-HCT, with pretreatment bone marrow material (BM) available. At HCT all pts were in CR. 99 pts (82.5%) were in first & 21 (17.5%) were in second CR. Donors were human leucocyte antigene (HLA)-matched related (n=17, 14.2%) or HLA-matched (n=69, 57.5%) or mismatched (>=1 antigen; n=34, 28.3%) unrelated. 35 (32.7%) developed acute graft-*versus*-host disease (GvHD; >=grade 2) & 62 (64.6%) chronic GvHD (cGvHD). Median follow-up was 3.8 y for pts alive. Pts were characterized for *NPM1*, *FLT3*-ITD, *FLT3*-TKD & *CEBPA* mutations at diagnosis. Percentage of BM CD34+/CD38- mononuclear cells at diagnosis was measured using flow cytometry.
Results: A cutpoint of 5% defined a high burden of BM CD34+/CD38-cells. In multivariate analysis, the negative prognostic impact of a high CD34+/CD38-burden on OS could be confirmed (Hazard Ratio 2.00, [1.32-3.04, 95%CI], *P*=.001). Pts with a high CD34+/CD38-cell burden were more likely to have *de novo* disease (*P*=.055) & to be *CEBPA* wild-type (*P*=.11) by trend. None of the pts with high CD34+/CD38- burden were *CEBPA* mutated. A high diagnostic CD34+/CD38- burden associated with higher cumulative incidence of relapse (CIR, *P*<.001, Figure 1A) & shorter overall survival (OS, *P*=.018, Figure 1B).

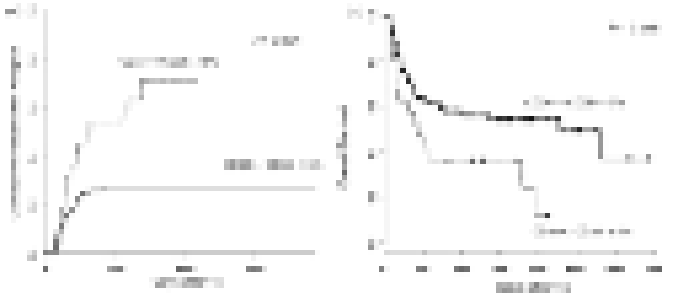

Figure 1. CIR (A) and OS (B) in RIC-HCT treated AML pts according to CD34+/38- burden at diagnosis.

Summary and Conclusions: A high CD34+/CD38-cell burden at diagnosis associated with increased relapse rates & shorter OS in RIC-HCT treated AML pts. The observed worse outcome is likely mediated by LICs within the CD34+/CD38-population, escaping the GvL effect of RIC-HCT. When confirmed an easy cutoff of 5% CD34+/CD38-BM mononuclear cells may help to identify pts at high risk of relapse & may enable studies for LIC targeting therapies in these pts.

P503

PROGNOSTIC SIGNIFICANCE OF IDH MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS UNDERGOING HEMATOPIETIC STEMCELL TRANSPLANTATION (HCT) AFTER REDUCED INTENSITY CONDITIONING (RIC)

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Background: Mutations in the Isocitrat-Dehydrogenase (*IDH*) 1 and 2 genes impact outcome in some AML subgroups. The prognostic significance in AML patients (pts) undergoing RIC-HCT, a post-remission therapy that is mainly based on an immunological graft-*versus*-leukemia effect and offers a potential cure for AML pts, who are ineligible for conventional conditioning, is still unknown.
Aims: To analyze the impact of *IDH* mutations in pts undergoing RIC-HCT.
Methods: We analyzed 143 AML pts (median age, 64 years [y]; range 33–75y) who received RIC (Fludarabine 30mg/m^2 at day -4 to -2 & 2 Gy total body irradiation day 0 [n=137]; Fludarabine 30mg/m^2, Cytarabine 2g/m^2, and Amsakrine 100mg/m^2 at day -12 to -9, Cyclophosphamide 40mg/kg at day -4 to -3, ATG/TG day -3 to -1 & 4 Gy total body irradiation day - 5 [n=4]; 2 Gy total body irradiation day 0 [n=2])-HCT at the University of Leipzig between May 2000 and August 2012, with pretreatment bone marrow or peripheral blood material available. Donors were human leukocyte antigen (HLA)-matched related (n=23; 16.1%) or HLA-matched (n=83; 58.0%) or mismatched (>=1 antigen; n=37; 25.9%) unrelated. The median follow-up for pts alive was 4.4y. 22.3% (n=32) developed acute graft-*versus*-host disease (GvHD; >=grade 2) and 55.8% (n=63) (17.7% (n=20) limited; 38.1% (n=43) extensive) chronic GvHD. Medical research council (MRC) genetic classification was: favorable (n=3; 2.2%) intermediate (n=99; 72.3%) or adverse (adv, n=35; 24.5%). All pts were characterized for *CEBPA* (n=18; 13.0%) and *NPM1* (n=32; 22.9%) mutations, *FLT3*-ITD (n=23; 16.7%) and *FLT3*-TKD (n=12; 9.0%) at diagnosis.
Results: We found an *IDH* mutation (*IDH1* or *IDH2* mutation) in 36 pts (25.2%). 15 pts (10.5%) had an *IDH1* mutation, 9 pts (6.3%) had the most frequently R132C mutation. 21 pts (14.7%) were found *IDH2* mutated, 14 pts (9.8%) had R140Q and 7 pts (4.9%) R172K mutations. The *IDH1*-SNP (rs11554137) was found in 25 pts (17.5%). The presence of *IDH* mutations associated significantly with higher age (*P*=.02) and by trend with higher hemoglobin (Hb) levels at diagnosis (*P*=.15). A mutation in the *IDH1* gene was associated by trend with higher age (*P*=.09) and *FLT*-TKD (*P*=.12) and significantly with lower white blood cell count (*P*=.04). Pts with an *IDH2* R140Q mutation associated with *NPM1* mutations (*P*=.04). The presence of the *IDH1*-SNP associated by trend with *CEBPA* mutations (*P*=.08) and with lower Hb (*P*=.07). Interestingly, none of the pts with the SNP had a *FLT3*-ITD (*P*=.013). In our cohort neither the presence of *IDH1* mutation nor the *IDH1*-SNP associated with overall survival (OS) or event-free survival (EFS). However, the presence of *IDH2* mutations were associated with longer EFS (*P*=.13) by trend. Analyzing subgroups we found a stronger impact of *IDH* mutations on outcome in pts with adverse karyotype. In this subgroup the presence of either *IDH1* or *IDH2* mutations were significantly associated with longer EFS (*P*=.02) and OS (*P*=.03) (Figure 1).

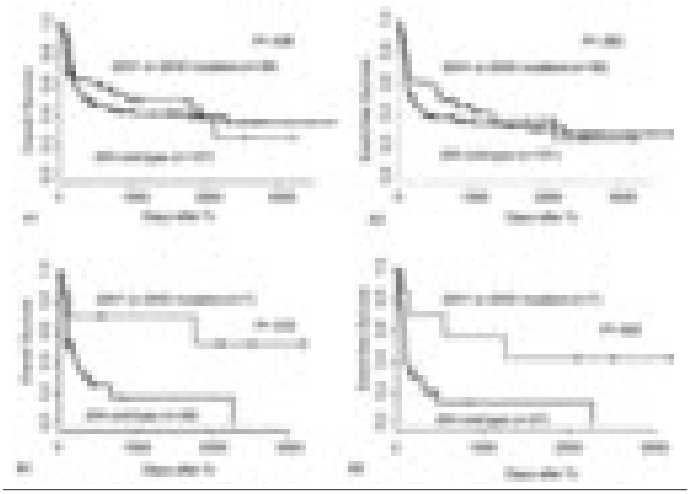

Figure 1. Overall survival (A1) and Event-free survival (A2) in RIC-HCT treated AML patients according to *IDH* mutation status. Figures B: overall survival (B1) and Event-free survival (B2) in RIC-HCT treated AML patients with an adverse karyotype according to *IDH* mutation status.

Summary and Conclusions: In the complete set of AML pts receiving RIC-HCT the presence of *IDH1* mutations did not impact survival, the presence of *IDH2* mutations associated with longer EFS by trend. We observed a stronger effect of *IDH* mutations in pts with adverse karyotype. Here the presence of either *IDH1* or *IDH2* mutations was significantly associated with longer survival. Thus especially pts with adverse cytogenetics harboring *IDH* mutations might benefit from RIC-HCT.

P504

IMPACT OF DOSE INTENSITY OF CONDITIONING ON THE OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA FROM AN HLA-IDENTICAL SIBLING

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Background: The established standard conditioning regimen of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for severe aplastic anemia (SAA) from an HLA-identical sibling is high-dose cyclophosphamide (CY; 200 mg/kg) with or without anti-thymocyte globulin (ATG). Since this regimen is

often associated with serious toxicities such as cardiotoxicity, the safety and efficacy of various less toxic conditioning regimens by reducing the dose of CY have recently been evaluated. However, because of the variation of those conditioning regimens, an optimal regimen has yet to be established.
Aims: The aim of this study is to evaluate the impact of the dose of CY on the outcomes of allo-HSCT for SAA from an HLA-identical sibling in order to explore an optimal conditioning regimen.
Methods: We retrospectively analyzed patients with SAA aged 16 year or older who underwent the first allo-HSCT from an HLA-identical sibling between 1989 and 2010 by using the data from the registry database of Japan Society for Hematopoietic Cell Transplantation. After excluding patients whose essential data of conditioning regimen were missing and patients who received myeloablative conditioning regimens such as those including TBI 8 Gy or greater, 204 patients (median age, 25.5 years (range 16-65)) were evaluable. Conditionings were defined as 1) high-dose CY (200 mg/kg or greater)-based regimen (n=118); 2) reduced-dose CY (100 mg/kg or greater but less than 200 mg//kg)-based (n=38); 3) low-dose CY-based (less than 100 mg/kg)-based (n=48). TBI (less than 8Gy) or local field irradiation were delivered in 106 (52%) patients, and ATG was given to 105 (51%) patients. The proportions of patients who received TBI or local field irradiation were not different among the 3 regimens. In contrast, the proportions of patients who received additional fludarabine were significantly higher in reduced-dose and low-dose CY regimens (26 (68%) of 38 and 47 (98%)) than in high-dose CY (1 (0.8%) of 118; P<0.01).
Results: Median follow-up period of 173 survivors was 49.7 months (range, 2.5-234.2). Five-year overall survival (OS) of all patients was 85.8% (95%CI, 80.5-91.0%). Major causes of death were graft failure in 7 patients, infection in 7, cardiotoxicity in 4, hemorrhage in 4, organ failure in 4, and graft-*versus*-host disease (GVHD) in 3. Although 5-year OS in patients receiving low-dose CY-based regimens (93.0% (95%CI, 85.1-100.0)) tended to be higher than that receiving high-dose CY-based (84.2% (95%CI, 77.1-91.3)) or reduced-dose CY-based regimens (83.8% (95%CI, 71.8-95.8)), the difference was not significant (P=0.214). Among other variables, age of 40 years or older at transplant was identified as the only factor affecting OS. Fatal cardiotoxicity occurred in four patients: 3 with high-dose CY, 1 with reduced-dose CY, but none with low-dose CY.
Summary and Conclusions: These results suggest that the dose of CY of the conditioning could be safely reduced by adding fludarabine in allo-HSCT for SAA from an HLA-identical sibling, leading to the comparable survival rate to that obtained by conventional CY dose and to a reduction of serious toxicities.

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Abstract withdrawn

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DECREASE OF INVASIVE FUNGAL DISEASE IN OUR CENTER WITH THE USE OF VORICONAZOLE AS ANTIFUNGAL PROPHYLAXIS IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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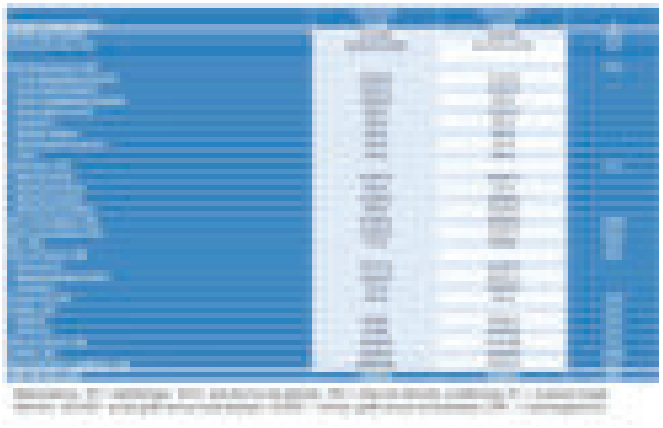
Background: Invasive fungal disease (IFD) causes significant morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). For decades, the most commonly antifungal used for prophylaxis of IFD was Fluconazole. But this agent is not effective against filamentous fungi. For this reason, some new triazole-drugs with anti-filamentous activity (as Voriconazole) have being used during the last years as antifungal prophylaxis for high-risk patients.
Aims: The aim of our study was to compare retrospectively the incidence of IFD in patients undergoing allo-HSCT in our institution using Fluconazole *vs* Voriconazole as prophylactic treatment.
Methods: In this retrospective study, we analyse 196 allo-HSCT consecutive patients (paediatrics and adults) submitted to this procedure in our institution. Patients were recruited from March 2001 to April 2008. A total of 98 patients received antifungal prophylaxis with Fluconazole and 98 with Voriconazole. Antifungal prophylaxis consisted on Voriconazole 200 mg/12 hours (5mg/kg/12h in children) or Fluconazole 200 mg/12 hours (5mg/kg/day in children) from day -1 or +1 until day +75 or +120 (depending on children or adults respectively). The temporary suspension was applied in those patients with toxicity, without reaching criteria for withdrawal, being able to continue the study drug if the suspected toxicity resolved in 7 days. AGA detection was done twice weekly from year 2004. It was considered positive when the index OD was ≥0.5 in 2 consecutive measurements or ≥0.8 on a single determination. When IFD was suspected a new determination of additional AGA and a high resolution TAC were done as well as a bronchoalveolar lavage. IFDs were classified like possible, probable or proven according to the revised criteria of the EORTC/MSG 2008. The criteria used to evaluate the toxicity were the latest version of the National Cancer Institute Common Terminology Criteria for Adverse Events.
Results: The baseline characteristics of these patients are shown in Table 1. We found differences in terms of: type of graft, regimen and type of conditioning and presence of cGVHD between both groups. The incidence of IFD was 16.3% in the Fluconazole group (n=16) *vs* 7.1% (n=7) in Voriconazole group (*P*=0.046). The median of FFS was 24.5 days and 106 days (*P*=0.003). According to the EORTC/MSG criteria, taking into account only patients who developed IFD, the percentage of possible IFD in the first group was 50% *vs* 14.8% in the second group. Probable: 37.5% *vs.* 71.4%; Proven: 12.5% *vs.* 14.28%. FFS at 180 days post allo-HSCT was 52.9% *vs* 75% (*P*=0.037); We also found significant statistical differences at 360 days post HSCT (83.7% *vs* 92.9%; *P*=0. 034). Deaths caused by IFD were 10.1% in Fluconazole (n=10) and 3.1% in Voriconazole (n=3) (*P*=0. 027). The median follow-up was 34 months. There was no significant difference in overall survival: 52.1% *vs* 52.6% (*P*=0.95). There were no differences in causes of death (*P*=0. 326). A total of 11 patients (11.22%) presented adverse effects in the first group *vs* 18 patients (18.36%) in the second one (*P*=0.065). The most frequent adverse effect for both groups was hepatic toxicity.

Table 1. Characteristics of the patients.



Summary and Conclusions: This study confirms that the use of Voriconazole as antifungal prophylactic treatment in patients undergoing allo-HSCT decreases the incidence of IFD and increases FFS *versus* Fluconazole. In spite of a greater toxicity in Voriconazole group, this toxicity was non severe and rapidly recovered with the withdrawal of the drug.

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LONG-TERM SURVIVAL IN PATIENTS RECEIVING RHEPO FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: We recently reported the efficacy of erythropoietin therapy following allogeneic hematopoietic cell transplantation (allo-HCT) on erythroid reconstitution and transfusion requirements.
Aims: As some meta-analyzes suggest increased mortality in patients with cancer receiving erythropoiesis-stimulating agents (ESA) particularly when no concomitant chemotherapy is administered, we assessed long-term follow-up of patients included in the study.
Methods: In this trial, 131 patients given myeloablative or non-myeloablative allo-HCT were randomized 1:1 between two arms: the control arm (no erythropoietic therapy) *vs* the EPO arm (erythropoietin β 500 U/kg/week). Patients were also stratified in 3 cohorts: 42 patients underwent myeloablative HCT (cohort A), while patients in the cohorts B (n=39) and C (n=50) were given non-myeloablative conditioning. RhEPO was administered once a week, from day 28 in cohorts A and B, whereas patients in cohort C received rhEPO from day 0. Treatment duration was initially planned for 16 weeks but there were rules for decreasing or withholding rhEPO according to the Hb level.
Results: The total number of injections of rhEPO until day 126 was 12.6±4.6. After day 126, 19 patients received maintenance therapy with the lowest possible dose of rhEPO. Median times to reach Hb ≥13 g/dL, ≥12 g/dL or +2 g/dL were shorter in the EPO arm and this resulted in a reduction of transfusion requirements in the EPO arm. After a median follow-up of 489 days, we did not observe any fatal TE or CV event and 1 patient in each arm died of an unknown cause. Indeed, 1-year and 5-year OS were 58% and 36% in the control arm and 63% and 40% in the EPO arm (p=0.30). Progression-free survival (PFS) was also similar in the two arms: 1-year and 5-year PFS were 47% and 32% in the control arm and 56% and 32% in the EPO arm (p=0.68). We also observed comparable relapse/progression rate: 31% in both arms at 1 year and 41% in the control arm *vs* 38% in the EPO arm at 5 years. Multivariate analyses with EPO use, conditioning, EBMT risk score and disease risk index were performed for OS and relapse/progression and only the disease risk index was significantly associated with a worsened survival, and with a trend to more relapse.

Summary and Conclusions: In conclusion, rhEPO following allo-HCT did not have an impact on survival in long-term analyses.

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SEQUENTIAL THIRD-PARTY MESENCHYMAL STROMAL CELL THERAPY FOR REFRACTORY ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Refractory acute graft-*versus*-host disease (aGVHD) remains the main therapeutic challenge of allogeneic stem cell transplantation (allo-SCT), and although several therapeutic approaches have been assessed, the outcome for steroid-resistant patients remains poor. Mesenchymal stromal cells (MSC) are multipotent progenitors that can be isolated and expanded from bone marrow (BM) and other sources and have shown *in vitro* and *in vivo* immunomodulatory properties. Based on this fact, MSC therapy is being explored in a number of immune-based diseases, including GVHD. Prior data from our group (Perez-Simon *et al.* Haematologica 2011) indicate that BM-MSC from third party donors is a feasible approach and a number of patients needed several doses to achieve a response and the latter was higher when MSC therapy was started early.
Aims: To evaluate the feasibility and efficacy of four sequential doses of cryopreserved third-party BM derived MSC for refractory aGHVD.
Methods: We have treated 28 patients with steroid-refractory aGVHD with cryopreserved third-party BM-derived MSC. Fifteen patients were treated in a multicenter open label phase II trial (EudraCT: 2010-020947-11) and the experience was further expanded using the same criteria in a compassionate use program at the Hospital Universitario de Salamanca. Inform Consent was obtained from all patients and donors and the studies (both the trial and the compassionate use) were approved by the local Ethics Committees and the Spanish Medicines Agency (AEMPS) and GMP cell production fulfilled all the legal requirements. MSC were isolated and expanded from 50 mL of BM following the same IMDP protocol (06-076) in two GMP facilities, and further cryopreserved. Patients received a median dose of 1.1x10e6 MSC/Kg of recipient body weight on days 1, +4, +11, and +18. The response of aGVHD to MSC therapy was evaluated according to standard criteria.
Results: All patients (n=28) received at least 2 doses, whereas 24 received 3 and 21 received the initially scheduled 4 doses on days 1, 4, 11 and 18. There were no adverse events related to the MSC infusion in the 101 procedures performed, with the exception of a cardiac ischemic event in a patient with high cardiovascular risk and comorbidities (prior ischemic cardiac disease, diabetic, with concomitant renal failure and gastrointestinal hemorrhage).
Response of aGVHD at 60 days after the first MSC dose was evaluable in 26 patients. Of them, 18 patients (69.2%) responded to MSC administration and 8 did not. Of the responding patients, 11 achieved a complete response and 7 a partial response. Median time to response was 28 days (range 9-58 days) in the responding patients. We did not find association of MSC response to any clinical or laboratory parameter evaluated (age, sex, disease status at transplantation, conditioning regimen, GVHD prophylaxis, ECOG, absolute lymphocyte counts, among others).
Regarding infectious diseases, in the 60 days after the first MSC administration, 10 patients (35.7%) experienced *de novo* CMV reactivation, 3 patients (10%) were diagnosed of invasive aspergillosis and bacterial infections were reported in 9 patients (32.1%)
Summary and Conclusions: The use of sequential doses of cryopreserved BM-derived MSC on days 1, 4, 11 and 18 in steroid-refractory aGVHD patients is a safe procedure and may offer a higher rate of complete responses.

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PLASMA PROHNPS ARE SPECIFIC EARLY MARKERS OF NEUTROPHIL ENGRAFTMENT IN PATIENTS UNDERGOING HIGH-DOSE CHEMOTHERAPY, AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Neutropenia is invariably a consequence of myeloablative conditioning before hematopoietic stem cell transplantation (SCT) as well as high-dose chemotherapy. The risk of life-threatening infections correlates with the length of neutropenia. Several plasma proteins such as lysozyme have been investigated as early markers of bone marrow recovery, but these lack specificity which renders them clinically less useful.
Human neutrophil peptides (HNPs) are abundant antimicrobial peptides exclusively synthesized by late promyelocytes and myelocytes. HNPs are synthesized as 10kD proforms with a common propeptide. These are cleaved off during the promyelocytic stage of differentiation and the mature peptides, HNPs, are stored in azurophil granules. Myelocytes are the main produces of proHNPs, but lack the ability to process to mature HNPs and instead secrete proHNPs unprocessed to the bone marrow plasma, and from here to blood.
Aims: We hypothesized that proHNPs may serve as early and specific markers of granulopoiesis in patients undergoing hematopoietic SCT or high-dose chemotherapy.
Methods: Plasma samples were obtained daily from patients undergoing allogeneic SCT (n=11), autologous SCT (n=16), or high-dose chemotherapy (n=14). Furthermore, plasma samples were taken from patients with AML prior to chemotherapy (n=19) and healthy controls (n=39). ProHNPs, MPO, neutrophil gelatinase-associated lipocalin (NGAL), and lysozyme were measured by ELISA.
Results: In accordance with their short life span, neutrophils disappeared rapidly from circulation after myeloablative conditioning or high-dose chemotherapy. In all patients, plasma levels of proHNPs, MPO, NGAL, and lysozyme declined with the neutrophils counts. Of the investigated proteins, only proHNPs consistently disappeared from circulation along with neutrophils. In general, the plasma content of all proteins investigated started to rise before neutrophils could be detected in circulation. However, only proHNPs invariably preceded the rise in neutrophils. In patients undergoing allogeneic SCT, proHNPs rose five to six days before neutrophil counts. One patient developed septic shock during neutropenia. This was reflected in rising plasma levels of lysozyme and NGAL (Figure 1), which are expressed in non-myeloid tissues. In contrast, proHNPs and MPO followed neutrophil engraftment. Patients undergoing autologous SCT were treated with G-CSF to speed up neutrophil recovery and in these, proHNPs rose approximately two days before neutrophils were detected. As for patients receiving induction or consolidation chemotherapy, proHNPs predated neutrophils by two to four days. Patients with acute myeloid leukemia had significantly lower levels of plasma proHNPs than healthy controls, indicating that proHNPs in contrast to MPO are not produced by leukemic blasts.

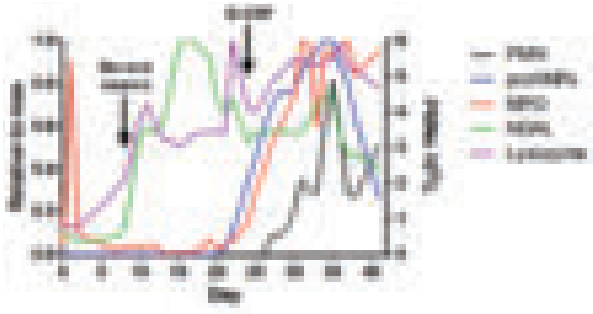

Figure 1.

Summary and Conclusions: ProHNPs are secreted exclusively by promyelocytes and myelocytes, *i.e.* myeloid cells not part of a leukemic clone in AML and ALL and can thus be taken as markers of normal hematopoiesis. In coherence with this, only proHNPs completely disappeared from circulation after myeloablative conditioning or high-dose chemotherapy and consistently returned along with normal granulopoiesis. Detection of proHNPs in plasma after chemotherapy induced bone marrow aplasia can thus been seen as an unequivocal indicator of reemerging granulopoiesis. Clinically, plasma proHNP could be an important tool to distinguish late take from primary graft failure in hematopoietic SCT patients, since measurement of proHNPs in plasma allows early detection of donor cell engraftment.

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POOR PROGNOSIS OF PATIENTS WITH SEVERE SINUSOIDAL OBSTRUCTION SYNDROME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN JAPANESE POPULATION:ON BEHALF OF COMPLICATIONS WORKING GROUP OF JSHCT

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Background: Sinusoidal obstruction syndrome (SOS) is recognized as one of lethal complications after hematopoietic stem cell transplantation (HSCT). We previously showed that the incidence of SOS after allogeneic HSCT was 11.0% by using the Japan Society for Hematopoietic Cell Transplantation (JSHCT) database and the Seattle criteria (ASH meeting 2013, abstract #2051).
Aims: Herein, we focused on the clinical outcomes of severe SOS patients in order to clarify the prognosis of those patients.
Methods: From 117 transplant centers of the JSHCT 618 patients were diagnosed with SOS between 1999 and 2010. Additional data on the diagnosis and clinical presentation were collected, and survival information was updated. For 462 patients the diagnosis of SOS was confirmed based on the Seattle criteria (presence before day 30 after HSCT, of at least two of the following features: (1) jaundice (2) hepatomegaly and right upper quadrant pain and (3) ascites and/or unexplained weight gain (≥2%)). The Baltimore criteria (hyperbilirubinemia ≥2 mg/dl before day 21 after HSCT and at least, two of the following features: (1) hepatomegaly (2) ascites and (3) weight gain (≥5%)) was used for further risk assessment. Severe SOS was defined as SOS with renal failure (serum creatinine of ≥3xULN or dialysis dependent) and/or respiratory failure (oxygen saturation <90% on room air, oxygen supplement required, or ventilator dependent) at diagnosis. Complete response for SOS (CR) was defined as resolution of all the signs and symptoms of SOS diagnostic criteria.
Results: A total of 107 out of 462 patients met the Baltimore criteria. One hundred and sixty-eight patients were diagnosed as having severe SOS. The median time from the day of transplant to diagnosis of severe SOS was 12 days (range, 0-28). The signs and symptoms at diagnosis were weight gain (90%), jaundice (78%), hepatomegaly (63%), right upper abdominal pain (57%), ascites (56%), respiratory failure (68%), and renal failure (64%). CR rate was only 22% in severe SOS patients (28% and 10% in patients with SOS diagnosed by the Seattle but did not fulfill the Baltimore criteria and in patients who fulfilled the Baltimore criteria, respectively (P=0.01, Fisher's exact test)). The main causes of death for severe SOS patients were SOS-related mortality (61%), disease progression (12%), and others (27%). Probabilities of overall survival calculated by using Kaplan-Meier method with SOS treated as time-dependent variable at day 100 and 2 years after HSCT were 22% and 10% in patients with severe SOS and 50% and 32% in SOS patients without organ failure, respectively (27% and 12% in patients with severe SOS diagnosed by the Seattle but did not fulfill the Baltimore criteria and 14% and 5% in patients who fulfilled the Baltimore criteria, respectively, P <0.001, logrank test).
Summary and Conclusions: This retrospective survey showed that patients with severe SOS had extremely poor prognosis even if the Seattle criteria was applied. We should establish risk classification of SOS, strategies for SOS prevention and treatment for those patients in order to improve overall survival.

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HIGH-DOSE PREPARATION WITH THIOTEPA/ETOPOSIDE/ARA-C/MELPHALAN (TEAM) *VS* BEAM FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTOHSCT) IN LYMPHOMA: A RETROSPECTIVE STUDY FROM THE EBMT

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Background: Thiotepa is an alkylating agent approved for high-dose therapy for autoHSCT. Because of its excellent capacity to cross the blood-brain barrier, it is regularly used for autoHSCT for primary CNS lymphoma (PCNSL). However, although thiotepa-based myeloablation might have benefits over traditional BEAM preparation because of better brain availability and less pulmonary toxicity, clinical level information about thiotepa-based autoHSCT outside of the PCNSL field is sparse.
Aims: AIM of the present retrospective study was to provide information on the potential risks and benefits of high-dose regimens using thiotepa instead of BCNU together with etoposid, ara-C, and melphalan (TEAM) in autoSCT for distinct subtypes of lymphoma outside of the PCNSL setting.
Methods: PRIMARY OBJECTIVE was to compare the outcome of TEAM-based autoHSCT (TEAM) with that of BEAM autoHSCT (BEAM) separately for diffuse large B-cell lymphoma (DLBCL; excluding PCNSL), follicular lymphoma (FL), and Hodgkin's lymphoma (HL). PRIMARY ENDPOINT was progression-free survival (PFS), secondary endpoints were overall survival (OS), non-relapse mortality (NRM), incidence of relapse (IR), engraftment, and early toxicity. ELIGIBLE were patients >18 years who were registered with the EBMT and had received TEAM or BEAM for a first autoHSCT between 2003-2011 for FL, DLBCL, or HL. Baseline patient, disease, and transplant data were collected from MED-A forms. Centers with potentially eligible patients were contacted to provide additional treatment and follow-up information. STATISTICAL ANALYSIS was based on a 1:2 matched pair comparison using stratified Cox and Fine & Gray regression models for comparison. Matching factors were age (+/- 10 years), sex, lymphoma subtype, time from diagnosis to autoHSCT, remission status at autoHSCT, performance status (PS), and year of autoHSCT.
Results: 309 patients could be matched (TEAM 110, BEAM 199); 7 BEAM patients were used several times as match. Additional information was provided for 174 of these 302 eligible patients. After data retrieval, a total of 42 TEAM could be compared to 72 BEAM matches. Median age of TEAM was 47 years, and 57% were male. 44% had HL, 40% DLBCL, and 16% FL. With a median time from diagnosis of 18 months, remission status at autoHSCT was CR/PR1 in 36%, CR/PR >1 in 50%, more advanced disease in 14%. 14% of the patients had a reduced PS (Karnofsky <90%) at autoHCST. There was no difference with their BEAM matches. There was one non-relapse death in the TEAM group and none in the BEAM group. 3-year PFS, OS, and IR for TEAM *vs* BEAM were 64% *vs* 60%, 82% *vs* 80%, and 33% *vs* 40% without any significant differences. Tolerability of both regimens (TEAM *vs* BEAM) was comparable with nausea (64% *vs* 73%), mucositis (57% *vs* 62%), and infections (55% *vs* 63%) as predominant toxicities. Pneumonia/pneumonitis was reported in 2.3% and 8.3% of TEAM and BEAM patients (p not significant). Other non-infectious complications were rare (10% and 18% in the TEAM and BEAM groups; p not significant) and were mainly attributed to gastro-intestinal and skin events.
Summary and Conclusions: TEAM is a valuable alternative to BEAM in autoHSCT for HL, DLBCL, or FL with no significant differences in terms of safety and efficacy.
Supported by a restricted grant from Riemser AG, Greifswald, Germany.

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CO-TRANSPLANTATION OF HAPLOIDENTICAL TRANSPLANTATION AND UMBILICAL CORD BLOOD UNIT PROVIDE SIGNIFICANT SURVIVAL BENEFIT FOR PATIENTS WITH LEUKEMIA

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Background: In the absence of a HLA-matched sibling donor, a HLA-mismatched/haploidentical transplantation can be considered an alternative for allogeneic stem cell transplantation (SCT), although its application is associated with the increased risk of severe acute graft-*versus*- host-disease (aGVHD), prolonged length of hospital stay, and poor overall survival(OS).
Aims: We conducted a HLA-mismatched/haploidentical transplantation combined with a third party single umbilical cord blood (UCB), known as co-transplantation, and compared to the haploidentical transplantation without UCB.
Methods: The co-transplantation group included 95 patients who received HLA-mismatched donor cell combined with third-party single cord blood cell and the control group included 63 patients who received haploidentical sibling donor cell alone.
Results: Neutrophil reconstruction duration of the co-transplantation group was 13d, platelet reconstruction duration was 15d, which have no significant difference compared to the control group (P >0.05). Recurrence rate were 22.4% and 26.2% respectively and with no statistical difference in the two groups (P >0.05). However, the cumulative incidence of aGVHD III-IV in the control group was significantly higher than that of co-transplantation group (36.4%vs19.5%, P<0.05). In the co-transplantation group, 3-year OS, disease free survival (DFS) and treatment related mortality (TRM) were 67.3%, 61.7% and 19.2% respectively and the results were significantly better than that of the control group (OS 42.9%, DFS 35.2% and TRM 38.3%, P <0.05).
Summary and Conclusions: In our experience the co-transplantation of haploidentical transplantation and unrelated cord blood unit offers significant survival benefit for patients with hematologic malignancies.

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IMPACT OF CD3/T REGS RATIO IN DONOR GRAFT ON SURVIVAL RATES IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: The therapeutic efficacy of allogeneic stem cell transplantation (alloSCT) for hematological malignancies relies largely on the graft *versus* leukemia (GvL) effect exerted by the donor CD3 cells, but an uncontrolled graft-*versus*-host-disease (GvHD) bears a risk of complications. On the other hand, T regs cells (CD4+CD25high Foxp3+) are believed to maintain tolerance and to inhibit GvHD after alloSCT; also, the Foxp3 gene encodes a transcription factor that is a key for thymic development, so T regs cells could preserve an optimal microenviroment for the reconstitution of functional immunity after alloSCT. Moreover, when looking at post allotransplant patients' outcomes, there is no evidence that donor graft CD3/T regs ratio may determine an effect in terms of OS, NRM and relapse free survival rates so far.
Aims: In this study we analyzed the graft CD3+/Tregs ratio (gCD3/Tregs R) and determined its impact on acute GVHD (aGVHD) and survival rates (OS, NRM and Relapse) after myeloablative alloPBSCT.
Methods: We analyzed 102 consecutive patients (median age 39 yy) transplanted with unmanipulated peripheral blood stem cells from an HLA identical related donor (n=78) or an HLA identical unrelated donor (n=32); diagnoses were acute myeloid leukaemia (n=82), acute lymphoblastic leukaemia (n=20).
Results: The median CD3+ and Tregs dose administered was 240 (range (r):

67-550) and 13x10^6/Kg (r: 2-21), respectively; the median gCD3/Tregs R was 22 (r: 8-250). Patients were subdivided into a high gCD3/Tregs R(>36) group(n=46) and a low gCD3/Tregs R(<36) group (n=56). The incidence of aGVHD (grade II-IV) in the low gCD3/Tregs R (LR) group was lower than in the high gCD3/Tregs R (HR) group (10/56 or 18% *vs* 35/46 or 77%, p<.001). The OS, NRM and relapse rate at 3 years was 54, 29 and 34%, respectively. Comparing LR with HR group a statistically significant difference is demonstrated for OS and NRM rates at 3 years (65 *vs* 31%,p<.004; 3 *vs* 71%, p<.001, Table 1), respectively, but not for the R one (35 *vs* 30%, p=ns). Comparing aGVHD+ with aGVHD- group OS, NRM and relapse were always statistically significant different at 3 years (39 *vs* 65%,p<.005; 61 *vs* 7%,p<.001; 9 *vs* 53%,p<.002).
Summary and Conclusions: Taken together, our data may suggest that Tregs content is able to mediate protective effects against aGVHD, while preserving GvL effects as demonstrated by relapse rate comparison between H and LR groups. However, larger studies are needed to understand the real contribution of gCD3/Tregs R on survival rates.

P514

THE IMPACT OF PRETRANSPLANT IRON CHELATING THERAPY WITH DEFERASIROX ON THE OUTCOME IN PATIENTS WITH SEVERE APASTIC ANEMIA UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Pretransplant iron overload have shown the association with a higher risk of transplant-related mortality (TRM) and other complications including fungal infections, hepatic dysfunction after allogeneic stem cell transplantation (SCT). Although these negative impacts of iron overload on the transplant outcomes have been evaluated in the patients with various hematologic disorders including thalassemia, myelodysplastic syndrome, and acute leukemia, it has yet to be analyzed completely for the patients with severe apastic anemia (SAA) undergoing allogeneic SCT. Especially, the impact of pretransplant iron chelating therapy (ICT) on the transplant outcomes was uncertain.
Aims: The aim of this study was to evaluate the impact of pretransplant ICT with deferasirox on the survival outcome and infection rate in patients with SAA undergoing allogeneic SCT.
Methods: we retrospectively analyzed 159 adult patients with SAA who underwent allogeneic SCT from Mar. 2002 to Dec. 2012. They had available pretransplant serum ferritin data. Among them, 50 patients were received pretransplant ICT with deferasorox, when their serum ferritin was more than 1000 μg/L, whereas 59 patients had more than 1000 μg/L of serum ferritin but did not received ICT. Another 50 patients had less than 1000 μg/L of serum ferritin at transplant.
Results: Sixty-nine men and 90 women were assessed. Their median age was 34 years (range, 13-59 years). Primary engraftment was achieved in all, but 11 patients developed secondary graft failure. After a median follow-up of 47.1 (range, 6.1-124.9) months for survivors, the patients with more than 1000 μg/L of pretransplant serum ferritin have significantly increased risk of TRM (15.3% *vs* 3.5%. P=0.039), and lower overall survival (OS) (84.8% *vs* 96.6%, P=0.036) than those with the patients who had less than 1000 μg/L of serum ferritin. For 50 patients receiving pretransplant ICT with deferasirox, median serum ferritin levels decreased from 1995 μg/L at the initiation of ICT to 1240 μg/L before SCT, including 10 patients to less than 1000 μg/L. Median duration of ICT before SCT was 3.6 months, and median MDD (mean daily dose) was 14.8 mg/kg per day. In the comparison between iron overload patients with ICT and those without ICT, pretransplant ICT group showed a lower infection rate after SCT (34% *vs.* 59%, P=0.008). In terms of OS, the possible survival benefit of pretransplant ICT was observed in unrelated transplant setting (93.5% *vs.* 78.3%, P=0.090).
Summary and Conclusions: These results indicate that higher serum ferritin was associated with a negative impact on outcome after SCT in SAA. Pre-SCT ICT can reduce the incidence of infection after SCT. The possible survival benefit of Pre-SCT ICT was present especially in unrelated donor SCT.

P515

IMPACT OF MONOSOMAL KARYOTYPE ON THE OUTCOMES OF ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION FOR OLDER PATIENTS (>60 YEARS OF AGE) WITH AML/MDS

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Background: Karyotype at diagnosis has emerged as the most significant prognostic factor in adult patients (pts) with myeloid malignancies. Recently a distinct group of cytogenetic abnormalities, referred to as monosomal karyotype (MK) have been shown to have particularly poor prognosis. MK was defined (Breems, 2008) as a karyotype with at least two autosomal monosomies or a single autosomal monosomy in combination with at least one structural abnormality. A slight benefit for allogeneic hematopoietic cell transplantation (allo-HCT) compared to chemotherapy or autologous HCT has been demonstrated in young AML (≤60) pts with MK (Cornelissen, 2012). However, the curative potential of allo-HCT in older (≥60) pts AML/MDS with MK is not known.
Aims: 1. Evaluate the impact of cytogenetic abnormalities on the progression-free survival (PFS) of older patients (>60 years) with AML or MDS who underwent an allo-HCT at Mayo Clinic Arizona between 2003-2013.
2. Determine the cumulative incidence of relapse and non-relapse mortality (NRM) in different cytogenetic risk groups.
3. Evaluate other factors that impact PFS outcome in older patients who undergo allo-HCT for AML/MDS.
Methods: In this retrospective review, we analyzed outcomes for 75 AML (n=53) or MDS (n=22) pts ≥60 years old who underwent allo-HCT at Mayo Clinic Arizona between 2005 and 2013, based on cytogenetic grouping as follows: cytogenetically normal (CN, n=26), cytogenetically abnormal but not MK (CA, n=32), and MK (n=17). Univariate and multivariate statistical analyses were performed to evaluate the impact of these 3 cytogenetic groupings on outcome (primary endpoint progression-free survival). The median age for all pts was 66 (60-76), and median follow-up for surviving pts was 26 months (range 90 days – 7.4 years). Forty-one pts had high-risk disease (MDS >5% blasts or AML not in CR), while 34 were considered standard risk (MDS <5% blasts or AML in CR). The majority of pts had good performance status (KPS ≥90% in 70), but 36 pts (48%) had an HCT-CI score of ≥3. Conditioning was myeloablative (Bu-Cy) in 4, reduced toxicity FBM (Fludarabine, BCNU, Melphalan) in 41, and RIC/NMA (fludarabine/busulfan 6.2mg/kg or fludarabine/TBI200cGy) in 30. Donors were matched related in 20, matched unrelated in 38, and mismatched unrelated in 17. GVHD prophylaxis included tacrolimus in all pts combined with mycophenolate mofetil (37) or methotrexate (38); 53 pts received in-vivo T-cell depletion with rabbit anti-thymocyte globulin (rATG, Thymoglobulin, Genzyme, Cambridge, MA).
Results: The Kaplan-Meier estimate of PFS at 2 yrs was 50% (CN), 38% (CA), and 3% (MK); *P*=0.004 (Figure 1). The cumulative incidence of relapse at 1 yrs (with death without relapse as a competing risk) was 26% (CN), 19% (CA), and 68% (MK); *P*=0.01. Relapse was the major cause of death in all cytogenetic groups but accounted for a higher percentage of deaths in pts with MK (50%). In addition NRM at 1 year was significantly higher in MK pts primarily due to GVHD and other causes (13% (CN), 32% (CA), and 58% (MK)). In a Cox proportional hazards analysis adjusted for age (≤66 *vs.* >66), risk status (standard *vs.* high), conditioning (myeloablative [Bu-Cy+FBM] *vs.* RIC/NMA), use of ATG (yes/no), donor type (unrelated *vs.* related), and HCT-CI (0-2 *vs.* ≥3), the only factor predictive for inferior PFS was cytogenetic status (MK *vs.* CN, HR 3.6 [95% CI, 1.52 – 8.72]; P=0.004); (MK *vs.* CA, HR 2.56 [95% CI 1.19 – 5.48]; P=.017).

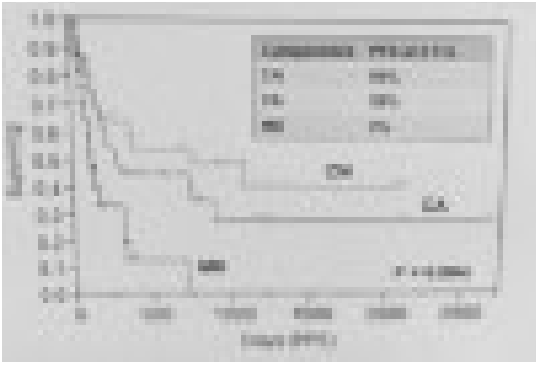

Figure 1.

Summary and Conclusions: Unlike younger patients ≤60 years(Cornelissen, 2012), older patients ≥60years with myeloid malignancies with MK who undergo Allo-HCT have very poor outcomes and limited curative potential. This is primarily due to high rates of relapse and NRM. Although larger studies will be required to confirm our results, novel transplant approaches or post-transplant strategies to prevent relapse should be a focus of future studies in this incurable pt population.

Hematopoiesis, stem cells and microenvironment

P516

THE RUNX-PU.1 PATHWAY PRESERVES NORMAL AND AML/ETO9A LEUKEMIC STEM CELLS

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Background: Runx transcription factors contribute to hematopoiesis and are frequently implicated in hematologic malignancies. All three Runx isoforms are expressed at the earliest stages of hematopoiesis; however their function in hematopoietic stem cells (HSCs) and leukemic stem cells is not elucidated.
Aims: Here, we aimed to investigate the functional effects of Runx factors on driving the expression of the hematopoietic transcription factor PU.1 in normal HSCs and leukemic stem cells.
Results: Mechanistically, using a knock-in mouse model in which all three Runx binding sites in the -14kb enhancer of PU.1 are disrupted we observed failure to form chromosomal interactions between the PU.1 enhancer and its proximal promoter. Consequently, decreased PU.1 levels resulted in diminished long-term HSC function through HSC-exhaustion, which could be rescued by reintroducing a PU.1 transgene. Similarly, in a mouse model of AML/ETO9a leukemia, disrupting the Runx binding sites resulted in decreased PU.1 levels. Surprisingly, leukemia onset was delayed and limiting dilution transplantation experiments demonstrated functional loss of leukemia initiating cells.
Summary and Conclusions: Our data demonstrate that Runx-dependent PU.1 chromatin interaction and transcription of PU.1 are essential for both normal and leukemia stem cells. Although low PU.1 levels have been considered as hallmark of leukemia, reducing PU.1 might also target the leukemic stem cell.

P517

THE TISSUE INHIBITOR OF METALLOPROTEINASES-1 (TIMP-1) MODULATES HUMAN HSPC FUNCTIONS THROUGH CD63/PI3K/AKT SIGNALING

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Background: Hematopoietic Stem and Progenitors Cells (HSPCs) reside within the Bone Marrow (BM) stem cell niche, a nurturing environment believed to protect HSPCs from external insults. However, recent findings showed that HSPCs actively sense pro-inflammatory factors. Here, we investigate the role of Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) within the BM microenvironment. First described as an inhibitor of Metalloproteinases (MMPs), TIMP-1. also displays cytokine-like functions. We recently found that TIMP-1$^{-/-}$ mice have decreased BM cellularity and that the engraftment capability of TIMP-1$^{-/-}$ HSCs is impaired, due to cell-cycle dysregulation. Strikingly, TIMP-1 is a key member of the inflammatory network and its expression is increased in response to pro-inflammatory cytokines: as such, TIMP-1 may play a key role in regulating emergency hematopoiesis under inflammatory stimuli.
Aims: As a contribution in dissecting the molecular pathways underlying the cross-talk between inflammatory factors and hematopoiesis, our study aims are: 1) to characterize the role of TIMP-1 in HSPCs proliferation and survival within the hematopoietic microenvironment; 2) to uncover TIMP-1's membrane receptor partner in HSPCs and elucidate the downstream signaling pathway ignited by TIMP-1 binding.
Methods: Human CD34$^+$ HSPCs were isolated from different sources, including BM, mobilized peripheral blood (mPB), and cord blood (CB). Investigations were performed at two levels: *1) TIMP-1 functional effect*: CD34$^+$ HSPC proliferation in the presence of different doses of TIMP-1 was tested *in vitro* by CFU-C and Long-Term Cultures (LTCs). TIMP-1 effects on cell proliferation and survival were assessed by PI staining and AnnexinV/PI staining, respectively. Primitive HSC potential was assessed by transplantation of TIMP-1-treated CD34$^+$ cells into immonodeficient mice (NOD/Shi-scid/IL-2Rγ*null* mice). In order to confirm the cytokinic nature of TIMP-1's effects, similar tests were performed in the presence of GM-6001, a synthetic MMP inhibitor. *2) TIMP-1 signalling pathway*: The expression of the tetraspannin receptor CD63 was assessed by flow cytometry, whereas its functional role was assayed by nucleofection of CD63-specific siRNAs in HSPCs. Downstream molecular targets of TIMP-1 (such as pAkt, CycD1, p27) were also confirmed by qPCR and flow cytometry.
Results: Our results suggest that rhTIMP-1 promotes CD34$^+$ cell survival and stimulates (in a dose-dependent manner) HSPC expansion (Figure 1A), without compromising their engraftment potential when transplanted into immunodeficient mice. In addition, clonogenic assays performed in the presence of GM-6001 confirmed that TIMP-1 effects are independent on MMP-inhibition (Figure 1B). The dissection of TIMP-1 signaling pathway indicated that the tetraspanin CD63 receptor is required for TIMP-1's cytokine functions in HSPCs and further investigation indicated that TIMP-1 stimulation induces PI3K recruitment and phopshorylation of AKT (Figure 1C), key modulators of survival/proliferation pathways in HSCs. Downstream targets of pAKT are also modulated, including the proliferation marker CycD1 (Figure 1D), which levels are increased upon exposure to TIMP-1.

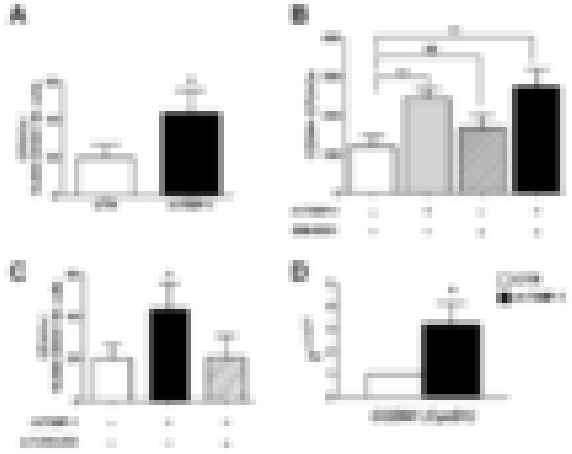

Figure 1.

Summary and Conclusions: These findings indicate that TIMP-1 promotes HSPC expansion and survival in human HSPCs via the activation of CD63/PI3K/pAKT signaling pathway. As such, TIMP-1 might be a key player in the modulating emergency hematopoiesis under inflammatory conditions.
RML and AC equally contributed.

P518

LACK OF GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) RESULTS IN B CELL LYMPHOPROLIFERATIVE DISORDER IN MICE

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Background: Glucocorticoids (GC) are widely used as immunosuppressive drugs and antitumor agents in some acute leukemias and multiple myeloma. Therapeutic doses of GC induce growth suppressive and cytotoxic effects on various leukocyte types including B cells. Molecular mechanisms of GC action include induction of GC target genes. Glucocorticoid-induced leucine zipper (GILZ) is a gene rapidly, potently and invariably up-regulated by GC treatment. It mediates a number of GCs effects, such as control of cell proliferation, apoptosis and differentiation. GILZ suppresses Ras/MAPK/Erk and NFkB pathways (Ayroldi *et al.*, Blood 2001; Bruscoli *et al.*, JBC 2012) and promotes TGF-b signaling in T cells (Bereshchenko *et al.*, Cell Reports, in press). It belongs to TSC22d family, members of which were recently found mutated in diffuse large B cell lymphoma patients (Pasqualucci *et al.*, NatGen 2011).
Aims: Here we address the physiologic role of GILZ in normal hematopoiesis, and evaluate its role in mediation of GC effects on various blood cells, using genetic approach.
Methods: Mice deleted for *gilz* gene were recently generated. We have monitored white blood cell counts in wild type (wt) and in gilz knock-out (KO) mice overtime. Development of lymphoid and myeloid lineages was evaluated both by peripheral blood (PB) cell counts (Hematocrit), and by flow cytometry analysis of bone marrow (BM), spleen and PB using Mac-1, B220, CD43, IgM and IgD staining. To test whether the effect of *gilz* gene deletion on hematopoiesis is self-intrinsic, we analysed hematopoiesis in BM chimeras generated by transplanting wt or gilz KO BM cells into lethally irradiated hosts.
Results: Young *gilz* KO mice showed normal body and lymphoid tissues weights and cell counts in PB, thymus, spleen, peripheral lymph nodes and BM. However, overtime *gilz* KO mice showed a 1.5-2 fold increase in white blood cell counts in PB. Increase in lymphocyte counts was due to accumulation of B220$^+$ cells, while the number of Mac-1$^+$ cells did not differ between wt and *gilz* KO mice. Flow cytometry analyis of B220$^+$ cell compartment in BM revealed an increase in the frequency and number of pre-B cells (IgM^{lo}IgD^{lo}), immature (IgM^{hi}IgD^{lo}) and recirculating B cells (IgM^{lo}IgD^{hi}) already in 8-week old mice. Frequency of Mac-1$^+$ cells was decreased in BM of *gilz* KO mice, although their

absolute number did not significantly differ between wt and *gilz* KO mice. Specific expansion of donor-derived B220$^+$ cells was also observed in BM of radiation chimeras, demonstrating the effect of GILZ deletion on B cells is self-intrinsic to hematopoietic cells. Preliminary data suggest that the defect start sas early as at common-lymphoid progenitor (CLP) stage. Treatment of purified B220+ cell with GC *in vitro* resulted in similar degree of apoptosis in wt and gilz KO cells, suggesting that the increase in B cells *in vivo* does not result from decreased sensitivity to the death induced by endogenous GC.
Summary and Conclusions: Our results show that lack of GILZ results in specific defect in B cell development, leading to the expansion of B220$^+$ cells compartment, associated with the expansion of early B cell progenitor cells and suggest that degerulation of GILZ expression may contribute to proliferation or differentiation of early B cells and pathologies of B cell lineage.

P519

TRUNCATION OF GM-CSF AND IL-3 BY DIPEPTIDYLPEPTIDASE 4 (DPP4) ALTERS THEIR RECEPTOR BINDING, SIGNALING AND FUNCTIONAL ACTIVITY IN NORMAL AND MALIGNANT HEMATOPOIETIC CELLS.
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Background: Dipeptidylpeptidase 4 (DPP4) is a serine protease that enzymatically cleaves select penultimate amino acids of proteins, resulting in functional alterations. Functional roles of full length (FL) *versus* Truncated (T) cytokines, as well as the mechanisms by which they may impart differing signaling and effects, have not been comprehensively elucidated. Importantly, the signaling alterations between full length and DPP4 truncated proteins, including the ability of the truncated protein to induce signaling that the full length factor can not, has not been investigated. We recently published that the number of cytokines, chemokines and growth factors that have putative DPP4 truncations sites has been dramatically underestimated and may have yet unappreciated clinical application.
Aims: 1) To determine the ability of DPP4 truncated GM-CSF (TGM) and IL-3 (T3) to reciprocally alter the receptor binding, signaling and functional activity of both the full length GM-CSF (FLGM) and IL-3 (FL3).
2) To investigate the signaling induced by FLGM and FL3 *vs* TGM and T3.
Results: We utilized the human, factor-dependent TF-1 cell line, as well as primary human cord blood (CB) cells and primary cells from patients with Acute Myeloid Leukemia (AML) to observe that DPP4 truncation of GM-CSF and IL-3 results in decreased colony stimulating factor (CSF) activity and in significant blocking of the functional ability of the full length CSF in normal and malignant hematopoietic progenitor cells (HPC) at less than a 1:1 ratio of the truncated protein to the full length. Receptor binding studies confirmed our hypothesis that DPP4 truncated GM-CSF (TGM) and IL-3 (T3) have enhanced receptor affinity and can compete to blunt the binding of their full length forms. *In vivo* studies demonstrated that both exogenously added TGM and T3 suppressed the effect of exogenously added full length GM-CSF (FLGM) or IL-3 (FL3) on progenitor cell numbers per femur and diminished HPC cycling. Mechanistic signaling studies show that the signaling induced by the full length *vs* DPP4 truncated proteins is altered with respect to miRNA expression, global protein quantitation, phosphorylation, acetylation, and reactive oxygen species (ROS) induction. Interestingly, miRNA, phosphorylation and global protein analysis showed that DPP4 truncated proteins were able to induce unique signaling that the full length counterparts did not induce, as well as signaling that overlapped with their full length counterparts, revealing the complexity of the signaling interactions and heretofore unknown activities of DPP4 truncated proteins.
Summary and Conclusions: These data show for the first time the ability of DPP4 trucanted proteins (TGM and T3) to reciprocally cross compete for receptor binding, and blunt the functional activity, of FLGM and FL3 on normal and malignant HPC, as well as induce differential signaling compared to that of their full length counterparts.

P520

GATA2 REGULATES DIFFERENTIATION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS
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Background: The bone marrow microenvironment comprises multiple cell niches derived from bone marrow mesenchymal stem cells (BM-MSCs). However, the molecular mechanism of BM-MSC differentiation is poorly understood. Transcription factor GATA2 is indispensable in hematopoietic stem cells (HSCs) as well as other hematopoietic lineages, suggesting that it may maintain BM-MSCs in the immature state and contribute to their differentiation.
Aims: We assessed the role of GATA2 in BM-MSC differentiation.
Methods: Murine BM-MSCs were established from *Gata2* conditional knockout mice (Charles *et al.* 2006), with MesenCult MSC Basal Medium (Stem Cell Technology). For GATA2 deletion, the BM-MSCs were infected with retroviruses expressing iCre (Goyama *et al.* 2008). Human BM-MSCs were established from BM mononuclear cells from healthy volunteers, with Dulbecco's modified Eagle medium containing 20% fetal bovine serum, and 10 ng/mL basic fibroblast growth factor (Peprotech). GATA2 loss- and gain-of-function analysis was conducted with specific siRNA and MSCV retrovirus vector, respectively. For transcription profiling, the Human Genome U133 Plus 2.0 Array (Affymetrix) was used. Gene Ontology (GO) analysis was based on DAVID software. A colony-forming assay was conducted with semisolid culture (Stem Cell Technology). Adipocyte differentiation was assessed by Oil Red O staining.
Results: Immunophenotypic analyses confirmed that established BM-MSCs expressed typical surface markers (CD29$^+$CD44$^+$Sca-1$^+$CD11b$^-$CD34$^-$CD45$^-$ and CD29$^+$CD44$^+$CD90$^+$CD105$^+$CD14$^-$CD34$^-$CD45$^-$ for murine and human BM-MSCs, respectively). Differentiation of murine GATA2-deficient BM-MSCs into adipocytes accelerated oil-drop formation and increased adipocyte-specific gene expression, including *Cebpb, Pparg,* and *Fabp4*. Further, GATA2 loss- and gain-of-function analyses in human BM-MSCs confirmed that decreased and increased GATA2 expression accelerated and suppressed, respectively, BM-MSCs differentiation into adipocytes and was accompanied by significant changes in the expression of representative adipocyte-related genes such as *CEBPA*, *CEBPB*, *PPARG*, and *FABP4*. Next, microarray analysis was conducted to characterize the GATA2 target gene ensemble in human BM-MSCs. An average of 2 independent analyses demonstrated that 90 and 189 genes were upregulated or downregulated by a factor of 2, respectively, with GATA2 knockdown. GO analysis identified significant enrichment of genes involved in cell cycle regulation. Concomitantly, flow cytometric analysis based on propidium iodide staining demonstrated that the number of G1/G0 cells increased after GATA2 knockdown. To test whether the ability of BM-MSCs to support HSCs was compromised by reduced GATA2 expression, GATA2 knockdowned BM-MSCs were cocultured with cord blood-derived CD34$^+$, cells, demonstrating that HSC frequency and colony formation were significantly decreased. Finally, because GATA2 expression is decreased in both HSCs and BM-MSCs in patients with aplastic anemia (Fujimaki *et al.* 2001, Xu *et al.* 2009), we evaluated whether the addition of various cytokines influences adipocyte differentiation. Among the cytokines, BMP4 suppressed adipocyte differentiation and significantly induced GATA2.
Summary and Conclusions: Decreased GATA2 expression induced BM-MSC differentiation, particularly into adipocytes, leading to a decreased hematopoietic supporting capacity. Further efforts to identify the regulatory mechanism of GATA2 in HSCs and BM-MSCs may lead to the development of novel therapeutic approaches for bone marrow failure syndrome.

P521

ISOLATION OF A CD34+CD38-CD45+CD117HI POPULATION HIGHLY ENRICHED IN HEMATOPOIETIC STEM CELLS (HSC) IN HUMAN EMBRYONIC AND FETAL LIVER: A MODEL TO HIGHLIGHT KEY REGULATORS OF *EX VIVO* HSC EXPANSION?
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Background: HSCs are characterized by their ability to both self-renew and differentiate to give rise to the different hematopoietic cell lineages. *Ex vivo* expansion of HSCs without alteration of self-renewal, homing in the bone marrow, and pluripotentiality represents a major therapeutic challenge, especially for treatment of malignant hemopathies, immune deficits or severe anemia. Unlike adult HSCs, which are quiescent, embryonic and fetal HSCs proliferate actively, especially in the fetal liver that is the major site of HSC amplification during embryonic development. Knowledge of the factors and mechanisms which are involved in the regulation of HSC amplification during their development in fetal liver should then provide clues allowing a better control of *ex vivo* expansion of these cells for therapeutic applications.
Aims: Undertake a functional and molecular characterization of populations enriched in HSCs in human embryonic and fetal liver (7 to 26 weeks of gestation - WG).
Methods: On the basis of the CD34$^+$CD38$^-$CD45$^+$, population, we tested: CD110 (Mpl receptor), CD93 (AA4.1) and CD117 (c-kit) as surface markers that could enrich this population in HSCs.
Results: Our results indicate that while CD110 and CD93 do not allow any HSC enrichment during embryonic stages, the CD34$^+$CD38$^-$CD45$^+$CD117^{hi} population presents remarkable potentialities as soon as 7 to 8 WG, which persist during fetal liver development. *In vitro*, long-term culture – initiating cell (LTC-IC) frequency on MS5 support is very high (1/4 to 1/2), and significantly superior to that observed for the CD34$^+$CD38$^-$CD45$^+$CD117$^-$ and CD34$^+$CD38$^-$

CD45+ control populations (1/3330 and 1/50, respectively). In addition, extended LTC-IC (e-LTC-IC) showed that not only CD34+CD38-CD45+CD117hi cells could be maintained in culture for more than 200 days (*versus* 33 days for CD34+CD38-CD45+CD117- cells) with a 8000 fold amplification of hematopoietic cells, but that a significant fraction of immature cells was maintained during passages. *In vivo* NSG engraftment is under investigation.
Summary and Conclusions: All together, our results highlight a very immature CD34+CD38-CD45+CD117hi population present early in the embryonic liver, highly enriched in HSPCs, endowed with high amplification potential *in vitro*, and which persists during development in the liver, in the bone marrow, and in the cord blood. Genome wide transcriptomic analysis of this population should reveal key regulators of HSC amplification that will provide a better context to expand HSC for cell therapy purpose.

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A ROLE OF C/EBP-ALPHA IN DEVELOPMENT OF PLASMACYTOID DENDRITIC CELLS IN THE THYMUS
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Background: While there is convincing evidence that conventional dendritic cells (cDCs) are derived from myeloid progenitors, the origin of plasmacytoid dendritic cells (pDCs) is less clear.
Aims: The aim of this project was therefore to determine the ancestry of pDCs under steady-state conditions *in vivo* by using a myeloid-specific reporter mouse model and to elucidate regulatory mechanisms involving the myeloid transcription factor C/EBPα in their early development.
Methods: The number of pDCs and their EYFP expression was determined by FACS analysis in spleen, thymus and lymph nodes of both, Cebpa^{Cre} EYFP as well as Mx1^{Cre} Cebpa$^{F/F}$ mice. The first model allows tracing of C/EBPα-positive myeloid progenitors and their progeny by EYFP expression, the latter results in a bone marrow specific knock-out (ko) of C/EBPα and a consecutive loss of all mature myeloid cells. DC formation was modelled *in vitro* by incubation of lineage negative bone marrow (BM) progenitors with FLT3L for 8 days. Apoptosis was detected by annexin V/propidium iodide staining and gene expression profiling was performed using Affymetrix GeneChip Mouse Gene 1.0 ST arrays.
Results: In Cebpa^{Cre} EYFP mice the percentage of EYFP-positive pDCs was increased in the thymus as compared to spleen and lymphnodes (21% *vs.* 13% and 12%; p<0.01) suggesting that a higher number of thymic pDCs is derived from Cebpa-positive myeloid progenitors. Accordingly, bone marrow-specific lack of Cebpa resulted in reduced numbers of thymic pDCs (0.12% in Cebpa wt *vs.* 0.04% in Cebpa ko mice; p<0.01), while the numbers of pDCs in spleen and lymph nodes were not affected. Analysis of pDC-specific surface molecules did not show any difference between myeloid-derived thymic pDCs and their EYFP-negative counterparts. Modelling dendritic cell formation *in vitro* by incubating BM progenitor cells with FLT3L revealed a critical role of C/EBPα in early DC development: while BM progenitors isolated from wt mice proliferated and differentiated into cDCs and pDCs, Cebpa ko BM progenitors displayed increased apoptosis and DC formation was almost absent. Gene expression analysis of FLT3L-stimulated BM progenitors showed not only C/EBPα-dependent upregulation of DC-specific genes, but also upregulation of TNFα and NF-κB-dependent genes including the anti-apoptotic factor Bcl2A1. Addition of TNFα to FLT3L during *in vitro* incubation reduced the percentage of apoptotic cells and partially restored DC formation of Cebpa ko BM progenitors.
Summary and Conclusions: C/EBPα significantly contributes to formation of pDCs in the thymus. Upregulation of Bcl2A1 expression via TNFα and NF-κB-signalling may be among crucial mechanisms of C/EBPα in early DC development.

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RECOMBINANT HUMAN IL-12 BUT NOT FILGRASTIM DECREASE ALL-CAUSE MORTALITY IN A PRIMATE MODEL OF ACUTE RADIATION SYNDROME: RESULTS FROM BLINDED VEHICLE-CONTROLLED GLP STUDY
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Background: Hematopoietic Syndrome of Acute Radiation Syndrome (HSARS) is life-threatening illness caused by rapid bone marrow ablation.
The Radiation Injury Treatment Network (RITN) recommends off-label use of leukocyte growth factors. A single published, randomized controlled study (Farese *et al.* Radiat Res. 2013) in non-human primates (NHP) showed that addition of 18 daily filgrastim injections (rHuG-CSF) to intensive, trigger-based medical management (antibiotics, blood products, intravenous fluids) improved survival by 38%. Significant delays in the initiation and potential shortages of full supportive care treatment can be expected in the mass casualty radiation disaster scenarios emphasizing importance of the development of countermeasures that can be effective as single agents without intensive medical management. Recombinant human interleukin-12 (rHuIL-12, HemaMax™) is being developed for mitigation of HSARS under the FDA Animal Rule using a NHP model of HSARS. Our previous randomized, blinded, placebo-controlled GLP study in the NHP model of HSARS showed that a single subcutaneous injection of rHuIL-12 (50ng/kg-500 ng/kg) 24-25 hours after lethal total body irradiation (700cGy; $LD_{90/60}$ significantly improved overall survival without the use of antibiotics, fluids or blood transfusions (log rank test p<0.05). rHuIL-12 also reduced incidence of severe neutropenia and thrombocytopenia, systemic infection and internal organ hemorrhage. Phase 1 randomized double-blind placebo controlled safety studies in 92 healthy human volunteers demonstrated that rHuIL-12 is safe and well-tolerated at unit dose levels up to 12µg (170ng/kg for a 70 kg adult).
Aims: Here we report the results from our second GLP randomized blinded vehicle-controlled NHP study that was designed to confirm to compare rHuIL-12 to rHuG-CSF for treatment of HSARS.
Methods: Rhesus macaques were irradiated (700 cGy) and treated with a single subcutaneous injection of vehicle or rHuIL-12 175ng/kg 24-25 hours after irradiation, daily subcutaneous injections of rHuG-CSF at 10µg/kg/d for 18 days starting 24-25 hours after irradiation, or combination of a single rHuIL-12 and 18 rHuG-CSF injections (26-36 animals/group; male:female=1:1) without antibiotics, intravenous fluids or any blood products.
Results: As shown in Figure 1, single injection of rHuIL-12 increased survival compared to rHuG-CSF (56% *vs* 31%, Logrank test p<0.05) while rHuG-CSF did not provide any survival benefit over the control group (36%). Combination of rHuIL-12 and rHuG-CSF was similar to the rHuIL-12 monotherapy group (58% survival). rHuIL-12 significantly decreased incidence of severe neutropenia (<100 cells/µl) and thrombocytopenia (<10,000 cells/µl) compared to vehicle and to rHuG-CSF. Combination of rHuIL-12 with rHuG-CSF resulted in further improvement of neutrophil and platelet counts. rHuIL-12 also decreased incidence of severe infections, while rHuG-CSF increased incidence of infections and mucositis. During the highest mortality period (Days 10-18) rHuIL-12 improved neutrophils, platelets, lymphocytes, red blood cells, and reticulocytes counts. rHuG-CSF increased neutrophil and platelet counts starting from day 14 only, had no effect on reticulocytes, and negative impact on lymphocytes and red blood cell.

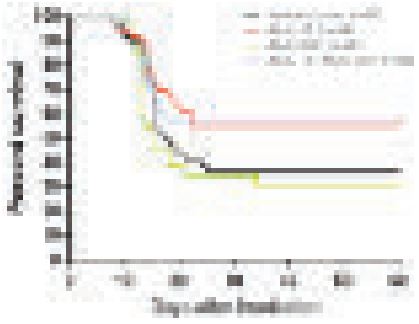

Figure 1. Survival of rhesus monkeys following lethal radiation and treated 24 hours post radiation with either vehicle, rHuIL-12, filgrastim (rHuG-CSF), or combination.

Summary and Conclusions: In conclusion, single subcutaneous injection of rHuIL-12 alone reproducibly improves survival in the NHP model of HSARS at dose levels shown to be safe in healthy human volunteers, while rHuG-CSF has no survival benefit in this model. These results support further development of rHuIL-12 as a medical countermeasure for HSARS.

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HISTONE DEACETYLACE INHIBITOR VORINOSTAT REDUCES KIT EXPRESSION BY DEREGULATION OF THE TRANSCRIPTIONAL COMPLEX AND INDUCTION OF HETEROCHROMATIN
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Background: Mast cell development and survival requires the binding of stem cell factor (SCF) to its receptor KIT. Mastocytosis is a disease defined by the abnormal increase in mast cell numbers due to a point mutation that render the KIT receptor ligand independent. Today there is no cure for mastocytosis which is lethal in its aggressive form. Vorinostat was the first histone deacetylase inhibitor to be approved for use in cancer therapy. The aim of this study is to investigate the effect of Vorinostat on KIT mutated mast cells and to define a mechanism for the effect obtained.
Aims: The aim of this study is to investigate the effect of Vorinostat on KIT mutated mast cells and to define a mechanism for the effect obtained.
Results: We found that Vorinostat induced apoptosis, dose and time dependently, in KIT D816V mutated mast cells. Vorinostat caused an efficient down-regulation of the KIT receptor, both on mRNA and protein levels as well as of the activity of pathways downstream of KIT. Analysis of transcription factors known to regulate KIT transcription showed that GATA1/2, Myb, ETS2, LOM2

and Sp1 all were downregulated after Vorinostat treatment. However, inhibiting the expression of these genes by RNAi failed to inhibit KIT expression to the same degree as Vorinostat treatment. When analyzing the histone modifications of the c-kit locus, using ChIP in a D816V mutated cell line, Vorinostat treatment induced repressive chromatin mark H3K9 while active chromatin mark H3K18 acetylation was reduced, indicating that the chromatin region had been closed from transcription. To investigate the clinical relevance, primary mast cells from three patients with mild to aggressive systemic mastocytosis was isolated by MACS separation and treated *ex vivo* with 5μM Vorinostat for 24h, whereby the mast cell population entirely vanished.
Summary and Conclusions: In this study we demonstrate that Vorinostat downregulates D816V mutated KIT, by mechanism of heterochromatin formation at the c-kit locus. These results can be translated into the clinic as we show that primary mastocytosis patient cells are sensitive to Vorinostat

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ASSOCIATION OF AN IMPAIRED BONE MARROW VASCULAR MICROENVIRONMENT WITH PRIMARY FAILURE OF PLATELET ENGRAFTMENT AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Primary failure of platelet engraftment (PFPE), defined as the engraftment of all peripheral blood cell lines aside from prolonged thrombocytopenia (platelet≤20×10^9/L or dependence on platelet transfusions) for more than 90 days after allogeneic hematopoietic stem cell transplantation (allo-HSCT), is a serious complication following allo-HSCT. Nevertheless, its underlying mechanisms remain to be elucidated. We previously reported that prolonged thrombocytopenia after allo-HSCT may be related to a reduction in ploidy and an immaturation of megakaryocytes(MKs) (Zhang X, *et al.* **Biol Blood Marrow Transplant**. 2011; 17:274-280). Recently, we have established a reliable assay for detecting the equivalents of the murine bone marrow (BM) microenvironment elements anatomically and phenotypically in human. Moreover, we found that the impaired BM microenvironment may contribute to the occurrence of secondary poor graft function post-HSCT(Kong Y *et al.* **Biol Blood Marrow Transplant**. 2013; 19: 1465-1473). In mice, the cross-talk between MKs and BM endothelial cells(BMECs) in vascular microenvironment regulate the MKs maturation and thrombopoiesis. We therefore hypothesized that the impaired BM microenvironment may hamper the MKs maturation, possibly translating to the occurrence of PFPE post-HSCT.
Aims: To evaluate whether abnormalities of the BM microenvironment are involved in the pathogenesis of PFPE after allo-HSCT.
Methods: In the present prospective nested case-control study, 20 patients with PFPE, 40 matched patients with good graft function (GGF) after allo-HSCT and 16 healthy donors (HDs) were enrolled. The cellular elements of the BM microenvironment, including the BMECs, perivascular cells and endosteal cells, were analyzed by flow cytometry as well as hematoxylin-eosin and immunohistochemical staining in BM trephine biopsies (BMB). Additionally, megakaryocytic-active chemokines, including stromal-derived factor-1(SDF-1) and vascular endothelial growth factor (VEGF) were detected in plasma of BM by enzyme-linked immunesorbent assay. The study was approved by the Ethics Committee of Peking University People's Hospital and written informed consent was obtained from all subjects.
Results: Although no significant different $CD34^+$ cells (0.25% *vs.* 0.26% *vs.* 0.28%, *P*>.05) and endosteal cells (15 per high-power field [hpf] *vs.* 16 per hpf *vs.* 20 per hpf, *P*>.05) were demonstrated among the PFPE,GGF patients and HDs. PFPE patients showed a remarkable decrease in the cellular elements of vascular microenvironment including BMECs (0.01% *vs.* 0.18% *vs.* 0.20%, *P*<.0001) and perivascular cells (0.01% *vs.* 0.12% *vs.* 0.13%, *P*<.0001) in comparison to GGF allo-HSCT recipients and HDs, respectively. Moreover, significant lower levels of SDF-1(3163pg/ml *vs.* 3928pg/ml, *P*=.0002) and VEGF (56pg/ml *vs.* 123pg/ml, *P*<.0001) were demonstrated in BM plasma of PFPE patients than that in GGF patients. Multivariate analyses revealed that BMECs (OR=171.57, *P*=.002) and the cytomegalovirus infection (OR=4.35, *P*=.009) were independent risk factors for PFPE.
Summary and Conclusions: Our results suggest that the impaired BM vascular microenvironment and its associated megakaryocytic-active chemokines may contribute to the occurrence of PFPE after allo-HSCT.
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REGULATION OF B LYMPHOPOIESIS BY SIGNAL-TRANSDUCING ADAPTOR PROTEIN-2, STAP-2
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Background: Hematopoiesis is changed in response to various types of stress, including infections and aging. Recent studies have shown that hematopoietic stem and progenitor cells (HSPC) in bone marrow (BM) are affected by pathogens and inflammatory cytokines. These cells express Toll-like receptors (TLRs) and B lymphopoiesis is arrested after exposure to TLR ligands such as LPS. Previously, we cloned signal-transducing adaptor protein-2 (STAP-2) as a c-fms/M-CSFR interacting protein. Like other adapter proteins, STAP-2 interacts with a variety of signaling or transcriptional molecules, and modifies their functions. We reported that STAP-2 combines with MyD88 and IκB kinase (IKK) to activate NF-κB and enhances the production of IL-6 and TNFα in macrophages *in vitro*. The disruption of STAP-2 attenuates dextran sodium sulfate-induced colitis via inhibition of macrophage recruitment. Although these findings suggest that STAP-2 is related to inflammatory and/or immune responses, little has been known about the role in B cell development in BM.
Aims: To evaluate the effects of STAP-2 on lymphopoiesis using gene-modified mice.
Methods: We generated transgenic mice (Tg) that overexpress STAP-2 under the control of the Eμ enhancer. The promoter could drive expression of the inserted cDNA in B lineage cells from pre-pro-B stage. All experimental procedures were conducted under specific pathogen-free conditions, according to protocols approved by Institutional Animal Care and Use Committees of Osaka University. Human BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital.
Results: With quantitative PCR, we first evaluated the expression level of STAP-2 in human BM. Although STAP-2 mRNA was ubiquitously observed, $CD34^+$ HSPC and $CD19^+$ B progenitors expressed the gene much higher than myeloid lineage cells. To study whether STAP-2 could regulate early lymphopoiesis in BM, several subsets of B progenitors in Tg and the deficient mice were analyzed with flow cytometry. As a result, the numbers of pre-pro-B and immature B cells were suppressed with statistical significance in Tg mice, while there were no differences between control and knock-out mice. In colony-forming unit (CFU) assays, we found that STAP-2 expression decreased the ability to generate CFU pre-B colonies. When HSPC (Lin^- $Sca1^+$ $cKit^{hi}$ cells or Lin^- $Sca1^+$ $cKit^{low}$ common lymphoid progenitors (CLP)) derived from Tg were cultured with OP9 stromal cells under B cell condition (1 ng/ml IL-7, 50 ng/ml Flt3-ligand and 20 ng/ml SCF), the production of $B220^+$ B cells were significantly suppressed (CLP cultures; 94.7±0.12% in WT *vs* 42.6± 9.66% in Tg). Moreover, in stromal-cell free cultures, HSPC from Tg arrested the development of B cells at the early stages before starting CD19 expression.
Summary and Conclusions: In this study, we show that the level of STAP-2 influences the early development of B cells at several stages, from pre-pro-B to immature B cells. STAP-2 was highly expressed in HSPC and B progenitors compared to myeloid lineage cells. STAP-2, which function is generally recognized under inflammatory condition, is speculated to interact with key molecules during B lymphopoiesis. Our findings indicate that STAP-2 involves the pathophysiology of B lymphocyte-related disorders caused by acute/chronic inflammation and aging. Further study would clarify the precise molecular mechanisms to suppress B cell generation in BM.

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IMPACT OF COLD SHOCK PROTEIN YBX1 ON HEMATOPOIETIC STEM CELL DEVELOPMENT AND FUNCTION
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Background: Y-box binding protein 1 (Ybx1) is an evolutionarily conserved DNA- and RNA- binding protein that is classified as a member of the cold shock proteins. Recent reports have highlighted its importance in regulation of early erythropoiesis and its interaction with evolutionary conserved signaling pathways such as Notch. This role in early hematopoietic development and the interaction with self-renewal associated signaling pathways suggests a requirement for Ybx1 in hematopoietic stem- and progenitor-cell (HSPC) development and function.
Aims: We aimed to assess for the impact of Ybx1 on HSPC development and function.
Methods: Expression levels of Ybx1 were analyzed by qPCR and intracellular flow cytometry on immunophenotypically defined murine bone marrow stem- and progenitor-cell populations. To characterize the requirement of Ybx1 on a functional level, we used a previously published conventional (straight) knockout mouse model (Lu *et al.*, Mol Cell Biol, 2005). In this model, homozygous inactivation of Ybx1 results in embryonic lethality around day E15, mostly due to malformation of the central nervous system while heterozygous animals ($Ybx1^{+/-}$) are born without any significant defects. Self-renewal and differentiation capacity were analyzed by plating fetal liver cells (FLC) or adult hematopoietic stem cells from the respective knockout mice in methylcellulose *in vitro* and by spleen colony formation (CFU-S12) and long-term reconstitution in lethally irradiated recipients *in vivo*.
Results: Ybx1 expression was massively up-regulated during differentiation

with highest levels detectable in multipotent progenitors (MPP) and committed myeloid progenitors (CMP, GMP and MEP). To assess for the role of Ybx1 in hematopoietic development we harvested FLC at day E13.5. FLC numbers were significantly decreased in Ybx1$^{-/-}$ fetuses compared to Ybx1$^{+/+}$ littermate controls. Immunophenotypically, Ybx1$^{-/-}$ showed a lower number of LT-HSC (CD34-LSK) but a higher amount of ST-HSC/MPP (CD34+LSK). Consistently, when injected into lethally irradiated recipient mice, an increased number of spleen colony forming units was detectable on day 12. When transplanted competitively for long-term reconstitution, Ybx1$^{-/-}$, FLC produced a significantly decreased chimerism, which is explained by the decreased number of LT-HSC. Even heterozygous (Ybx1$^{+/-}$) animals showed a slight trend towards a decreased repopulation capacity. To investigate whether this trend could be recapitulated in adult hematopoiesis, we performed immunophenotyping of adult stem- and progenitor-cell populations and functional experiments on adult HSC derived from Ybx1$^{+/+}$ or Ybx1$^{+/-}$ animals. Immunophenotyping of young (<8 weeks) and aged animals revealed no significant quantitative differences in HSPC populations. However, plating of Ybx1$^{+/+}$ or Ybx1$^{+/-}$, HSC in methylcellulose revealed increased differentiation for the heterozygous state after one week and consecutive reduction of colony formation in the second round of plating. Of note, TER119+ cells appeared to be significantly reduced in these platings, which is in line with the previously published requirement of Ybx1 for erythroid development. When injected into lethally irradiated hosts, Ybx1$^{+/-}$, cells produced slightly higher numbers of spleen colonies but a comparable chimerism during long-term reconstitution.
Summary and Conclusions: Taken together, our data provide first evidence for a role of cold shock protein Ybx1 in hematopoietic development, self-renewal and differentiation of hematopoietic stem- and progenitor cells.

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IMPACT OF COMMENSAL MICROBIOTA ON HEMATOPOIETIC STEM AND PROGENITOR CELL HOMEOSTASIS

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Background: Most of hematopoietic stem and progenitor cells (HSPCs) reside in the adult hematopoietic bone marrow (BM). However, at any given time, about 1% of HSPCs leave the BM, enter systemic circulation, perambulate peripheral tissues, and some re-home to the BM. It has been suggested that invading pathogens in peripheral tissues induce migration arrest of HSPCs and promote HSPC proliferation and differentiation to ensure local supply of myeloid effector cells. Besides pathogens, commensal microbiota is also known to control quantity and quality of the immune cell pool of peripheral tissues in steady-state. However, it remains unclear if commensal microbiota controls BM HSPC pools and their migration to peripheral tissues.
Aims: We characterize the size and function of HSPCs in the BM, blood, and peripheral organ (spleen) of germ-free (GF) mice in order to address the above issue.
Methods: We use C57BL/6 GF mice that are bread and housed in sterile isolators and recolonized C57BL/6 GF mice are used as controls. Sex- and age-matched mice are used in the studies. The number of colony-forming units in the peripheral blood, BM, and spleen are analyzed after 12-14 days of culture.
Results: We observe that the size as well as the function of HSPCs is similar in the BM of germ-free (GF) and conventional environment control mice. In contrast, lineage$^-$cKit$^+$Sca-1$^+$ (LSK) cells that contain HSPCs are significantly increased in circulation whereas numbers of LSK cells are reduced in the spleen of GF mice compared to controls. Accordingly, the number of immature hematopoietic progenitor cells that generate CFU-GEMM is significantly increased in the peripheral blood and significantly decreased in the spleen whereas it is similar in the BM of GF *versus* control mice.
Summary and Conclusions: These data suggest that commensal microbiota regulate egress from and/or homing of HSPCs to circulation and peripheral tissues in steady-state.

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IMMUNOMODULATION EFFECTS OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF REFRACTORY ACUTE GRAFT-*VERSUS*-HOST DISEASE

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Background: Mesenchymal stem cells (MSCs) have been considered as a promising strategy for the prevention and treatment of acute graft-*versus*-host disease (aGVHD), but the mechanism of MSCs ameliorating GVHD is still not fully understood. A few studies suggested that MSCs ameliorated GVHD via protecting and repairing the function of thymus.
Aims: This study explored immunomodulation effects of MSCs in the treatment of refractory aGVHD.
Methods: Twenty refractory aGVHD patients receiving MSCs treatment were enrolled in this study, and twenty grade II to IV aGVHD patients treated without MSCs were matched as non-MSCs group. MSCs were given at a median dose of 1×10^6, cells/kg once weekly until aGVHD got complete response (CR) or MSCs were infused for a total of 8 doses. Lymphocyte subsets of T-cell, B-cell, and NK-cell in peripheral blood were analyzed by flow cytometry before and 30, 60, 90, 180 and 360 days after the MSC infusion in MSC group and the corresponding period in the control group.
Results: A total of the 33 patients who survived more than 30 days after the study treatments were evaluated for the alterations in cellular immune compartment and cGVHD, including 15 cases in MSCs group and 18 in non-MSCs group. In MSCs group, patients had a significantly higher CD4$^+$/CD8$^+$ T-cell ratio at 60, 90 and 180 day after MSCs treatments, which subsequently equalized at 360 day compared with non-MSCs group. The MSC group presented higher percentages of CD4$^+$CD25$^+$ regulatory T cells (Treg) comparing with non-MSCs group, especially at 90 and 180 day, and approached at 360 day. The proportion of CD4$^+$CD45RO$^+$ and CD4$^+$CD45RA$^+$ naïve T-cells in MSC group were also higher than those in non-MSCs group at 60, 90 and 180 day, but not different at 360 day. The proportion of CD19$^+$ and CD16$^+$CD56$^+$ was not detected striking difference between the two groups. Four patients (26.7%) experienced cGVHD in MSCs group, compared with twelve (66.7%) in non-MSCs group (p=0.02).
Summary and Conclusions: In conclusion, our data indicate that MSCs might ameliorate aGVHD and induce longer-lasting immune tolerance by altering cellular immune compartments in peripheral blood. The alteration of the cellular immune compartments is associated with the protecting and repairing role of MSCs to thymus.

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ROLE OF STROMAL CELL-MEDIATED NOTCH SIGNALING IN HEMATOLOGICAL MALIGNANCIES

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Background: Stromal cells are essential components of the bone marrow (BM) microenvironment regulating and supporting the survival of different tumors, including B-cell acute and chronic lymphocytic leukemia (B-ALL and CLL), and acute myeloid leukemia (AML).
Aims: In this study, we investigated the role of Notch signalling in human BM-mesenchymal stromal cell (hBM-MSC)-promoted ALL, CLL and AML survival and chemoresistance.
Results: The block of Notch signalling through γ-secretase inhibitor (GSI) XII reverted the protective effect mediated by co-culture with BM-MSC. The treatment with combinations of anti-Notch neutralizing Abs resulted in the decrease of B-ALL cell survival, either cultured alone or cocultured in presence of BM-MSC from normal donors and B-ALL patients. The inhibition of Notch-3 and -4 or Jagged-1/-2 and DLL-1 resulted in a dramatic increase of apoptotic B-ALL cells by 3 days, similar to what is obtained by blocking all Notch signaling with the GSI XII. The same Notch receptors are involved in CLL survival except for Notch-1 that, in CLL, mediates a synergistic effect with other Notch receptors in inducing the anti-apoptotic phenotype. Some preliminary data showed that Notch system is involved in survival and chemoresistance of acute myeloid leukemia blasts.
Summary and Conclusions: Overall, our findings show that stromal cell-mediated Notch signaling has a role in promoting ALL, CLL and AML survival and resistance to chemotherapy. Therefore, the target of Notch pathway activation may represent a useful strategy to overcome drug resistance and improve the efficacy of conventional treatments.

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MUTATIONAL ANALYSIS OF BONE MARROW MESENCHIMAL STROMAL CELLS IN MYELOID MALIGNANCIES

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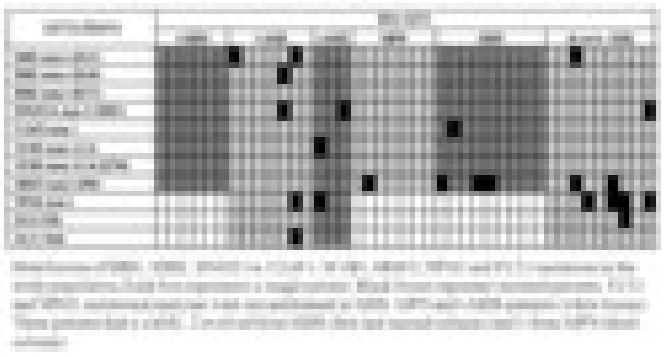
Background: Growing evidence suggest the involvement of the microenvironment in the pathophysiology of hematopoietic malignancies. Mesenchimal stromal cells (MSC) support hematopoiesis through the production and secretion of cytokines, cell-cell interactions and immunomodulating properties. Anomalies of multiple MSC features have been described in MDS and AML. These include significantly reduced growth, proliferative and differentiating capacities, premature replicative senescence, abnormal expression of surface molecules and chemokines, and reduced ability to support hematopoietic stem and progenitor cell growth in long-term culture assays.
Aims: Deep sequencing approaches have identified new recurrent mutations of genes involved in epigenetic and spliceosome machineries in AML and MDS. So far, the prevalence of somatic mutations of epigenetic and spliceosomal

genes in MSC compartment in patients with myeloid malignancies is not known. In this line, we investigated the frequency of recurrent mutations of epigenetic and spliceosomal genes, of FLT3 and NMP1 genes in BM-MNC cells and MSC isolated from 41 patients with myeloid malignancies.

Methods: The study population included 41 patients with myeloid malignancies, 9 *de novo* AML, 9 MDS, 7 MPN, 3 secondary AML (sAML, 2 evolved from MDS, 1 from MPN), and 13 t-MN (7 t-AML and 6 t-MDS). BM-MNCs were isolated from patients at the time of diagnosis. MSCs were expanded using Mesencult medium (Voden) in plastic-adherent cultures up to the second passage. Flow cytometry analysis confirmed the standard MSC phenotype (CD45-, CD73+, CD90+ and CD105+) in more than 99% of MSC population. DNA was extracted from BM-MNC and MSC using QIAamp DNA Mini Kit (Qiagen). The following hotspot mutations were studied on gDNA by Sanger sequencing (Life technologies): IDH1 R132, IDH2 R140 and R172, DNMT3A R882, U2AF1 S34 and R35, SF3B1 exons 13–16, and SRSF2 exon 1. FLT3 mutations (ITD and TKD) were studied by RT-PCR and RFLP RT-PCR, respectively, whereas NPM1 exon 12 mutations were detected by HRM, followed by Sanger sequencing of positive cases, in patients with *de novo* and *therapy-related* AML.

Results: In BM-MNC, FLT3 ITD and FLT3 TKD mutations were found in 2 patients (1 *de novo* AML and 1 t-AML respectively), while NPM1 exon 12 mutations were present in 6 AML (4 *de novo,* 3 COSM158604 and 1 COSM1319219; 1 sAML, COSM158604; 1 t-AML, COSM28937). IDH1 R132 mutations were found in one patient with *de novo* AML (R132C) and in two with t-AML (R132H and R132L). One t-AML patient presented an IDH2 R140Q mutation, while no mutations were detected at the codon R172. Three DNMT3A R882 mutations were found in one *de novo* AML (R882H), one t-AML (R882H) and one sAML post Polycythemia Vera (R882C). One U2AF1 S34Y mutation was found in a MDS, while a SF3B1 K666N mutation was detected in one AML following a primary MDS. Six SRFS2 P95 mutations were found in two *de novo* AML (P95L and P95R), three MDS (P95L, P95H and P95 frameshift mutation) and one MPN (P95H), Table 1. We found no mutations for any of the studied genes in the MSC compartment, both in carriers of mutations in the hematopoietic compartment and in wild-type patients.

Table 1.



Summary and Conclusions: In our cohort of patients, common mutations of genes involved in epigenetic regulation and spliceosome machinery are absent in the mesenchimal compartment of leukemic bone marrows and are restricted only to the malignant hematopoietic clone. Further investigation is required to ascertain the role of methylation and missplicing in the microenvironment dysfunction observed in myeloid malignancies.

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ADDITION OF PLERIXAFOR TO G-CSF IMPROVES THE GRAFT CONTENT IN SIDE POPULATION (SP) WITH IMMATURE PHENOTYPE IN PATIENTS THAT FAILED TO RESPOND WELL TO G-CSF ALONE.

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Background: Plerixafor the bicyclam compound that inhibits the binding of stromal cell derived factor-1α to its cognate receptor CXCR4 has been used worldwide since its approval. Plerixafor induces rapid release of $CD34^+$ cells into circulation following hematopoietic stem and progenitor cells mobilization (HSPC) and is particularly useful to optimize HSPC mobilization in poor mobilizer patients (PM). Best demonstration of Plerixafor efficacy in terms of collection of cells with great potential to hasten hematological recovery following myelo-suppressive chemotherapy has been shown initially in model of xenotransplantation (Broxmeyer *et al.* J Exp Med 2005 and Hess *et al.* Biol Blood Marrow Transplant 2007). Another convincing example is provided by clinical allogeneic transplantation setting resulting in sustained donor derived hematopoiesis using Plerixafor mobilized HSPC (Devine *et al.* Blood 2008).

Aims: We describe in the present study the phenotype of cells subsets among mobilized HSPC grafts.

Methods: We analyzed apheresis product (AP) derived from graft samples following mobilization of healthy donor (n=1) and patients (n=9). Patients were diagnosed with hematologic malignancies [Myeloma (n=4), Non Hodgkin Lymphoma (n=4), Hodgkin Lymphoma (n=1)]. Patients and donor characteristics are summarized in Table 1. Four patients were mobilized with G-CSF+Plerixafor, among them 2 served as internal control (AP with G-CSF alone on D1 of apheresis and following addition of Plerixafor on Day 2). Two patients and the healthy donor received G-CSF alone while 3 other received Pegylated G-CSF formulation. Phenotypic study of AP was performed by flow cytometry based on CD34, CD38, CD133, CD90 membrane antigens expression and on the proportion of cells with Side Population (SP) activity in order to assess quality of the graft in terms of immature cells content including cells with long term hematopoietic potential (ongoing study).

Results: The percentage of cells with SP phenotype is similar in each arm. However, the phenotypic distribution of these cells seems to be different depending on the patients status [good mobilizer (GM) *versus* PM] and on the type of mobilizing agent used (Table 1). In GM patients, SP cells are mostly present in $CD34^+$, cells population and express immature phenotype ($CD34^+CD38^{low}$, >50%) with parallel expression of CD90 and CD133 markers. In PM patients who required Plerixafor use, SP cells are also present in $CD34^-$ cells fraction (data not shown) and less than 10% of SP cells from the $CD34^+$ fraction display the $CD34^+CD38^{low}$ phenotype (n=2 Pts) comparatively to G-CSF or Pegylated+G-CSF AP counterparts. Interestingly, Plerixafor in one of 2 Pts controls appears to overcome the cells subsets proportion observed with G-CSF alone while leading to increased immature phenotype of the cells present in the grafts (Table 1).

Table 1. This table summarizes donor and patients characteristics. Immature phenotype of $CD34^+$ cells subsets content in graft collected after G-CSF alone, G-GSF+Plerixafor or Pegylated G-CSF injection is detailed.

Summary and Conclusions: The original description of substantial primitive cells subsets with SP phenotype reported by our group brings promising data in the knowledge of functional and phenotypic characterization of G-CSF+Plerixafor mobilized autologous cells in transplantation setting. Thus, this preliminary observation highlights the role of this agent in collection of cells with sustained hematopoiesis potential as suggested in previous clinical study and will probably be confirmed by ongoing more complete evaluation set up in our laboratory.

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THE IRON CHELATOR DEFERASIROX AFFECTS REDOX SIGNALING IN HEALTHY HEMATOPOIETIC STEM CELLS

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Background: Hematopoietic stem cells (HSCs) constitute a reservoir of undifferentiated cells that can be committed, upon appropriate stimuli, in the haematic lineages. Although residing in a bone-marrow hypoxic microenvironment (niche) and mainly relying on anaerobic glycolysis, HSCs are endowed with mitochondria. Recently, specific interest has been focused on HSCs mitochondria and on their role as reactive oxygen species (ROS) generators during the early phases of commitment. The iron chelator deferasirox (DFX), but not defer-

oxamine (DFO), both used to treat complications related to transfusional iron overload in myelodisplastic syndrome patients (MDS), is able to induce hematological responses in a consistent percentage of patients but signalling pathways responsible of such a feature are still poorly understood. Consolidated evidences highlight the importance of redox signalling in the homeostasis of fundamental processes in cell adaptive biology and particularly in controlling the balance between self-renewal and differentiation of stem cells. In this setting, reactive species of oxygen (ROS) would act as secondary messengers, modulating the expression of master transcription factors and regulatory proteins leading or (pre)conditioning stem cells towards differentiation.
Aims: In the present study we investigated the effect of DFX and DFO on ROS production in HSCs in order to identify a molecular mechanism explaining the differential effect of iron chelators in rescuing altered hematopoiesis.
Methods: Human HSCs, isolated upon informed consent from peripheral blood of G-CSF-treated healthy donors by immuno-selection against the specific markers CD133 and CD34, were treated with 100 μM DFX or DFO for 24 hours.To completely abrogate ROS production, cells were co-incubated with diphenil iodide (DPI) 100μM, a known inhibitor of the main sources of ROS: the flavo-oxidases of the respiratory chain and the NADPH oxidases. Cell viability was determined by trypan blue staining. ROS levels were analyzed by laser scanning confocal microscopy (LSCM) and flow cytometry after the incubation at 37°C for 15 minutes with the intracellular H_2O_2 specific probe dichlorodihyrofluorescein-diacetate (H_2DCFDA)10μM. Oct4, sox2 and sox17 transcript levels were measured by real time PCR. β-catenin and BMI1 protein levels were assessed by western blotting. Data were presented as mean±s.e.m. and were compared by unpaired Student T-Test; a $p<0.05$ was considered significant.
Results: DFX treatment of HSCs resulted in a significant up-regulation of ROS level whereas no significant change was observed following by DFO treatment and this was observed in HSCs but also in other cell types, thus suggesting a not specific effect on blood cells. This effect seemingly is independent on the DFX iron-chelating property but pertains to additional pharmacological properties that warren further investigation. Moreover, the increase of ROS production in turn led to the activation of oct4, sox2 and sox17 gene expression and the reduction of β-catenin and BMI1 regulatory proteins. These molecular events triggered by DFX were accompanied by an up-regulation of CD71, marker of erythroid progenitors; moreover, an increase of mitochondrial mass, inversely proportional to CD34 expression, was also observed.
Summary and Conclusions: Our data would suggest a novel mechanism by which DFX treatment, through ROS signalling activation, influences key factors involved in self-renewal/differentiation of HSCs. In this scenario, the modulation of ROS, because of their ability to restore the hematopoietic function, could be taken in account as potential further pharmacological target in MDS treatment.

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INTERACTIONS BETWEEN MULTIPOTENT MESENCHYMAL STROMAL CELLS AND HEMATOPOIETIC PROGENITOR CELLS ALTER AFTER INTERLEUKIN 1 BETA ADMINISTRATION

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Background: The stromal microenvironment regulates the maintenance, proliferation and maturation of hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). Multipotent mesenchymal stromal cells (MMSCs) have been shown to produce mature stromal cells and maintain HPCs. Based on this property, MMSCs can be used as a model to study the cross-talk between stromal cells and HPCs. The functions of interleukin-1 β (IL-1 β) – proinflammatory cytokine - and its effects on cells of non-hematopoietic origin are poorly understood. It was demonstrated that IL-1 β stimulates growth of the stromal microenvironment *in vivo* and MMSCs *in vitro*.
Aims: The aim of this study was to investigate the effect of IL-1 β on the ability of MMSCs to support HPCs *in vitro*.
Methods: MMSCs were isolated from the bone marrow of 26 donors (13 males and 13 females) ranging in age from 18 to 56 years (median: 32). The samples were collected in the Department of Bone Marrow Transplantation of Hematological Scientific Centre after informed consent and during aspiration of hematopoietic stem cells for allogeneic transplantation. MMSCs were cultivated in standard conditions in α MEM supplemented with 10% fetal bovine serum without or with 4 pg/ml IL-1 β, and the maintenance of HPCs on MMSC layers was estimated using cobblestone area forming cell (CAFC 1-2wk) and long-term culture initiating cell (LTC-IC) assays. The proliferative potential of the MMSCs was assessed by determining the total cell production and clonal efficiency. The relative expression level of various genes in the MMSCs was analyzed using RT[2] Profiler PCR Array (Qiagen) and RQ-PCR.
Results: The total MMSCs production increased 1.3–2 fold after addition of IL-1 β to the culture medium. The clonal efficiency was increased (1.5–2 fold) in populations of MMSCs cultivated with IL-1 β that could be related to the increased frequency of proliferating cells in the MMSC population. Treatment of MMSCs with IL-1 β resulted in enhanced ability of these cells to maintain HPCs, as detected using CAFC and LTC-IC assays. The frequency of CAFC 1-2wk increased in 1.57±0.13 fold (p=0.0004) and LTC-IC in 1.34±0.35 (insignificantly). Gene expression analysis in MMSCs treated with IL-1 β revealed that the relative expression level of ICAM1 was up-regulated (3.08±0.84, p=0.01), while ANXA2 (0.72±0.05, p=0.01) and JAG1 (0.74±0.4, p=0.03) were down-regulated. No differences were detected in the expression of ANG1, VCAM1, and PTHR, which play roles in the adhesion of HSCs. The expression level examination of chemokines and growth factors demonstrated that SDF1 was down-regulated (0.75±0.1, p=0.05) and FGF2 was up-regulated, although not statistically significant (1.3±0.2, p=0.47), but no differences were detected in the expression of thrombopoietin (THPO) or insulin-like growth-factor 1 (IGF).
Summary and Conclusions: The results revealed the participation of IL-1 β in the regulation of hematopoietic stromal niches. IL-1 β enchances the ability of MMSCs to maintain hematopoietic progenitors *in vitro*, and this effect could be explained by increased proliferative potential and cumulative cell production as well as by modulation of the expression of genes participating in the interaction between HPCs and the stromal microenvironment, *e.g.*, adhesion molecules (ICAM1) and growth factors (FGF2 and SDF1).

Red cell Biology

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MODIFIED ACTRIIB-FC FUSION PROTEIN (ACE-536) AMELIORATES ANEMIA IN A MODEL OF SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is a hereditary disorder caused by a single point mutation in the β-globin gene resulting in the production of sickle hemoglobin variant (HbSS). In the deoxygenated state, HbS is labile and can undergo auto-oxidation to form hemichromes and polymerize to generate rigid and misshaped erythrocytes. The combination of hemichrome formation and HbS polymerization leads to membrane damage that alters membrane structure and contributes to the very short half-life of sickle erythrocytes. The membrane damage manifest in a redistribution of membrane lipids leading to expression of phosphatidylserine on the outer leaflet of the erythrocyte membrane. Externalized phosphatidylserine promotes adhesion to both macrophages and activated endothelial cells, contributing to both reduced HbS cell lifespan and vascular occlusion. In an attempt to correct the anemia mediated by the short half-life of the red cells there is a tremendous increase in eryrthrocytosis resulting in an increase in peripheral reticulocytes from about 2% to 50% of total red cells.
Aims: An effective treatment for SCD would decrease the anemia and limit the generation of a hypoxic environment thereby reducing the generation of sickled erythrocytes. Additionally, reducing the propensity of the erythrocyte to adhere to the vasculature and macrophages would be expected to reduce the obstruction of blood vessels, ischemic events and pain crises.
Previously, we have shown that RAP-536 (murine ortholog of ACE-536) increases the production of red cells in both normal and thalassemic mice. Importantly, in β-thalassemia mice RAP-536 has been shown to reduce hemichromes on erythrocyte membranes, decrease reactive oxygen species, reduce hemolysis and improve red cell half-life.
In the present study, we extended the evaluation of RAP-536 in the murine model of sickle cell disease (β^S/β^S).
Methods: SCD mice were dosed subcutaneously with RAP-536 (1 mg/kg, twice weekly) or TBS vehicle (VEH) control (N=5/group). Non-symptomatic compound heterozygote (β/β^S) littermates were dosed similarly (N=5/group) and used as controls.
Results: At study baseline, SCD mice had reduced RBC number (-28%, P<0.01) and hemoglobin (-14.5%, P<0.05) and increased reticulocytes (+50%, P<0.001) compared to compound heterozygote mice. Following one month of treatment, RAP-536 significantly increased RBC number (+15.2%, p<0.01), hemoglobin (+9.28%, p<0.05) compared to VEH treatment in SCD mice. Importantly the increased RBC occurred while reticulocytes (-13.5%, p<0.05) decreased, which is potentially consistent with an increase in red cell half-life. RAP-536 treatment of SCD mice for 6-weeks resulted in a substantial decrease in phosphatidylserine (PS) exposure in peripheral blood cells as determined by scramblase enzyme assay (-14%, p=0.08) and annexin-V assay (-18.75%, N.S) suggesting a trend towards improved membrane phospholipid asymmetry compared to vehicle treatment.
Summary and Conclusions: In summary, RAP-536 reduces the anemia and red blood cell pathology in a murine model of SCD. ACE-536 is currently being tested in Phase 2 clinical trials in MDS and β-thalassemia patients, and merits evaluation as a therapy for SCD.

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MACROPHAGE MIGRATION INHIBITORY FACTOR INHIBITS OVEREXPANSION OF IMMATURE ERYTHROID CELLS IN THE SPLEEN DURING CHRONIC PSYCHOLOGICAL STRESS

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Background: Psychological stress is a part of modern style of life and stress response can affect multiple organs. Recently, we have reported that chronic psychological stress stimulates extramedullary erythropoiesis. Because inappropriate activation of stress erythropoiesis may predispose to leukemia transformation, the balance between stimulatory and inhibitory regulators of this process is critical at any time. Considering that macrophage migration inhibitory factor (MIF) is secreted in response to stress as well that this cytokine inhibits erythroid differentiation, we assumed that MIF has a role in the control of stress-induced erythropoiesis.
Aims: This study was undertaken to determine whether MIF is involved in the regulation of erythropoiesis during chronic psychological stress using MIF-knockout (MIF-KO) mouse model.
Methods: Adult male C57BL/6 wild type (WT) and MIF-KO mice were subjected to 2 h daily restraint stress for 7 or 14 consecutive days. The number of spleen erythroid progenitors *burst forming units-erythroid (*BFU-E) and colony-forming unit-erythroid (CFU-E) was determined using colony assays whereas analysis of CD71/Ter119 profile of spleen erythroid progenitors was performed by flow cytometry. In WT mice, the spleen expression of MIF was assessed by Western blot and immunohistochemistry.
Results: Chronic restraint stress significantly increased the number of BFU-E and CFU-E cells in the spleen of WT mice. The effect of stress on CFU-E- derived colonies was more prominent in MIF-KO mice suggesting inhibitory role of MIF in these progenitors. Furthermore, analysis of erythroid precursors revealed that both 7 and 14 days of repeated stress elicited a marked increase in the percentage of CD71+Ter119- and CD71+Ter119+ cells, while 14 days of restraint in addition increased the percentage of CD71-Ter119+ cells in WT animals. However, following 14 days of stress exposure, MIF-KO mice demonstrated a significant increase in the percentage of CD71+Ter119+ cells accompanied with a decrease in the percentage of CD71-Ter119+ cells. In addition, according to forward scatter and CD71 expression, total Ter119+ cells were divided into three subpopulations: more immature (E1), less immature (E2) and mature (E3) cells. The results showed that 7 and 14 days of restraint stress increased the percentage of E1 and E2 in WT animals. The same, but significantly profound effect of stress on E1 and E2 cells was observed in MIF-KO mice, with decreased percentage of E3 following 14 days of stress. To further confirm a role of MIF in regulation of stress-induced erythropoiesis, we evaluated expression of this cytokine in the spleen of WT mice. Western blot revealed enhanced MIF expression in the animals restrained for 7 or 14 days. Also, numerous MIF-immunoreactive cells were detected within the red pulp of stressed mice.
Summary and Conclusions: Taken together, obtained results demonstrate for the first time that MIF functions as a negative regulator of extramedullary erythropoieis induced by chronic psychological stress. These findings are important in a view of the fact that chronic psychological stress may be involved in the pathogenesis of hematological malignancies.

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PATHOPHYSIOLOGY OF ANEMIA IN THE VK*MYC MOUSE MODEL OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a disorder characterized by proliferation of plasma cells (PC) in the bone marrow (BM) and by overproduction of monoclonal immunoglobulin detectable as a serum spike (M-spike). Anemia is a common complication of MM and the underlying pathophysiological mechanisms are not completely elucidated. Previous studies suggested both a direct cytotoxic effect of MM PC that leads to apoptosis of erythroid precursors and the functional impairment of their migratory and proliferation capacity induced by bone marrow microenvironment perturbation. Other studies in patients strengthened the importance of inflammatory cytokines that, released by MM PC and monocytes-macrophages, promote the production of the liver hormone hepcidin. Hepcidin targets and degrades the iron exporter ferroportin (Fpn), favoring iron retention in macrophages and thereby iron-restricted anemia, as occurs in all forms of anemia of chronic inflammatory diseases.
Aims: To address the pathophysiology of anemia in the Vk*myc mouse, which spontaneously develops a myeloma-like disease (Chesi M *et al.* 2008).
Methods: We followed disease progression by evaluating the M-spike incidence and size from 35 to 85 weeks of age and by BM histological examination. We measured the hematological and iron parameters, serum erythropoietin and liver hepcidin expression. In BM and spleen we evaluated cellular sub-populations by flow cytometry using different markers combination: CD138 and B220 for B cells, CD11b and F4/80 for monocyte/macrophages, CD44, TER119 and CD71 for erythroid precursors. We determined the iron phenotype by measuring the iron importer transferrin receptor (CD71) and the iron exporter Fpn.
Results: Vk*myc mice slowly develop the disease with M-spike evident by 35 weeks of age. Both the percentage of affected mice and the size of the monoclonal component progress overtime. At 80 weeks, disease indexes mean values are: M-spike size 15%; BM PC expansion 5%, as evaluated by flow cytometry (CD138+B220-) and 25%, as evaluated by histology and no significant PC increase in the spleen. MM PC show increased expression of CD71 and no reduction of Fpn as compared to non malignant PC. Starting from 50 weeks Vk*myc mice show reduction of hemoglobin (Hgb) levels and of red blood cells (RBC) count as compared with wt counterparts. With exception of few cases, anemia of Vk*myc is mild. Both Hgb levels and RBC count indirectly correlate with M spike and PC expansion in BM. Both mild and severe anemias are not the consequence of defective erythropoietin concentration or of abnormal iron metabolism. Hepcidin levels are not statistically different between wt and Vk*myc animals and are not correlated with Hgb or M-spike. With exception of pro-erythroblasts, all the erythroid precursors are statistically reduced in Vk*myc BM with respect of wt counterparts. In parallel the same population is increased in the spleen.
Summary and Conclusions: Vk*myc model is characterized by a limited MM cells expansion and mild anemia. In this condition, which likely reproduces the

initial stages of the human disease, we excluded a role of inflammation and hepcidin in the pathogenesis of anemia. The reduction of BM erythroid precursors may explain reduced Hgb levels and RBC count, while spleen erythropoiesis likely mitigates anemia. Loss of erythroid precursors could result from BM microenvironment perturbation, which is under investigation. Iron sequestration by MM PC high CD71 might contribute to erythropoiesis restriction.

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THE SPECTRUM OF ADULT'S PARVOVIRUS B19 INFECTION IN HOSPITAL ACCORDING TO THE CLINICAL BACKGROUND: REPORT OF A SINGLE CENTER EXPERIENCE AND PROPOSALS FOR THE CLINICAL PRACTICE.

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Background: Parvovirus B19 (PVB19) infection which is usually asymptomatic in healthy adults can lead to a wide spectrum of clinical and biological manifestations depending mainly on the underlying status of the patient, such as chronic hereditary hemolytic anemia or immunodeficiency.
Aims: So far, there is no gold standard for the diagnosis of PVB19 infection and physicians use indifferently either sero-diagnosis, molecular methods or both.
Methods: All cases of PVB19 infection, based indifferently on a positive serological test (positive IgM) and/or by a positive PCR in the peripheral blood and/or the bone marrow, registered from September 2007 to September 2013 in our tertiary referral university hospital were included. When available, frozen stored sera were retrospectively tested to allow complementary PVB19 serology or PCR according to the initial test performed. Demographic characteristics, clinical data, complete blood count (CBC) and reticulocytes count at the time of PVB19 infection diagnosis were collected retrospectively for each patient. All bone marrow smears were retrospectively examined blindly by an expert in order to assess the presence or not of pure red cell aplasia (PRCA) and especially the presence of typical giant pronormoblasts.
Results: Eighty-three PVB19-infected patients at least on one positive criteria, namely IgM antibodies, positive blood PCR and/or positive bone marrow PCR, were included. These patients were dispatched homogeneously in three equilibrated groups statistically independent: 29 patients had no underlying predisposing condition, 25 patients had an underlying hereditary hemolytic anemia (mainly sickle cell disease) and 29 were immunocompromised patients (solid-organ transplant or hematopoietic stem cell transplant, treatment with immunosuppressors, HIV positive patients…). Classical PVB19-related symptoms, namely arthromyalgia and non-specific cutaneous manifestations (macular erythema and/or edema), were less frequent in immunocompromised patients than in patients with no underlying predisposition condition ($p<0.01$) but couldn't be assessed in patients with an underlying hereditary hemolytic anemia due to some confounding factors. All the patients with chronic hemolysis or immunodeficiency displayed anemia compared to only 41.4% of patients with no underlying predisposing condition. As expected, the median reticulocyte count was low (18.5×10^9/l) yet with a large range (0-323). Bicytopenias were displayed by patients belonging to all the three groups and pancytopenias were noticed mainly in the immunocompromised patient group. The classical PRCA was observed in only 9 out of the 28 bone marrow smears that were performed. Interestingly, the presumably pathognomonic feature of PRCA, namely red inclusion in the nucleus of giant pronormoblasts, was found in a single case. Positive IgM were found in 93% of patients with no underlying predisposing condition whereas only 58% of blood PCR were positive in this subgroup. Conversely, both serology and PCR were positive in almost every patient with hereditary hemolytic anemia. In the group of immunocompromised patients, bone marrow PCR analysis, mostly performed by physicians, allowed a positive diagnosis in 91% of cases.
Summary and Conclusions: We report here for the very first time the various characteristics of documented PVB19 infection cases observed in 3 distinct adult populations. The extensive analysis of hematological and clinical features at time of PVB19 infection matched with the available virological parameters lead us to propose some recommendations for the diagnosis of the PVB19 infection according to the clinical background that may be helpful to hematologists in their clinical practice.

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CONTINUOUS ERYTHROPOIETIC ACTIVITY OF EPOETIN BETA PEGOL IN A GENE-MODIFIED MOUSE MODEL FOR ERYTHROPOIETIN-DEFICIENCY ANAEMIA

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Background: The erythroid growth factor erythropoietin (EPO) is secreted from renal EPO-producing cells (REPs) locating in peritubular interstitial spaces of kidneys under hypoxic/anaemic conditions. Therefore, damage in REPs by kidney diseases often causes aneamia. To treat the renal anaemia, erythropoiesis-stimulating agents (ESAs) have been widely used since more than 20 years ago. Recently, long-acting ESAs were developed in order to avoid frequent administration of ESAs. Epoetin β pegol (C.E.R.A.) is a methoxy polyethylene glycol-conjugated recombinant EPO (rHuEPO), and possesses the longest half-life among currently available ESAs.
Aims: /Methods: The continuous effects of C.E.R.A. on *in vivo* erythropoiesis were investigated by using inherited super-anaemic mice (ISAM), which exhibit adult-onset chronic anaemia due to insufficient expression of the *EPO* gene in REPs.
Results: ISAM showed the phenotype of pure red-cell aplasia associated with high concentrations of iron in the liver, spleen and serum. The iron concentrations were decreased to the normal range 3 days after a single subcutaneous injection of C.E.R.A. or rHuEPO (3 μg/kg for each). As well, elevated concentrations of serum hepcidin, which negatively regulates iron utilization for haemoglobin synthesis, were reduced greater than 75%. Haemoglobin levels in the C.E.R.A.-injected group reached around 9 g/dL at 7 days after administration, and the improved level was maintained at least a week. In contrast, the haemoglobin concentration of rHuEPO group remained low (approximately 3 g/dL). Interestingly, the cardiac hypertrophy, which was caused by the chronic anaemia of ISAM, was significantly improved by C.E.R.A. administration along with correction of anaemia.
Summary and Conclusions: The erythropoietic activity of ESAs is enhanced-by improved retentivity in blood. Additionally we propose that ISAM provide a useful model system for assessment of ESAs.

P540

CATION LEAK ANALYSIS OF ABCB6 MUTANTS IN FAMILIAL PSEUDOHYPERKALEMIA

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Background: Isolated Familial Pseudohyperkalemia (FP) is a dominant red cell trait characterized by cold-induced slow 'passive leak' of red cell K+ into plasma, first described in a large Scottish family from Edinburgh (Stewart GW, *et al.*, 1979). Although in freshly obtained blood samples plasma [K+] was normal, it was increased when measured in blood stored at or below room temperature. This trait was unaccompanied by clinical symptoms or signs except for mild abnormalities of red cell shape. FP Lille was later described in a large Flemish kindred with morphologically normal red cells (Dagher G, *et al.*, 1989; Vantyghem MC, *et al.*, 1991). In this family, red cell K+ efflux measured in the presence of ouabain and bumetanide was normal at 37°C, but greatly increased at 22°C and 9°C. FP Lille mapped to 2q35-q36 (Carella M, *et al.*, 2004). Subsequently, FP Chiswick and FP Falkirk cases with remarkable increased MCV were reported (Haines PG, *et al.*, 2001).
Functional gene mapping and sequencing analysis of the candidate genes within the 2q35-q36 critical interval in three multigenerational FP families with 20 affected individuals identified two novel heterozygous missense mutations in the ABCB6 gene that cosegregated with disease phenotype (Andolfo I. *et al.*, 2013). The two genomic substitutions altered two adjacent nucleotides within codon 375 of ABCB6, a porphyrin transporter that in erythrocyte membranes bears the Langereis blood group antigen system (Krishnamurthy PC, *et al.*, 2006; Helias V, *et al.*, 2012).
Aims: We want to understand the pathogenetic mechanism of cation leak in FP by functional studies of ionic flux of ABCB6 mutants.
Methods: cDNAs encoding full-length wildtype ABCB6 were cloned in pcDNA3.1 vector. The point mutations c.1123C>T (p.R375Q) and c.1123C>T (p.R375W) were introduced into pcDNA3.1-ABCB6 by site-directed mutagenesis. pcDNA3.1-ABCB6-WT, pcDNA3.1-ABCB6-R375Q and pcDNA3.1-ABCB6-R375W were transfected into HEK-293 cells for 72h. The cells were maintained at 37°C and 30°C to evaluate the effects of the temperature. HEK-293 cells contents of Na+ and K+, at 72 hours after transfection, were determined by atomic absorption spectrometry as previously described (De Franceschi L. *et al.*, 2007). Furthermore, 72 hours after transfection, the cells were incubated in a medium containing Rubidium, oubain and bumetanide at 37°C and 30°C. Rubidium was determined in cell lysate and K+ was determined in the supernatant by atomic absorption spectrometry.
Results: ABCB6 mutants R375Q and R375W, expressed in human cell line

HEK-293, lead to less net accumulation of Na+, and to greater loss of cell K+ than produced by overexpression of wildtype ABCB6. The ionic flux alterations are more evident at 30°C. In particular, the mutation R375W shows more significant K+ content alterations in respect with mutation R375Q.
Summary and Conclusions: Here, we performed the first functional study to understand the pathogenetic mechanism of FP. Our findings demonstrate that missense mutations in ABCB6 lead to cellular K+ efflux as exhibited in erythroid cells of patients with FP. Moreover, this method will be used as tool to demonstrate the causative role of variations found in FP patients.

P541

GENETIC MARKERS AND FETAL HEMOGLOBIN (HBF) PRODUCTION: ASSOCIATION STUDY IN DELTABETA THALASSEMIA

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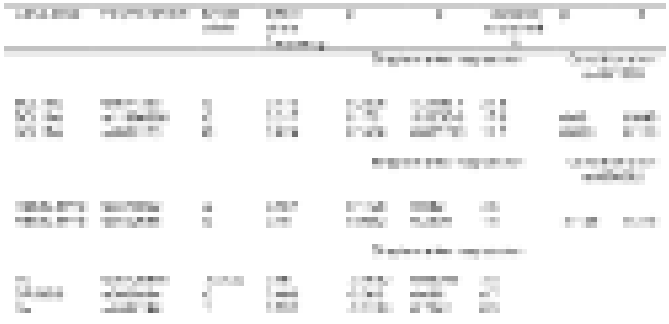
Background: HbF is a strong and heritable modifier of disease severity for individuals with β-like thalassemias. Several loci have been described carrying polymorphisms that modulate HbF levels. BCL11A seems to be the most important locus outside the β-globin cluster. It codifies for an HbF silencer that binds to several locations in the β-globin cluster, but most of the studies dealing with BCL11A function focus on a binding region located 1Kb upstream of the δ gene. It has been previously described that HbF production in carriers of the *(δβ)º Spanish* deletion is almost entirely done by the (δβ)º chromosome. Since this deletion eliminates the former BCL11A binding region, an HbF association study in these carriers could be very informative, in order to better understand the role of BCL11A and other HbF modifiers.
Aims: To characterize associations with fetal hemoglobin levels at the BCL11A, HBS1L-MYB, CSNK2A1 and β-globin loci, in a population of 111 *(δβ)º Spanish* deletion carriers.
Methods: We recruited DNA and hematological data from 111 carriers (>5 years old) of the *(δβ)º spanish* deletion. Six of them showed different α^+ thalassemic mutations. We genotyped polymorphisms at the BCL11A, HBS1L-MYB, CSNK2A1, OR51B6, γ and ψβ loci. All DNA genotyping was performed using TaqMan Assays, except for the γ promoter variants which were detected by sequencing (-158 xmnI at γ^G and -AGCA deletion at γ^A). 8 out of 14 polymorphisms passed all the quality controls and were analyzed in a linear regression framework. HbF (g/dl) was $\log_{10}$ transformed and used as a quantitative trait. Statistical analyses were carried out with PLINK (single marker regression, conditional analysis and LD calculations) and SPSS (stepwise linear regression). All analysis tested additive inheritance models and included α-globin locus status as covariate.
Results: Results for single marker regression are shown in Table 1. The most significant SNP at the BCL11A locus was rs4671393 (p=4.219E-7). In the HBS1L-MYB region, only rs9376092 was associated with HbF levels (p=0.049). Surprisingly, none of the markers in the HBB locus showed a significant statistical association, but the AGCA deletion was close to reaching a significant p-value. In addition, conditional analysis on rs4671393 revealed a marginal independent association of other SNP at the BCL11A locus (rs11886868). We performed a stepwise analysis to further understand if rs11886868 and the AGCA deletion had a significant and independent effect on HbF levels when multiple loci are taken into account. This model explained 31.5% of the phenotypic variation in HbF levels with just 2 SNPs (20.7% by rs4671393 and 10.8% by rs9376092). None of the "borderline" alleles (rs11886868 and the AGCA deletion) reach enough significance to be included in the final model.

Table 1. Single marker regression and conditional analysis.



Summary and Conclusions: This is the first HbF association study at different loci performed in δβ thalassemia patients. All cases have the same thalassemic determinant, avoiding possible confounding factors (like influence of the type of mutation on HbF levels).
In our cases, most of the HbF is produced by the chromosome carrying the deletion. This may help to explain why we did not find an association between HbF and -158 xmnI. An observable effect could exist just if this SNP is located in the chromosome that produces HbF, but currently we do not have any way to detect if -158 xmnI is in CIS with the *(δβ)º Spanish* deletion.
Finally, our results suggest that, in our patients, BCL11A should exert most of its effect on the β-globin locus through a different binding point that the one previously mentioned (1Kb upstream of the δ gene).

P542

MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST IN PATIENTS WITH HEREDITARY SPHEROCYTOSIS

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Background: Hereditary spherocytosis (HS) is an hemolytic anemia due to defects in the red cell membrane proteins (band 3, spectrin, ankyrin, or protein 4.2) that cause loss of membrane surface area, reduced deformability, trapping and destruction of RBCs in the spleen. Naturally occurring autoantibodies (NAbs) against band 3 are thought to be involved in the removal of senescent and damaged erythrocytes. Mitogen-stimulated direct antiglobulin test (MS-DAT) is a functional and quantitative method for the detection of anti-RBC antibodies in mitogen-stimulated whole blood cultures, found positive in some DAT-negative autoimmune Hemolytic anemia (AIHA), and in a fraction (about 40-50%) of patients with chronic lymphocytic leukemia and myelofibrosis.
Aims: To investigate the occurrence of anti-erythrocyte antibodies by MS-DAT in HS, and to relate their presence with the degree of hemolysis and with the type of membrane defect.
Methods: Ninety-one consecutive HS patients (51 male and 40 female, median age 38 yrs, range 2-87 yrs) were investigated; 13 patients had been splenectomized at the moment of the study. Diagnosis was made on the basis of clinical history, physical examination and the results of laboratory tests: complete blood counts, blood smear examination, reticulocyte counts, hemolytic parameters, eosin-5′-maleimide (EMA)-binding test, and red blood cell osmotic fragility tests. All patients underwent SDS-PAGE analysis of the red cell membrane proteins. MS-DAT was performed, by competitive solid phase ELISA, stimulating whole blood with mitogens (PHA, PMA and PWM). Two-hundred and ten healthy blood donors were studied as control group and 43 patients with AIHA were included as positive controls for MS-DAT.
Results: We found that 61% of HS cases were MS-DAT positive: 29 (53%) with band 3 deficiency, 17 (30%) spectrin deficiency, and 9 (17%) with no detectable defect (Figure 1, open symbols for splenectomized cases). The amount of RBC-bound IgG was clearly greater in HS compared to controls (P<0.0001), albeit lower than that observed in AIHA patients (P<0.0001); there was no difference in IgG bound among various HS groups. Since almost all (42/43) AIHA patients showed RBC-bound IgG values higher than 250 ng/mL, 26 HS patients (13 band 3 and 8 spectrin deficiency, and 5 no detectable defect) with values above this threshold were considered separately in the analysis. These cases displayed a significantly higher number of spherocytes (11%) than patients with RBC bound-IgG 150-250 (6.5%, P=0.04) and MS-DAT negative cases (5%, P<0.01); they showed also a more evident hemolytic pattern and a significantly greater number of reticulocytes compared with MS-DAT negative cases ($230x10^3$/mmc *vs* $125x10^3$/mmc, median value, P<0.01).

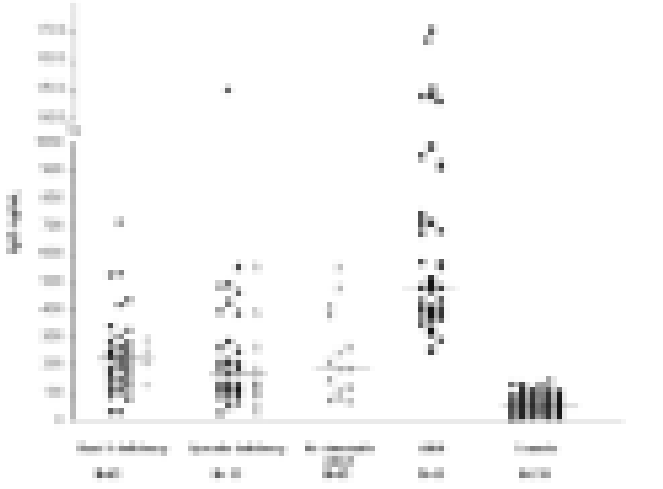

Figure 1.

Summary and Conclusions: These findings suggest that in 60% of patients with HS, mitogen-stimulation is able to induce the production of auto-antibodies which might be responsible for immune-mediated destruction of erythro-

cytes, independently of the membrane structural alteration. These auto-antibodies may be naturally occurring anti-band 3 antibodies, as suggested by their low concentration compared with that found in AIHA, and their pathogenic role in enhancing the hemolytic process in HS.

P543

TWO NOVEL MISSENSE MUTATIONS IN PIEZO1 IN SIX PATIENTS WITH DEHYDRATED HEREDITARY STOMATOCYTOSIS

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Background: Hereditary stomatocytosis is a rare cause of hereditary hemolytic anemia. It concerns a heterogeneous group of disorders characterized by altered cellular hydration state. Dehydrated hereditary stomatocytosis (DHSt), also known as hereditary xerocytosis, is among this group of so called channelopathies of the red blood cells. It is an autosomal dominant disorder and recently mutations in *PIEZO1* have been linked to this disease. *PIEZO1* encodes a mechanosensitive ion channel protein. As result of mutation DHSt red blood cells exhibit impaired potassium and sodium balance leading to their dehydration. DHSt is characterized by mild to moderate hemolytic anemia and may present with pseudohyperkalemia and perinatal edema.
Aims: We performed DNA sequence analysis of *PIEZO1* in six DHSt patients from three unrelated families. The diagnosis DHSt was established using osmotic gradient ektacytometry by Laser-assisted Optical Rotational Cell Analyzer (LoRRca, Mechatronics, Hoorn, The Netherlands) showing a characteristic left shift of the curve.

Results: DNA analysis revealed four different mutations in *PIEZO1*, two of which were novel (Table 1, novel mutations in bold).

Table 1. *PIEZO1* genotypes of the six DHSt patients.

	Family 1			Family 2		Family 3
PIEZ01	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**
Allele 1	c.6262C>G p.Arg2088Gly	c.6262C>G p.Arg2088Gly	c.6262C>G p.Arg2088Gly	c.1276C>T p.Cys426Arg	c.1276C>T p.Cys426Arg	c.7367G>A p.Arg2456His
Allele 2	normal	c.6495-6508delAGA p.2166-2169delLysl	normal	normal	normal	norma

The two novel mutations c.6262C>G and c.1276C>T both segregated with disease in the respective families. Furthermore, both Arg2088 and Cys426 concern residues that are highly conserved in PIEZO1 (http://genome.ucsc.edu/). Moreover, *in silico* analysis by Polyphen and SIFT predict that substitution with, respectively, glycine and arginine is not tolerated. Interestingly, the p.(Cys426Arg) mutation is the most N-terminal mutation identified so far in PIEZO1.
In addition to the p.Arg2088Gly mutation, a second mutation was identified in patient 2 from family 1. This mutation predicts the deletion of one out of four lysine residues between amino acids 2166-2169 and was previously reported in association with DHSt. The mutation was inherited from the patient's mother who was completely asymptomatic and hematologically normal. Notably, the clinical phenotype of patient 1 was more severe when compared to that of his brother (patient 3) and father (patient 1).
Summary and Conclusions: Molecular characterization of six DHSt patients from three unrelated families revealed four mutations in *PIEZO1*. Two of these mutations have not been previously reported: p.(Arg2088Gly) and p.(Cys426Arg). Our findings further establish the correlation between mutations in PIEZO1 and DHSt, a rare channel disorder of the red blood cell.

P544

THE REDUCTION OF PEROXIREDOXINS ACTIVITY ARE CORELATED WITH THE INCREASE OF REACTIVE OXYGEN SPECIES (ROS) AND CELL HEMOLYSIS OBSERVED IN PATIENTS WITH HEMOLITIC ANEMIAS

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Background: Oxidative stress modulation is tightly related with the physiopathology of various hematologic diseases, especially with hemolytic anemias, such as sickle cell disease (SS), glucose 6-phosphate dehydrogenase (G6PD) deficiency and β thalassemia. In these diseases, the cellular environment is extremely pro-oxidant, which results in oxidative damages and consequently in cell hemolysis that promotes the formation of more reactive oxygen species in other cells. In this context, the efficiency of the cellular anti-oxidant system is vitally important. Among the enzyme able to decompose peroxides (Catalase, Glutathione and Peroxiredoxins), the Peroxiredoxins (PRDX) draw attention for their abundance and great reactivity with their substrates. In the red blood cells, the PRDX2 is the third most abundant protein and probably one of the main protectants of the cell.
Aims: This study evaluated the ROS levels and the gene and protein expression of Peroxiredoxins in reticulocytes and erythrocytes in patients with hemoglobin SC disease, sickle cell disease, β thalassemia and glucose 6-phosphate dehydrogenase (G6PD) deficiency and compared the results with the ones found in healthy volunteers.
Methods: Peroxiredoxin gene and protein expression was evaluated in G6PD deficiency (n=8) hemoglobin SC disease (n=18), sickle cell disease (n=17) and β thalassemia (n=12) patients, and 15 healthy volunteers were included in this study. The ROS levels were measured by flow cytometry using the DCFH-DA reagent. The gene expression and protein production were evaluated using quantitative real time PCR and Western Blotting, respectively. The statistical method used was Mann Whitney an p value<0,05 was considered with statistical significance.
Results: Our results showed a significant increase of ROS in patients with β thalassemia, hemoglobin SC disease and sickle cell disease. The results also showed that the gene and protein expression of PRDX1 were raised in β thalassemia and reduced in patients with sickle cell disease. The *PRDX2* analysis did not show significant differences in the diseases, however the western blotting analysis showed a decrease of this protein in SS patients, suggesting a post-transcriptional mechanism in this disease. *PRDX5* did not show differences in any diseases. The gene and protein expression of PRDX6 was reduced in β-thalassemia and sickle cell disease. In hemoglobin SC disease and G6PD deficiency, protein and transcription analysis of PRDX1, 2 and 6 did not exhibit any significant difference. However, PRDX 1 and 2 was found as a dimer in the patients when compared with controls individuals, where these proteins are found in their active (reduced) state. Futhermore, we found a presence of a high molecular weight structure, indicating a decamer formation only in the patients.
Summary and Conclusions: Our results showed an interesting correlation among oxidative stress in hemolytic anemia patients with the production of peroxiredoxins enzymes. The decrease of expression of these enzymes could be an important event in the increase of hemolysis observed in these patients. Moreover the presence of a dimer and decamer structure observed only in the patients, could indicate that the activity of these proteins were reduced and that the decamer formation could be associated with the chaperone activity observed in these proteins in high levels of oxidative stress. The modulation of these proteins could be used as potential therapeutic targets for the attenuation of the damage caused by the oxidative stress, consequently improving the patients prognostic. This study is supported by CAPES and FAPESP.

P545

HYPERTHERMIA INDUCES ERYPTOSIS IN HEREDITARY SPHEROCYTOSIS

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Background: In patients with hereditary spherocytosis (HS) anemia frequently gets worse throughout the course of febrile infectious diseases, due to hemolytic crisis. Actually, the incidence of this complication is 67.2% in our series of HS patients. This observation and usual clinical practice suggest a potential harmful action of temperature on erythrocytes, which would affect their survival. Circulating erythrocytes can undergo premature self-destruction through a mechanism known as eryptosis. A variety of clinical disorders are associated with excessive eryptosis. This process can be triggered by the increase of intracellular calcium or high oxidative stress and can be accelerated by different environmental factors.
Aims: To evaluate whether the temperature increment on erythrocytes may be one factor responsible for anemia worsening during febrile processes.
Methods: Erythrocytes from 9 HS patients and 11 normal controls (NC) were incubated at different temperatures, mimicking either the physiologic body temperature (36.5ºC) or a febrile state (38.5ºC). Flow cytometry was used to analyze signs of eryptosis, such as cell shrinkage (forward scatter) and phosphatidylserine (PS) translocation. Intracellular calcium content, as well as reactive oxygen species (ROS) and reduced glutathione levels (GSH), were also determined by flow cytometry.
Results: In NC, percentages of eryptotic cells and PS exposure either at 36.5

or 38.5°C showed no significant difference, while HS erythrocytes showed a significant increase in eryptotic cells (23.3±4.4% *vs.* 40.4±7.0%, for 36.5 and 38.5°C, respectively; n=9; p <0.01) and PS exposure (6.0±1.0% *vs.* 13.5±2,2%; n=9; p <0.01). Temperature increase did not trigger significant changes in ROS or the GSH levels either in HS or in NC erythrocytes. Erythrocytes from HS patients showed significantly higher intracellular calcium content than NC after incubation at 38.5ºC (p <0.05): mean fluorescence intensity was 56.1±6.5 *vs.* 60.4±1.1 in NC (n=3) and 77.3±7.4 *vs.* 143.7±44.4 in HS (n=6), after incubation at 36.5 and 38.5ºC, respectively. Calcium sequestration in the environment significantly decreased the programmed cell death induced by temperature in HS erythrocytes, since the increment of eryptotic cells and PS externalization were significantly lower in free calcium assays.

Summary and Conclusions: Our results showed that high temperature of incubation induced eryptosis of HS erythrocytes, which resulted more sensitive to temperature changes than red blood cells from controls. An increase in intracellular calcium content seems to be an important factor responsible for the eryptosis induced by high temperature, and could explain the worsening of anemia during hyperthermia in these patients. To our knowledge, this is the first report showing a direct harmful effect of hyperthermia on erythrocytes of HS patients.

P546

HAMP GENE MUTATION ASSOCIATED WITH JUVENILE HEMOCHROMATOSIS IN BRAZILIAN FAMILY

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Background: Juvenile hemochromatosis (JH) is a rare form of iron overload that frequently causes cardiomyopathy, hypogonadotrophic hypogonadism and endocrine dysfunctions before the age of 30. JH types 2A and 2B are caused by mutations in *HJV* (OMIM 602390) and *HAMP* (OMIM 606464), respectively. *HJV* mutations are more frequent than *HAMP* mutations.

Aims: to identify the causal mutation of the JH phenotype in a Brazilian family.

Methods: Bidirectional sequencing for each *HFE*, *HJV* (hemojuvelin), *HAMP* (hepcidin), *TFR2* (transferrin receptor 2) and *SLC40A1* (ferroportin) exons and intron-exon regions was performed in three members of a Brazilian family. Nuclear magnetic resonance (NMR) using T2* evaluation was made.

Results: The index case was a 43-year-old Brazilian female who had previous secondary amenorrhea at age 29, osteoporosis, rheumatoid arthritis, osteoarthritis of knees, modification of skin color, and heart failure 8 months ago (left ventricular ejection fraction - LVEF by MRI of 42.8% - NV 56-78%). Her laboratory tests were: hemoblogin (Hb) 11.7 g/dL, transferrin saturation (TS) 100%, serum ferritin (SF) 7,350 ng/mL and MRI using T2* evaluation showed liver iron concentration (LIC) of 30.67 mg Fe/g dry (NV<3.0 mg/g) and myocardium iron concentration (MIC) of 3.40 mg/g (NV<1.1 mg/g).Her sister, a 40-year-old, had articular pain, hearing impairment, skin hyperpigmentation, and secondary amenorrhea (Hb 12.0 g/dL,TS 98.0%, SF 4,880 ng/mL, LIC=39.57 mg/g; MIC=2.96 mg/g and LVEF by MRI of 36%). Her brother, a 33-year-old, had skin hyperpigmentation, and sexual impotence (Hb 14.6 g/dL, SF 4,800 ng/mL, TS 96%, LIC=39.57 mg/g; MIC=4.95mg/g and LVEF by MRI of 42.8%). Secondary causes of iron overload were excluded and, in the family investigation, healthy parents had a history of consanguinity. In the 3 patients, *HAMP* sequencing revealed a substitution in homozygosis G >A at position +14 of the 5'-UTR region (g.47G>A) creating a new AUG codon, which leads to a shift of the reading frame. No other mutation was identified, except heterozygous genotype for the *HFE* p.H63D alteration in the three patients.

Summary and Conclusions: This *HAMP* mutation on 5'-UTR region, firstly found in a Portuguese family (Matthes T, 2004, Blood) and now identified in a Brazilian family, can be an important region during molecular investigation for patients with JH phenotype.

P547

HB CERVANTES, HB MARAÑÓN, HB LA MANCHA AND HB GOYA: DESCRIPTION OF FOUR NEW HEMOGLOBINOPHATIES

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Background: Thalassemias are the most common monogenic disorders worldwide and represent a serious health problem in areas where their incidence is higher. The α-thalassemias are due to a deficiency or absence of synthesis in the α-chain of hemoglobin (Hb). The defects in the post-translational modifications produce hyper-unstable Hbs that are not detected by most of electrophoretic or chromatographic methods available so far. Structural hemoglobinopathies are due to mutations that cause the change of the amino acid sequence of the protein chain. Some of these variants have altered electrophoretic mobility. They do not have a clinical impact but can cause interference in the analytical determination of some parameters such as the glycosylated hemoglobin in diabetic patients.

Aims: We shown 4 new α-chain Hb variants. Two variants are hyper-unstable and the other 2 are structural variants that have an altered electrophoretic mobility.

Methods: The first 2 families were studied by presenting microcytosis and hypochromia with normal Hb A2 and Hb F without iron deficiency. The other 2 families were studied by presenting a peak of abnormal Hb during routine analytical assays. Haematological data were obtained on a haematology analyzer (Coulter LH750). HbA2 and F quantification and the separation of abnormal Hb were performed by ion-exchange HPLC (VARIANT). The abnormal Hb also was separated by capillary electrophoresis (Sebia). The study of the globin chains was performed by reversed-phase HPLC. The most frequent mutations were ruled out by α-*globin StripAssay* (ViennaLab). The molecular characterization was performed by specific sequencing in an ABI PRISM 3100 Genetic Analyzer.

Results: The haematological parameters and the genotype of the studied subjects are summarized in Table 1. The electrophoretic and chromatographic studies are recruited in Figure 1.

Table 1. Hematological parameters and genotype of the studied subjects. M=Male; F=Female; α^{C}=Cervantes; α^{M}=Marañón; α^{LM}=La Mancha; α^{G}=Goya.

Family	Age (Years)	Sex	Subject	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA2 (%)	Hb F (%)	Hb X (%, by HPLC-Variant)	Genotype
A	54	M	I1	16.5	78.7	24.9	2.7	1.3	--	$\alpha^{C}\alpha/\alpha\alpha$
	25	F	II1	13.8	77.2	24.8	3	0.6	--	$\alpha^{C}\alpha/\alpha\alpha$
	21	M	II2	15.9	81	26.3	2.7	0.4	--	$\alpha^{C}\alpha/\alpha\alpha$
B	14	F	I1	13.9	65.9	21.2	2.6	0.3	--	$\alpha^{M}\alpha/-\alpha^{3,7}$
C	52	M	I1	15.6	93.8	31.1	1.8	0.3	21.9	$\alpha^{LM}\alpha/\alpha\alpha$
D	2	M	I1	12.6	85.4	27.6	2.4	1.8	13.5	$\alpha^{G}\alpha/\alpha\alpha$

Figure 1. Electrophoretic and chromatographic studies.

Summary and Conclusions: In the Hb Cervantes, a residue that interacts with the Alpha-Hemoglobin-Stabilizing Protein (AHSP) that maintains the stability of entire globin chain is affected. For Hb Cervantes, its hyper-instability is due probably to its lower affinity for the AHSP. In the case of the Hb Marañón, a residue that generates an adequate hydrophobic environment for the distal union with the heme group and the α chain, is unstable causing an α-thalassemia phenotype. In our patient, Hb Marañón is also associated with α+-thalassemia deletion which causes a severe phenotype with significant microcytosis and hypochromia. The Hb La Mancha has the same mobility as Hb D, so it could be confused with other variants that do have a clinical expression. Then, it is needed a molecular level characterization by sequencing to study this variant. Finally, Hb Goya has the same mobility as Hb J. A lower percentage of the variant was obtained due to possible instability component, though the patient did not show features of anemia. In the Hb Goya, the helix E is affected. All these variants reveal the complexity and variety of disorders that can be found in the genes encoding Hb. Some variants, such as Hb Cervantes and Hb Marañón, with a significant clinical impact if associated with other forms of α-thalassemia may lead to more severe forms of this set of conditions, as the Hb H disease. Other as Hb Goya and Hb La Mancha, although clinically silent may interfere with various routine analytical assays as the quantification of glycated Hb. In any case, given the variety of alterations already described in Hb genes, we should be aware to this type of emerging diseases.

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PDGF, PDGFR AND TGF GENE EXPRESSION PROFILE IN PATIENTS WITH BETA-THALASSEMIA AND SICKLE CELL DISEASE WITH AND WITHOUT PULMONARY HYPERTENSION

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Background: Pulmonary hypertension (PH) is one of the major complications in patients with hemoglobinopathies. Platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) pathways are among those involved in the development of PH. The expression of PDGF-A, PDGF-B, PDGFR-A, PDGFR-B and TGF-β genes in peripheral blood have not been investigated in relation to PH in patients with inherited hemolytic anemia.
Aims: We sought to quantify the expression of study of PDGF-A, PDGF-B, PDGFR-A, PDGFR-B and TGF-β genes in peripheral blood sample in hemoglobinopathies patients with and without PH in comparison with healthy individuals.
Methods: We enrolled 55 consecutive patients with hemoglobinopathies, including 11 with thalassemia major (TM), 22 with thalassemia intermedia (TI) and 12 with sickle cell disease (SCD). A group of 16 healthy individuals served as controls. PH was defined as a peak tricuspid regurgitation velocity (TRV) >2.9m/s by trans-thoracic echocardiography. Real-time PCR was used to quantify mRNA gene expression of PDGF-A, PDGF-B, PDGFR-A, PDGFR-B and TGF-β by mean of SYBR green detection (CFX96, Biorad). Results were compared to healthy individuals and analyzed by using t-test. There are expressed as: mean value±SEM (p<0,05).
Results: Expression of all studied genes except PDGF-A was significantly higher in TM patients than controls (*PDGFR*-A, 1,2±0,1 *vs* 0,07±0,007, *PDGFR-B*, 0,3±0,03 *vs* 0,23±0,05, *PDGF-B*, 10,3±1,3 *vs* 7,5±0,5 and *TGF*-β, 62,6±15,3 *vs* 59,6±4,7). Moreover, SCD patients expressed higher levels of *PDGFR*-A (1,85±0,2 *vs* 0,07±0,007) and *PDGF-B* (15,6±2,2 *vs* 7,5±0,5) and lower levels of *PDGFR*-B (0,08±0,007 *vs* 0,23±0,05) compared to controls. No statistical significant difference was revealed for *PDGF-A* and *TGF*-β. Interestingly, TI patients with PH presented a significant lower expression of *PDGFR*-A and *PDGFR*-B (0,0003±2,4E-5;0,03±0,002 vs0,07±0,004; 0,23±0,05), *PDGF*-A (0,3±0,002 *vs* 0,4±0,002) and TGF-β (7,9±0,8 *vs* 59,6±4,7) compared to controls. No statistical significant difference was revealed for *PDGFR-B*. TI patients independent the present of PH demonstrated lower expression levels of *PDGFR*-A (0,0003±2,4E-5 *vs* 1,9±0,02) and *TGF* β (7,9±0,8 *vs* 85,4±2,3) compared to total patients with hemoglobinopathies.
Summary and Conclusions: Patients with thalassemia intermedia and PH expressed statistical significant lower levels of PDGF-A, PDGFR-A, PDGFR-B and TGF-β genes in peripheral blood compared to control group. Moreover thalassemia major patients over-expressed PDGF-B, PDGFR-A, PDGFR-B and TGF-β genes compared to control group.

P549

INFLUENCE OF HBG2 XMNI POLYMORPHISM ON HBF LEVELS IN PORTUGUESE BETA-THALASSEMIA CARRIERS

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Background: Increased levels of Hb F can ameliorate the clinical manifestations and severity of sickle cell disease and β-thalassemia (β-thal) hemoglobinopathies. The identification of modulators that are responsible for the reactivation and/or maintenance of Hb F levels in adults have been extensively studied because an understanding of these modulations is important for the development of new therapies. Recent genetic association studies found three major loci involved in HbF variance, including the -158C/T SNP (XmnI, rs7482144) located 5' upstream of *HBG2 (*chr. 11p15), and several SNPs within *BCL11A* intron-2 (chr. 2p16) and in the *HBS1L-MYB* (HMIP) intergenic region (chr. 6p23). Some of these polymorphisms, including *BCL11A* rs766432, *HMIP* rs9399137 and *HBG2* rs7482144, have been associated with the HbF levels in β-thal carriers of different populations.
Aims: To evaluate whether genetic variability of SNPs *BCL11A* rs766432, HMIP rs9399137 and *HBG2* rs7482144 are associated with HbF levels in β-thal carriers of Portuguese descent.
Methods: Sixty seven subjects of Portuguese origin with β-thal *minor*, aged 2-77 years, with HbF levels ranging from 0.2% to 9.5% and heterozygous for one of the following mutations in *HBB* gene [c.118C>T (p.Glu40term); c.48G>A (p.Trp16term); c.92+6T>C; c.126_129 delCTTT; c.92+1G>A], were recruited. HbF and HbA2 levels were determined by HPLC (Variant 2 Bio-Rad Laboratories, Hercules, CA, USA). SNPs rs766432 and rs9399137 were genotyped by allelic discrimination assays using TaqMan probes (Applied Biosystems, Foster City, USA), and rs7482144 by PCR-RFLP with XmnI endonuclease. HbF level was log-transformed to normalize the distribution and statistical analyses were performed by using the PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/). Informed consent was provided by all the participants.
Results: The minor allele frequencies observed for the three polymorphisms in the total sample were: 0.139 for *BCL11A* rs766432-C, 0.25 for HMIP rs9399137-C, and 0.152 for *HBG2* rs7482144-T. Genotype distributions were in agreement with the Hardy-Weinberg equilibrium (p>0.05). The distribution of log transformed HbF values (mean) according to SNP genotypes showed lower values in homozygotes for the major ancestral allele *versus* genotypes with the derived allele (*BCL11A* rs766432: 0.024 *vs.* 0.258; HMIP rs9399137: 0.009 *vs.* 0.186; HBG rs7482144: -0.073 *vs.* 0.499). Linear regression under an additive genetic model to test the association between SNPs and HbF levels showed statistical significance for SNPs *BCL11A* rs766432 (β=0.29; p=0.009), HMIP rs9399137 (β=0.18; p=0.043) and *HBG2* (XmnI) rs7482144 (β=0.49; $p=1.12 \times 10^{-7}$), after adjustment for age and sex. However, conditional analysis demonstrated that after conditioning on the most highly associated SNP rs7482144 (XmnI), no significant interaction with HbF levels was observed for *BCL11A* rs766432 (p=0.11) or HMIP rs9399137 (p=0.27).
Summary and Conclusions: Our results suggest that the increase of HbF levels in Portuguese β-thal carriers is highly associated with the *HBG2* (XmnI) polymorphism. However, our data do not replicate previous findings showing statistically significant associations between the HbF trait and *BCL11A* rs766432 or HMIP rs9399137 polymorphisms.

P550

MOLECULAR CHARACTERIZATION OF THE MUTATIONS CAUSING G6PD DEFICIENCY AMONG EGYPTIAN CHILDREN WITH GENOTYPE - PHENOTYPE CORRELATION

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Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect. It is considered a health problem, especially in Asia, Middle East and Mediterranean countries with many biochemical and clinical phenotypes. The effective management of G6PD deficiency is to prevent haemolysis by avoiding oxidative stress with fava beans is the commonest. No data is available about the effect of non-fava beans diet in those patients. Molecular characterization of G6PD deficiency variants is essential, since the biochemical characterization has lost its significance due to the individual variability
Aims: was designed to determine the frequency of the common mutations causing G6PD deficiency in Egyptian children, as well as making genotype-phenotype correlation for the identified mutations affecting G6PD gene on Xq28.Also to investigate the challenge of non-fava beans diet on occurrence of hemolysis.
Methods: Quantitative analyses for enzymatic activity, G6PD electrophoresis and molecular typing were performed on 108 G6PD-deficient children. A polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) technique was used to detect the G6PD enzyme mutation with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Patient's medical records were reviewed as regard age at diagnosis, demographic data, the offending agent which precipitated the first attack, history of blood transfusion and G6PD level at diagnosis. Challenging of patients with haemoglobin level ≥12 gm/dl with intake of non- fava beans diet with monitoring of those patients by complete blood count, reticulocytic count markers of hemolysis.
Results: The G6PD Genotypes detected were; *Mediterranean variant mutation* in 53% (from which 54.7% had G6PD 1311T silent polymorphism), Cairo mutation in 13% and African mutation in 16% of cases. Chatham mutation in 4%, Santmaria in 1% and Asahi mutation in 1% of cases and these 3 molecular defects were first to be described in Egyptian G6PD deficiency. Of the studied patients 83% were symptomatic; 64% demonstrated acute haemolytic crisis with necessity of blood transfusion induced mainly by ingestion of fava beans and 61% had history of neonatal jaundice. Acute haemolytic anemia was found in 79% of Mediterranean variant, 56% of African variant, 61.5% of cairo variant, 50% of Chatham variant, although none of the patients with santmaria and Asahi variants had developed haemolytic crisis.The G6PD enzyme level was significantly lowered in Mediterranean and African mutation compared to other mutations and was not correlated with disease severity. With prospective follow up of patients after ingestion of legumes rather than fava beans taken in small amount (5-20 gm) per day for 3 successive days no attacks of hemolysis were developed as indicated by colour of urine, CBC, reticulocytic count and markers of hemolysis (indirect bilirubin, LDH).
Summary and Conclusions: G6PD deficiency *Mediterranean* mutation is the most common mutation among Egyptian children with G6PD deficiency followed by African and Cairo mutation. This is the first report of G6PD Santamaria, chatham and Asahi among our Egyptian population. Ingestion of small amount of legumes rather than fava beans was not associated with haemolysis in G6PD deficient children.

Thalassemia and sickle cell disease

P551

EXTRAMEDULLARY HEMATOPOIESIS (EMH) IS ASSOCIATED WITH A THALASSAEMIA INTERMEDIA-LIKE PATTERN OF MYOCARDIAL AND LIVER IRON LOADING IN REGULARLY POLYTRANSFUSED THALASSAEMIA PATIENTS

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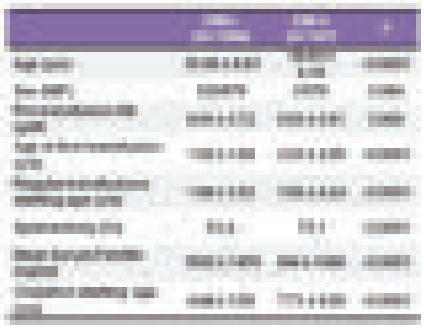
Background: Extramedullary hematopoiesis (EMH) is an incidental finding in regularly and historically polytransfused thalassaemia patients but no study has evaluated if it is a marker of a peculiar pattern of iron loading.
Aims: We studied the relationship between EMH and Magnetic Resonance Imaging (MRI) findings.
Methods: 1266 thalassemia patients (pts) regularly transfused (655 F; 31.25±8.86 years) consecutively enrolled in the Myocardial Iron in Thalassemia (MIOT) Network were considered. MRI was used to assess the presence of EMH by SPGR sequences, to quantify cardiac and hepatic iron overload by a multiecho T2* approach, and to assess cardiac function, volumes and pulmonary diameter by SSFP sequences. Myocardial fibrosis was evaluated by LGE technique.
Results: EMH was detected in 167 pts (13.2%). The Table 1 shows demographic and haematological comparisons between EMH- and EMH+ pts. No significant differences were found in the chelation regimens between the two groups. EMH+ pts had significant less cardiac iron overload than EMH- pts (13.2 *vs* 28.3% of pts with global heart T2* <20 ms; P=0.003). Biventricular volumes, cardiac index, ejection fractions, atrial areas and presence of myocardial fibrosis were comparable between the two groups. EMH+ patients had a significantly higher LV mass index (62.3±13.2 *vs* 58.63±13.19; P=0.001) and a significantly higher pulmonary artery diameter (24.7±4.2 *vs* 23.6±3.8; P=0.002). The MRI LIC was significantly lower in the EMH+ patients than EMH- pts (6.23±8.13 *vs* 9.23±11.71 mg/g/dw; P=<0.0001). Considering the 482 (38.1%) patients with MRI LIC≥7 mg/g dw, the EMH+ group had a significant lower frequency of global heart T2*<20 ms (18.4% *vs* 40.8% p=0.007).

Table 1.



Summary and Conclusions: In this large cohort of regularly transfused thalassemia patients, EMH was not rarely observed and was associated to a heart thalassemia intermedia like pattern (reducedcardiac and liver iron loading and stigmata of high cardiac output state) despite the transfusional regimen.

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MAGNETIC RESONANCE IMAGING T2 AND R2* TECHNIQUES REFLECT RENAL HEMOSIDEROSIS IN PATIENTS WITH THALASSEMIA AND SICKLE-CELL DISEASE; CORRELATIONS WITH SERUM FERRITIN AND MARKERS OF HEMOLYSIS

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Background: Iron overload is a significant problem in hemoglobinopathies. Recently, magnetic resonance imaging (MRI) gradient echo (T2*), the reciprocal of T2* (known as R2*) and spin echo (T2) techniques have been developed to quantify tissue iron in the liver and the heart. Patients with thalassemia or sickle-cell disease (SCD) may develop kidney insufficiency. The significance of iron overload in the kidneys of these patients is poorly understood.
Aims: To evaluate kidney R2* and T2 MRI as surrogates for kidney iron of patients with different hemoglobinopathies.
Methods: We prospectively studied 158 patients: 85 had thalassemia major (TM), 27 thalassemia intermedia (TI), 37 SCD and 9 double heterozygous SC/β-thalassemia (HbS/β-thal). All patients had MR examinations of the kidneys, liver and heart. For T2* mapping a gradient echo, multi echo FSPGR sequence was used, while for T2 mapping a T2-prepared single shot SSFP technique with multi echoes was performed. The normal reference values of T2 in our lab was <80 msec, while for R2* normal range we used the one previously described by Schein *et al.* (J Magn Reson Imaging 2008;28:698-704), which was 21.3±5.8 Hz with a 95% confidence upper limit of 33.7 Hz. Regarding renal function, patients were divided in the 5 chronic kidney disease (CKD) stages of the KDIGO classification, according to eGFR evaluated by the CKD-EPI formula.
Results: Kidney T2 values were (mean±SD) 100.9±11.7, 99.4±13.7, 99.1±13.4 and 105.9±14.9 msec and for patients with TM, TI, SCD, and HbS/β-thal, respectively; 7/85(8.2%) TM, 2/37(5.4%) SCD, 1/27(3.7%) TI patients and none with HbS/β-thal had T2 values lower than the normal limit of <80 msec. Kidney R2* values were 17.5±11.4, 22.0±19.7, 18.1±14.7 and 22.2±18.8 Hz respectively. Compared to historic controls, 3/85 (3.5%) TM, 4/27 (14.8%) TI, 2/37 (5.4%) SCD and 1/9 (11.1%) HbS/β-thal patients had R2* >upper normal limit of 33.7 Hz. There was no difference between the different groups regarding T2 or R2* values. eGFR values were 107±19, 117±10, 100±33 and 105±27 ml/min/1.73m^2, respectively. Patients with TI had higher eGFR than SCD and TM patients (p=0.014 and p=0.001, respectively). The number of TM, SCD, HbS/β-thal and TI patients with CKD stage 3-5 was 4 (4.7%), 4 (10.8%), 1 (11.1%) and 0, respectively. There was no correlation between T2 or R2* values and eGFR or serum creatinine in all patients groups. This may be due to the different T2 or R2* values between the two kidneys of the same patient (p=0.004 and p=0.08, for T2 and R2*, respectively). Patients with low T2 values (<80 msec) had higher serum ferritin and lower Hb (p=0.009 and p=0.046, respectively). Patients with high R2* values (>33.7 Hz) had higher serum LDH and reticulocyte counts (p=0.022 and p=0.033, respectively). Renal R2* (r=-0.256, p=0.001) but not T2 values correlated also with liver T2* values. In TM patients there was a negative correlation of T2 values with ferritin (r=-0.290, p=0.009) and LDH (r=-0.331, p=0.003), a positive correlation of R2* with ferritin (r=0.402, p=0.001) and LDH (r=0.361, p=0.001) and a negative correlation with Hb (r=-0.240, p=0.03).
Summary and Conclusions: Our study provides evidence that T2 and R2* reflects kidney iron deposition in 3-14% of patients with different hemoglobinopathies. More interestingly, in TM patients both MRI techniques correlated with serum ferritin and LDH. Further studies, possibly with the performance of renal biopsies, will reveal the best MRI technique for detecting iron overload in the kidneys of patients with hemoglobinopathies.

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HEPATIC FIBROSIS AND CIRRHOSIS IN PATIENTS WITH THALASSAEMIA MAJOR IS ASSOCIATED WITH PRESENT AND HISTORICAL IRON OVERLOAD, AGE, AND ACTIVE HEPATITIS C INFECTION

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Background: Patients with transfusion-dependent thalassaemia are at risk of cirrhosis, which can result in liver failure and hepatocellular carcinoma. Standard clinical methods for identifying hepatic pathology (ultrasound, biochemistry) are insensitive to fibrosis. Liver biopsy is invasive and unsuitable for screening. Lack of suitable screening tests has hampered estimates of the prevalence of and risk factors for hepatic fibrosis among patients with thalassaemia. Transient Elastography (TE) enables reliable non-invasive evaluation of liver stiffness and hence, risk of cirrhosis. The Enhanced Liver Fibrosis (ELF) Score comprises a set of extracellular matrix markers and has excellent correlations with liver fibrosis.
Aims: To estimate the prevalence and risk factors for fibrosis, we used TE and ELF to prospectively screen chronically transfused, chelator-treated patients with thalassaemia without previous diagnosis of cirrhosis.
Methods: Adults with transfusion dependent thalassaemia without known cirrhosis were invited to undergo TE and ELF measurements. Concurrent/ historical ferritin and HCV viral load, and recent (<1year) liver ultrasound and T2* MRI data were collected. Prevalence of cirrhosis and fibrosis defined by TE and/or ELF score was calculated, and risk factors for fibrosis evaluated.
Results: We screened 63 patients (mean age 43y, 46% male, 89.9% presently using deferasirox) for liver fibrosis by TE, of whom 61 also had ELF scores measured. Mean concurrent (2013) ferritin was 776.5ng/uL; mean ferritin in 1998, 2003 and 2008 was 734.5ng/uL, 1041.9ng/uL and 1091.1ng/uL respec-

tively; a significant increase was seen between 1998-2003, and a significant reduction from 2008-2013, coinciding with the introduction of deferasirox. Prevalence of HCV Ab was 54%, including 27% with detectable viral load. TE and ELF score were correlated (r=0.47, p=0.0001). By TE, 18/63 (29%) had fibrosis, including 7 (11%) with cirrhosis. By ELF score, 96.7% of patients had evidence of fibrosis, including 34.4% with severe fibrosis. Interestingly, concurrent liver T2* was not correlated with fibrosis risk by either measure. Controlling for confounders by multiple linear regression, TE score was associated with age (p=0.002), presence of detectable HCV virus (p=0.002), and both current (p<0.012) and historic (1998) ferritin (p=0.012) concentrations (R^2=0.457). By multiple regression, ELF score was associated with age (p=0.005) and historic (1998) ferritin levels (p<0.001).

Summary and Conclusions: Hepatic fibrosis and cirrhosis are prevalent in adults with transfusion dependent thalassaemia. Although concurrent ferritin is important, historic iron overload and active hepatitis C viral infection also mediate fibrosis risk. Chronically transfused patients with a history of prior iron loading and/or untreated hepatitis C virus should undergo specific screening for cirrhosis, even if ferritin levels are presently controlled.

P554

FIBROSCAN FOR THE DETECTION OF LIVER FIBROSIS IN HAEMOGLOBINOPATHY PATIENTS REQUIRING CHRONIC TRANSFUSION THERAPY

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Background: Iron overload from chronic transfusion therapy is a major risk factor for the development of hepatic fibrosis in haemoglobinopathy patients. Whilst liver biopsy remains the gold standard for assessment of hepatic fibrosis, this test is invasive and subject to sampling variation. Transient elastography (FibroScan) is a novel, rapid, non-invasive test which uses liver stiffness measurements (LSM) to assess hepatic fibrosis. To date few studies have examined the utility of FibroScan in patients with transfusional siderosis.
Aims: This study aimed to examine the relationship between LSM and liver iron concentration (LIC) as measured by MRI in a cohort of haemoglobinopathy patients receiving regular transfusion therapy.
Methods: 27 patients who receive monthly blood transfusion for their haemoglobinopathies had LSM using FibroScan. Scans were deemed successful if at least 10 valid LSM were obtained and the interquartile range/median was <30%. These results were correlated with liver iron concentration measured by MRI in the same patients as part of the ongoing TIMES study - an epidemiological study to assess the prevalence of iron overload using MRI in patients with transfusional siderosis.
Results: Of 27 patients evaluated 23 have thalassaemia major and 4 have sickle cell disease. Four have hepatitis C. The mean age was 33 (range 18-56), the mean ferritin was 1844µg/l (range 344-8036) and the mean LIC was 8.7mg/g (range 0.5->43.0). All patients are currently on iron chelation therapy. The majority of patients (n=22) had no/minimal fibrosis (LSM<7.5kPa) with a mean LIC in this group of 8.0mg/g. However 4 patients had scans consistent with advanced fibrosis/cirrhosis (all with LSM >10.5kPa). Two of these patients have hepatitis C whilst another had a markedly elevated LIC (>43mg/g). However one patient, with sickle cell disease, had elevated liver stiffness despite a low LIC and no other clear risk factors for liver disease. There was no correlation between liver iron content as assessed by MRI and LSM (Figure 1).

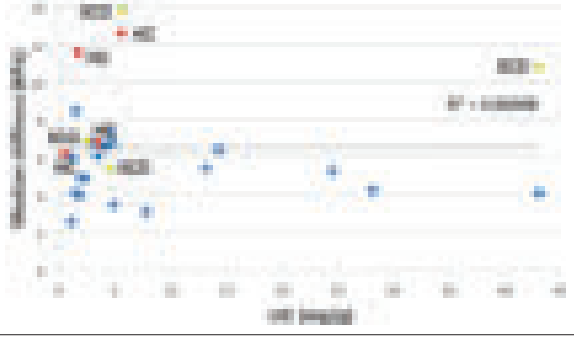

Figure 1. LSM *vs* LIC. Patients with hepatitis C are shown as HC; those with sickle cell disease are shown as SCD.

Summary and Conclusions: FibroScan is likely to prove useful for monitoring for the development of fibrosis in patients with iron overload from chronic transfusion therapy, particularly in those with additional risk factors such as hepatitis C. LSM appear to be independent from LIC. Significant liver fibrosis may be detected in patients without current evidence of high iron load, perhaps due to previous inadequate iron chelation. In one patient with sickle cell disease in our cohort, the elevated LSM may be unrelated to iron, a possible cause being the effect of sinusoidal obstruction incurred during previous sickling crises. Further studies are needed to confirm the utility of FibroScan in patients with transfusional iron overload.

P555

EFFECT OF SPLENECTOMY ON CARDIAC IRON AND FUNCTION IN DIFFERENT TRANSFUSION-DEPENDENT PATIENTS

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Background: The main therapeutic rationale for splenectomy in transfusion-dependent patients with hemoglobinopathies is to decrease blood consumption and transfusion requirement. However, since the spleen is a large physiologic iron depot, splenectomy may have a possible role of in determining extrahepatic iron overload.
Aims: This study aims to observe retrospectively the effect of splenectomy on cardiac iron and function in different groups of transfusion-dependent patients.
Methods: 1735 transfusion-dependent patients enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. 14 patients had sickle-thalassemia, 23 patients had sickle-cell disease (SCD), 179 had thalassemia intermedia (TI) and 1519 had thalassemia major (TM). Cardiac iron was assessed using a multislice multiecho T2* approach. Left ventricular ejection fraction (LV EF) was quantified by cine sequences.
Results: The frequency of splenectomy was: 21.4% in sickle-thalassemia, 65.2% in SCD, 84.9% in TI and 55.1% in TM (P<0.0001).
Splenectomized TM patients were older than non-splenectomized patients (34.3±7.9 yrs *vs* 27.2±7.8 yrs; P<0.0001). In each hemoglobinopathy, cardiac T2* and LV EF were comparable between splenectomised and non-splenectomized patients (Figure 1).

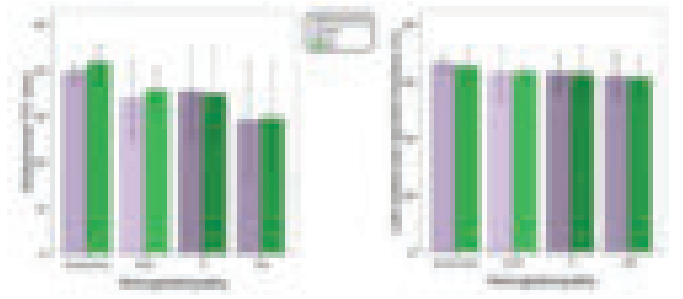

Figure 1.

Summary and Conclusions: Regardless by the type of hemoglobinopathy, in regularly transfused patients splenectomy was not associated with increased cardiac iron and reduced cardiac function.

P556

CORTISOL RESPONSE TO LOW DOSE *VERSUS* STANDARD DOSE ACTH STIMULATION TESTS IN CHILDREN AND YOUNG ADULTS WITH THALASSEMIA MAJOR

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Background: Beta Thalassemia major patients with repeated blood transfusion have high prevalence of endocrinopathies due to iron overload
Aims: To evaluate Cortisol response to low dose *versus* standard dose ACTH tests in children and young adults with thalassemia major
Methods: We examined the adrenocortical function in 23 thalassemic patients (10 children and 13 young adults) aged 8-26 years. Serum cortisol and (DHEA-S) concentrations were determined in each subject before blood transfusion both in basal condition and after low dose (LD) (1 mcg), followed by standard dose (SD) (250 mcg, respectively) with synthetic corticotrophin β 1-24 ACTH (Synacthen)
Results: Using a peak total cortisol cutoff level of 550 nmol/L and increments

of 200 mcg above basal cortisol, adrenal insuffi ciency (AI) was demonstrated in 8 patients (34.7%) after the LD ACTH and in 2 patients (8.7%) after SD cosyntropin (ACTH) test, but none of the controls. Using a peak total cortisol cutoff level of 420 nmol/L and increments of 200 mcg above basal cortisol, AI was demonstrated in 5 patients (21.7%) after the LD ACTH and in 2 patients after SD ACTH test (8.7%), but none of controls

Summary and Conclusions: The use of LD ACTH test diagnoses more adrenal abnormalities *versus* SD ACTH in thalassemic patients. The relatively high prevalence of AI in thalassemic adolescents and young adults necessitates that these patients have to be investigated for AI before major surgery and those with impaired cortisol secretion should receive stress doses of corticosteroids during the stressful event.

P557

VENOUS THROMBOEMBOLISM IN SICKLE CELL DISEASE: PREVALENCE AND PATIENT CHARACTERISTICS.

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Background: Although sickle cell disease (SCD) is recognised to be a hypercoagulable state, there is a dearth of data evaluating the prevalence of venous thromboembolism (VTE) in patients with this condition. The characteristics of SCD patients presenting with deep vein thrombosis (DVT) or pulmonary embolism (PE) are poorly defined, and nature of VTE and clinical outcomes are largely unknown.

Aims: To describe the prevalence, risk factors and presentation of VTE in a large cohort of SCD patients.

Methods: Medical records of all 855 adult SCD patients (all of African descent) regularly attending clinic at King's College Hospital, London, were retrospectively reviewed to identify cases of VTE. Records were reviewed from the earliest date of attendance to the last patient record or death. Within the cases a subset of young adult patients was identified, defined as those ≤25 years old. Patients were excluded if there was no accessible documentation regarding their first VTE as this precluded confirmation of the event.

Results: Amongst the 855 patients, 40 cases of VTE were identified; 30 patients were included. Of 223 patients below 25 years old, 10 young adults with VTE were identified, and 9 included as a subset. The prevalence of VTE in the total cohort was found to be 3.5%, and 4.0% in the young adult subset. The mean length of follow up was 8.1 years (range 3 months – 15.1 years). The mean age at first VTE in the young adult subset was 19 years (range 16-25 years), and 31.9 years (range 16-50 years) for the group as a whole. Regarding the site of the first VTE, 56.7% suffered a PE (of which 76.5% were isolated and 23.5% were associated with symptomatic DVT), 23.3% presented with proximal DVT without PE and 16.7% with distal DVT alone. One patient was found to have an inferior vena cava thrombus on echocardiogram. Overall 43.3% of VTEs were unprovoked (44.4% in the young adult subgroup). 23.3% of patients had recurrent VTE events. 17 patients underwent thrombophilia testing of which 5 were positive. 8 patients also had raised Factor VIII levels (range 201-369 IU/dL, mean 324.4 IU/dL). Conventional VTE risk factors were present in 80.0% of patients; SCD-specific risk factors (including history of chronic leg ulcers, avascular necrosis, splenectomy, HbSC genotype or pulmonary hypertension) were present in 56.7%.

Summary and Conclusions: These data demonstrate that the prevalence of VTE in this SCD population is much greater than that of the non-SCD black population. Strikingly, the prevalence of VTE in young adults with SCD is almost a hundred-fold greater than might be expected for this age group when compared to the non-SCD black population. Epidemiological studies have stated the rates of DVT in the general population to be 2-3 times greater than that of PE; in this SCD population PE was more common than DVT. However, the proportion of unprovoked and provoked VTE remains comparable to a non-SCD population. 26.7% of patients were found to have raised Factor VIII levels; this has previously been identified as an important risk factor in black patients and in this study was also associated with recurrent VTE.

This study indicates that SCD represents a major risk factor for VTE, particularly in young adults. This may have significant implications for both prophylaxis and the management of VTE in these patients.

P558

SICKLE CELL DISEASE IN AN URBAN ENVIRONMENT IN THE UNITED KINGDOM: DESCRIPTION OF AN ADULT COHORT

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Background: Sickle cell disease (SCD) is the fastest growing serious genetic disease in the UK and Europe with an estimated 12500 affected individuals in the UK. Due to improved comprehensive care, an affected newborn in the UK can now expect to live to adulthood, but an increased lifespan has led to an increased burden of end-organ damage in adults.

Aims: To describe the prevalence and incidence of, and associations between, end-organ complications in an adult SCD cohort in an urban setting in the UK.

Methods: Data were gathered from electronic hospital records captured during steady-state clinic visits of patients from 01/01/2001 to 31/12/2012. End-organ complications were defined using local guidelines and criteria outlined in Ballas *et al.* 2010.

Results: 796 patients have been registered for the 13 year study period. These patients were followed up for a total of 5341 patient years, with a mean follow-up per patient of 6.7 years (range 1-13). Despite the transitory nature of our cohort, patient numbers have increased over the study period from 225 in 2000 to 515 in 2012. The proportions of the different genotypes have remained stable (HbSS 57–65%, HbSC 27–36%, HbSβ^+ 4-5%, and HbSβ^0 1%). Mean age increased from 32 (in 2000) to 36 (in 2012) years (range 16-83), the proportions of the more severe sickle genotypes decreasing with increasing age. 34 patients were identified who had died during the study period, of these 26 had HbSS, 6 HbSC and 2 HbSβ^+. Mean age of death was 41.3 (median 42, range 20 – 79) years. End-organ complications assessed included avascular necrosis, osteomyelitis, acute chest syndrome, tricuspid regurgitant jet velocity ≥2.5 ms, sickle cell lung disease, stroke, retinopathy, gallstones, leg ulcers, priapism, renal impairment (albumin:creatinine ratio ≥4.5 and creatinine clearance <60ml/min). The total number of end-organ complications per patient was calculated using the date last seen as the end point of data collection. Analysis was limited to patients with HbSS and HbSβ^0 (analysed together as sickle cell anaemia, SCA) and HbSC. 48% of patients had no evidence of sickle-related end organ complications at the end of the study period, 26% had 1 complication; 16%, 2 complications; 6%, 3 complications; 3%, 4 complications, and 1% ≥5 complications. The prevalence and incidence of all complications was higher in SCA compared to HbSC, except for sickle retinopathy (prevalence 18% in SCA *vs* 50% in HbSC). Patients with SCA had significantly more complications than patients with HbSC ($p<0.0001$); this persisted when corrected for age ($\beta=0.58$ $p<0.0001$). The number of patients without end-organ complications reduces with increasing age for both sub-groups. However within SCA, patients in their 6th and 7th decades appear to have fewer complications, forming a sub-group with a milder phenotype (see Figure 1). 51% of patients with SCA have at least 1 complication by the end of their 4th decade.

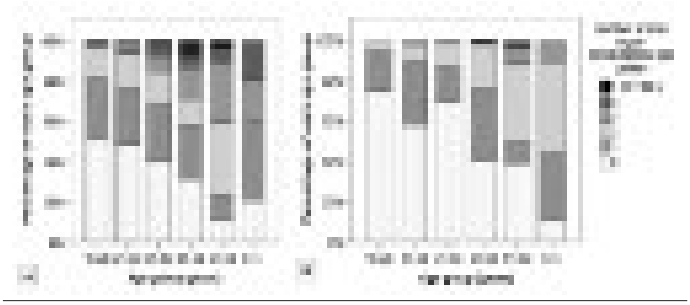

Figure 1.

Summary and Conclusions: The patterns of morbidity and mortality in this UK urban adult sickle cohort are comparable to that previously described in an US cohort. However, the increasing usage of interventions such as hydroxyurea and blood transfusion may alter these patterns of disease presentation and progression.

P559

OPHTHALMOLOGIC COMPLICATIONS IN A SICKLE CELL DISEASE COHORT WITH IN NORTH LEBANON

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Background: Sickle cell disease (SCD) is a hereditary hemoglobinopathy characterized by vaso-occlusion, hemolytic anemia, recurrent infections and end organ failure. Recurrent vaso-occlusion potentially affects the vascular beds of all organs including those of the eye. Previous studies have shown that ophthalmological findings in SCD are often detected before patients report any visual symptoms, underscoring the importance of early screening. To date, no data on the epidemiology and the prevalence of eye complications in the Lebanese SCD population has been reported.

Aims: The primary objectives of this study are to prospectively determine the prevalence of ophthalmological complications in a SCD cohort and the importance of early screening in the detection of asymptomatic eye findings. The secondary objectives are to delineate possible risk factors for ophthalmic complications in the above group.

Methods: A SCD cohort followed in a single comprehensive hemoglobinopathy center in North Lebanon will undergo a detailed ophthalmological exam

including best-corrected visual acuity (BCVA) measurement, slit-lamp examination, direct and indirect ophthalmoscopy and retinal photographs. Fluorescein angiography (FA) will be performed on patients with reduced BCVA or retinal photograph abnormalities. Frequencies of events will be calculated, and Pearson's test used for correlation analysis.
Results: 104 eyes for 52 patients, 86.5% with sickle cell anemia (HbSS) and 13.5% with sickle β thalassemia (S/βT), 52% males and 48% females, mean age at time of testing 13.8 years (age range, 3-44 years) were examined. 88.5% of the patients had history of at least one blood transfusion, and 26.9% and 69% were on iron chelation therapy and hydroxyurea, respectively. BCVA of the right eye was <20/100, 20/100-20/50, and >20/50 in 2.3%, 4.7% and 93% of cases respectively, whereas BCVA of the left eye was <20/100, 20/100-20/50 and >20/50 in 0%, 2.3% and 97.7%, respectively. Among the 44.2% of patients with conjunctival involvement, comma shaped vessels were found in 56.5% of cases, pallor was found in 56.5% of cases and hyperemia in 4.3% of cases. Only 1 patient had evident stage 1 hyphema. Among the 52% of patients with posterior segment manifestations, salmon patch hemorrhages were found in 11.5%, black sunbursts in 38.4%, hairpain loops in 30.7% and seafans in 3.8% of cases. 4/50 patients (8%) had proliferative sickle retinopathy (PSR); 3 (75%) aged 3-9 years had stage 1 and 1 (25%) aged 34 years had stage 4 PSR. Enlarged fovea was seen only in 3 (6%) patients.
Summary and Conclusions: Ophthalmologic findings are quite frequent among Lebanese patients with SCD and proliferative sickle retinopathy is seen at a very young age. This study underscores the importance of early screening and therapeutic intervention for ophthalmologic complications in improving patients' outcome in SCD.

P560

SOLUBLE FMS-LIKE TYROSINE KINASE-1 IN YOUNG PATIENTS WITH SICKLE CELL DISEASE: RELATION TO HEMOLYSIS-ASSOCIATED COMPLICATIONS

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Background: Many mechanisms contribute to the complex pathophysiology of sickle cell disease (SCD), with dysfunction of the vascular endothelium as a major underlying pathology. Soluble fms-like tyrosine kinase-1 (sFLT-1) is a member of the vascular endothelial growth factor receptor (VEGFR) family. By adhering to and inhibiting VEGF and placenta growth factor, it induces endothelial dysfunction suggesting a possible role in the pathogenesis of SCD-associated vasculopathy and pulmonary hypertension.
Aims: To determine the levels of sFLT-I in young SCD patients compared with age- and sex-matched healthy controls and evaluate its role as potential marker for hemolysis associated complications.
Methods: Thirty-five children and adolescents aged 3.2-18 years (median 12.1 years) with SCD in the steady state were studied stressing on the frequency/severity of sickling crises, transfusion history, hydroxyurea therapy, hematological profile and serum ferritin, and echocardiography assessment of pulmonary hypertension. sFLT-I was measured by enzyme linked immunosorbent assay (ELISA).They were compared to 35 age and sex matched healthy controls.
Results: sFLT-1 was significantly higher in SCD patients in the steady state (median 130pg/ml) compared to controls (median 70 pg/ml), p<0.001. Levels of sFLT-1 were significantly higher in patients with history of manifest or silent stroke (p=0.007) and in the presence of pulmonary hypertension (p<0.001). SCD patients who experienced sickling crisis during the study period had higher sFLT-1 levels than patients in steady state (p<0.001). Hydroxyurea-treated patients had lower sFLT-1 levels than untreated patients (p=0.005). Significant positive correlations were observed between sFLT-1 and transfusion index(r=0.431, p=0.01), WBC count (r=00639, p<0.001), serum indirect bilirubin(r=0.513, p=0.002) and LDH (r=0.335, p=0.049) and serum ferritin (r=0.412, p=0.014) while sFLT-1 was negatively correlated to hemoglobin (r=-0.599, p<0.001) and HbF (r=-0.476, p=0.004). Multiple regression analysis revealed that sickling crisis (p<0.001), pulmonary hypertension (p<0.001), HbF (p=0.03) and indirect bilirubin (p=0.046) were independently related to sFLT-1. ROC curve analysis revealed that the cutoff value of sFLT-1 at 132.5 pg/mL could predict the occurrence of sickling crisis with 100% sensitivity and specificity of 100% [AUC 1.0, p <0.001] and sFLT-1 cutoff value at 127.5 pg/mL could differentiate patients with and without pulmonary hypertension with a sensitivity of 100% and specificity of 74%[AUC 0.958, p <0.001]
Summary and Conclusions: sFLT-1 may be considered a potential biological marker for vascular dysfunction and disease severity in SCD. sFLT-1 levels may contribute to the pathogenesis of SCD-associated pulmonary hypertension and would help in early crisis prediction as well as monitoring the response to hydroxyurea therapy.Further longitudinal studies with large number of patients are needed to verify these preliminary results.

P561

GROWTH DIFFERENTIATION FACTOR-15 IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL DISEASE: RELATION TO HEMOLYSIS, IRON OVERLOAD AND VASCULAR COMPLICATIONS

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Background: Growth differentiation factor-15 (GDF-15) is a member of the transforming growth factor-β superfamily, it is strongly expressed in the placenta and during erythroblast maturation.However, GDF-15 is expressed in various tissues and is highly inducible during macrophage activation in response to several inflammatory cytokines and after tissue injury. GDF-15 expression at the high levels contributes to pathological iron overloading through a mechanism of incomplete hepcidin suppression. Sickle cell syndromes are characterized by increased levels of erythropoiesis, although the primary defect in sickle cell involves destruction of mature erythrocytes.
Aims: To determine the serum levels of GDF-15 in children and adolescents with SCD and assess its relation to markers of hemolysis, iron overload and vascular complications.
Methods: GDF-15 levels were measured by enzyme linked immunosorbent assay (ELISA) in 35 SCD patients aged 3.2-18 years (median12.1years) in the steady state and were compared to 35 healthy age and sex matched controls. The results were correlated to clinical and laboratory variables including age,sex, frequency and severity of sickling crises, previous cerebral stroke,transfusion history, hydroxyurea therapy, hematological profile and serum ferritin and echocardiography assessment of pulmonary hypertension.
Results: The median (IQR) of GDF-15 level in SCD was 2750 (1200) pg/ml compared to 90(120) pg/ml in controls, (p<0.001). GDF-15 levels were significantly higher in patients who had serum ferritin ≥2500 µg/L (p<0.001), in patients with previous manifest or silent cerebral stroke(p=0.022), and with splenectomy (p=0.018). GDF-15 level were not significantly related to age,sex,frequency of sickling crises,pulmonary hypertension,or hydroxyurea therapy. GDF-15 levels were positively correlated with transfusion index (r=0.874, p<0.001), LDH (r=0.773, p<0.001), serum indirect bilirubin (r=0.432,p=0.009) and serum ferritin (r=0.790,p<0.001), while GDF-15 levels were negatively correlated to hemoglobin (r=-0.621,p<0.001) and HbF% (r=-0.837,p<0.001). Multiple regression analysis revealed that transfusion index (p=0.001), LDH (p=0.042) and serum ferritin (p=0.006) were independently related to GDF-15 levels in SCD patients.
Summary and Conclusions: Increased GDF-15 in SCD reflects the importance of ineffective erythropoiesis in the pathophysiology and clinical severity of anemia in SCD. GDF-15 levels are significantly related to markers of hemolysis and iron overload in SCD and may provide utility for identifying patients who are at increased risk of thrombotic events. Further longitudinal studies including larger number of patients are needed to verify the practical utility of GDF-15 measurement and its potential to predict the clinical severity and outcome in SCD patients.

P562

PITUITARY ABNORMALITIES IN SHORT ADOLESCENTS AND YOUNG ADULTS WITH SICKLE-CELL DISEASE (SCD) AND RECURRENT VASO-OCCLUSIVE CRISIS

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Background: Growth failure is the most frequent endocrine abnormality observed in patients with SCD. Decreased synthesis of IGF-I might be secondary to a disturbed GH-IGF-I axis and defective GH secretion has been reported in some patients. Infarction, atrophy, and hemorrhage may occur in the pituitary gland in SCD during or following the vaso-occlusive crisis.
Aims: To define the possible abnormalities of pituitary gland in sickle-cell disease (SCD) we measured the circulating concentrations of insulin-like Growth factor –I (IGF-I) and studied the Magnetic Resonance Imaging (MRI) of the pituitary gland in 7 adolescents and young adults with SCD with short stature (HtSDS <-2) and history of recurrent painful crisis.
Methods: Seven patients with SCD (age: 24.2 +/- 4.5 years) and short stature (HtSDS=2.5 +/- 0.4) and history of severe and recurrent vaso-occlusive crisis (at least 3 in the past 3 years) were studied. All were transfusion – dependent, with full pubertal development (Tanner's stage 5) (euogonadal). They were regularly transfused since early childhood and underwent chelation therapy using desferrioxamine which was replaced by deferasirox for the last 4 -5 years.
Results: In the 7 patients with SCD circulating IGF-I were decreased (IGF-I SDS=-2.1+/-0.5) compared to adults standards. Pituitary MR imaging showed abnormalities in 4/7 of these patients in the form of heterogeneous appearance of the anterior pituitary, presence of single or multiple hypointense foci due to hemosiderin deposition in the pituitary (4/7) and significantly decreased (2/7) or increased volume (1/7). These lesions can be explained by hemosiderosis of the gland and/or ischemia during the vaso-occlusive crisis (Figure 1).

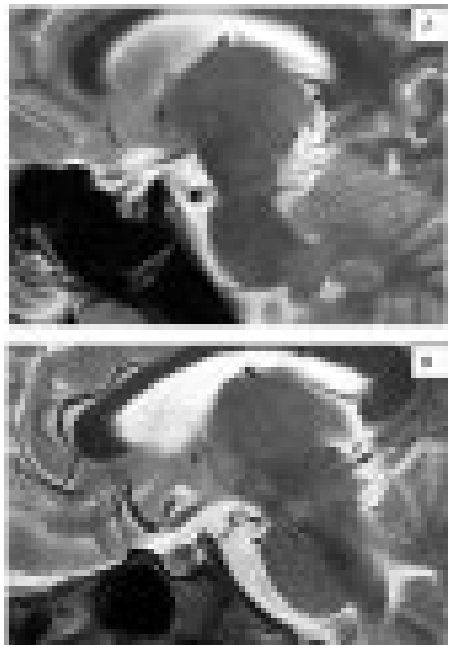

Figure 1. Two pituitary MR images showing deposits of hemosidrin: A) one hypointense lesion; B) many spots.

Summary and Conclusions: Pituitary MR imaging showed significant abnormalities of the anterior pituitary gland in SCD patients with short stature and significant history of vaso-occlusive crisis. This study demonstrated the value of MRI imaging of the pituitary to support investigating of the GH-IGF-I axis in these patients.

P563

OUTCOMES OF A COHORT OF CHILDREN WITH HEMOGLOBINOPATHY RECEIVING ORAL IRON CHELATORS FOR TREATMENT OF TRANSFUSIONAL IRON OVERLOAD IN TURKEY: 24-MONTH FOLLOW-UP

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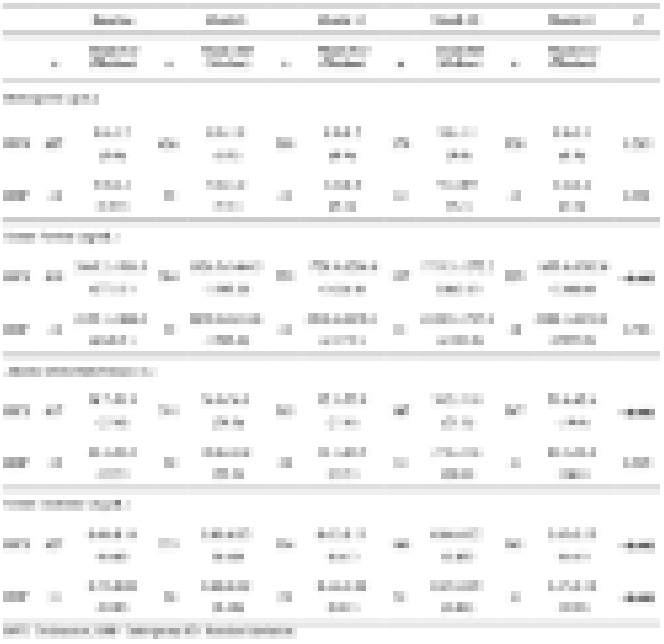
Background: Optimal management of transfusional iron overload (TIO) is still an important indicator of success in treatment of hemoglobinopathies. Outcomes of chelation therapy may directly affected by efficacy, tolerability, and safety of chelators.

Aims: This study aimed to evaluate outcomes of children with hemoglobinopathy receiving oral iron chelators (OIC) for treatment of TIO in Turkey.

Methods: This is a nation-wide, prospective, multi-center, non-interventional study in Turkey which is still ongoing (planned follow-up time: 3 years). Patients aged 2-18 years with β-thalassemia major (bTM) or sickle cell anemia (SCA) who were suffering from TIO and under OIC treatment were included. All patients and/or their parents provided informed consent. Patients' data including clinical and demographic characteristics, laboratory and imaging results related to TIO, and serious adverse events were recorded. Herein, 24-month follow-up results of the study are presented.

Results: Patients (n=474) from 30 centers representing the profiles of Turkish patients were enrolled, of whom 450 (50.7% female; mean age, 9.5±3.8 years) had a primary diagnosis of bTM and 24 (33.3% female; mean age, 10.1±4.2 years) had a primary diagnosis of SCA. Results of some laboratory parameters on the 24-month follow-up are summarized in Table 1. Of the patients, 391 (82.5%) completed 24-month follow-up. There were 209 (53.5%) patients with a hemoglobin level of ≤9 g/dL on month 24. Serum ferritin levels were significantly decreased with DFX therapy; however, no significant change was observed with DFP therapy. The rate of patients treated with DFX and serum ferritin levels of <1000 ng/mL was increased from baseline to month 24 (18.7% *vs.* 38%). Additionally, the mean DFX dose was increased from 26.3±14.1 to 29.0±9.0 mg/kg/day on month 24. The mean level of alanine aminotransferase (ALT) was significantly decreased in patients receiving DFX, whereas no significant change was observed in those receiving DFP; only 18 patients (17 patients receiving DFX and 1 patient receiving DFP) experienced an increase in ALT level of 3 times the upper limit of normal (ULN). Most of these patients had also relatively higher ALT level at baseline. The mean creatinine level was increased both with DFX and DFP therapies. However, no patient had a creatinine level of over ULN.

Table 1. Results of selected laboratory parameters.



Summary and Conclusions: This study comprises the overall prospective largest pediatric cohort including bTM and SCA patients suffering from TIO and under OIC treatment. More than half of the patients had a hemoglobin level of ≤9 g/dL after 24-month follow-up, revealing that such patients still do not receive optimal transfusion regimens according to current guidelines. 24 months follow up results revealed that DFX therapy reduced serum ferritin significantly but DFP therapy did not. There was no clinically significant change in liver or kidney function in this growing population of patients receiving DFX or DFP. In the majority of patients, serum ferritin level remained above the current guideline recommendations and hence optimization of OIC treatment is needed through active dose adjustments and proper monitoring.

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NON-TRANSFERRIN BOUND IRON REVISITED: NOW THAT ITS POTENTIAL TOXICITY IS ESTABLISHED, HOW CAN ITS MEASUREMENT HELP TO GUIDE CHELATION

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Background: It is now possible to measure plasma non-transferrin bound iron (NTBI) readily, reproducibly and at reasonable cost, particularly its labile (chelatable) plasma iron (LPI) component that can infiltrate cells and raise labile cell iron (LCI) and thereby promote formation of noxious radicals.

Aims: Can those measurements provide indications for chelation efficacy/adequacy in maintaining LPI at sub-toxic levels?

Methods: 90 Thalassemia major patients (48 female/42male) from two centres (49 from Corinth and 41 from Patras) receiving chelation therapy, either as monotherapy, or in different combinations were evaluated. The Corinth patients were studied twice one year apart. Compliance was evaluated by history and pharmacy records. Blood samples were drawn after a 24 hour hold of chelator and then at 2 hours after an observed dose. LPI was determined in plasma with the FeROS™ assay (Aferrix, Tel Aviv).
Results: Baseline LPI compared to Compliance showed an R squared of 0.12. The p value was 0.001 indicating that poorer compliance is associated with higher baseline LPI. 60% (56/90) had normal trough LPI (<0.45μM) and of the remaining 34, 50% (17/34) normalized within 2 h of chelation. In 35 (24 on DFP+DFO, 11 on DFX+DFP) that were internally compared, LPI in the DFP+DFO group was significantly lower ($p<0.05$) than in the DFP+DFX group. Detailed regression analysis indicated that DFP+DFO achieved 0.15 μM lower LPI compared to DFP+DFX (adjusted for baseline LPI). DFP+DFO was 9 times more likely to reduce their baseline LPI levels compared to DFP+DFX and 10 times more likely to normalize their baseline LPI levels.
Summary and Conclusions: LPI provides a convenient and immediate measure of chelation efficacy. It is feasible to monitor adequacy of treatment and patient compliance similar to the use of HbA1C in diabetes management. Repeating such measurements every 3 months should be useful as preventive/corrective guidance in patient management.

P565

CHARACTERIZATION OF RARE ALPHA-GLOBIN GENE MUTATIONS IN THAILAND

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Background: Hemoglobin Constant Spring is the commonest α-globin variant worldwide. Other α-globin variants are very rare. Amplification and direct sequencing of the α-globin genes are much more problematic than those of the β-globin gene as the sequences of α1-globin and α2-globin genes are almost identical.
Aims: We developed a simple protocol using a common forward primer and gene-specific reverse primers to separately amplify α1-globin and α2-globin genes followed by direct DNA sequencing to characterize genetic determinants of α-globin variants in Thailand.
Methods: We performed hemoglobin analysis using isoelectric focusing (IEF) and high performance liquid chromatography (HPLC) during 2012-2013. Cases showing unidentified hemoglobin variants were subject to molecular analysis. Direct sequencing on α1-globin and α2-globin gene was performed when α-globin variants were suspected or β-globin gene analysis yielded negative results. Hematological and hemoglobin analysis profiles of α-globin variants in this cohort were compared to those of our uncommon β-globin variants previously published (Int J Hematol 2009;89:568-71) to determine the phenotypes suggestive of α-globin mutations.
Results: In 41 unrelated individuals, 11 types of mutations of the α-globin genes were identified. Mutations on α1-globin gene are much more common (87.8% of cases) than those on α2-globin gene: 24 Hb Hekinan [α1 27(B8) Glu>Asp], 5 Hb Q-Thailand [α1 74(EF3) Asp>His], 2 Hb Lansing [α1 87(F8) His>Gln], 2 Hb Owari [α1 121(H4) Val>Met], 2 Hb G-Georgia [α2 95(G2) Pro>Leu], 1 Hb Port Phillip [α1 91(FG3) Leu>Pro], 1 Hb Westmead [α2 122(H5) His>Gln], 1 Hbl [α2 16(A14) Lys>Glu], 1 Hb J-Singapore [α2 78(EF7) Asn>Asp], 1 IVS-I-117 (G>A) on α1-globin gene, and 1 insertion of 21 bp on α1-globin gene causing a duplication of amino acid residues 93-99. When iron deficiency anemia was excluded, these heterozygous α-globin variants demonstrated normal or α^+-thalassemia phenotypes. This is the first report of Hb Lansing, Hb Owari, Hb G-Georgia, Hb Port Phillip, Hb Westmead, Hbl, Hb J-Singapore and IVS-1-117 mutation in Thai population. All cases with Hb Hekinan, Hb Owari, Hb G-Georgia and Hb Westmead were clearly seen on IEF, while they were undetectable on HPLC, except for 2 compound heterozygosities for HbHekinan and Southeast Asian deletional α^0-thalassemia. Unknown variants of HbA_2 were able to be found in Hb Hekinan, Hb Q-Thailand, Hb G-Georgia and Hb Port Phillip. When compared with uncommon β-globin variants, the presence of unknown variants of HbA_2 (sensitivity 30%, specificity 100%), the presence of HbBart's or HbH (sensitivity 5%, specificity 100%), unknown variants identified only on IEF (sensitivity 70%, specificity 75%), and unknown Hb variant levels of ≤30% on both IEF and HPLC (sensitivity 73%, specificity 100%) were phenotypes suggestive of heterozygous α-globin variants. Using combined characteristics as criteria for α-globin variant testing yielded sensitivity of 90.2% and specificity of 75%. Hb Lansing mutation was previously reported on α2-globin gene, but it was found on α1-globin gene in our study. Interestingly, cases of Hb Lansing on α1-globin gene had Hb Lansing levels of <10% on HPLC and normal oxygen saturation on pulse oximetry, while carriers of Hb Lansing on α2-globin gene in the previous report had HbLansing levels of ≥10% on HPLC and falsely low oxygen saturation.
Summary and Conclusions: This is the largest series of rare α-globin mutations. The majority of them were missed on HPLC. HbHekinan is the most common α-globin variant in Thais accounting for 58.5% of cases. Some distinct hemoglobin analysis characteristics are suggestive of α-globin over b-globin variants. The percentages of Hb variants, as well as their phenotypes, were also determined by the presence of mutations on either α1 or α2 gene and co-inherited α gene deletions.

P566

DETECTION OF B THALASSEMIA CARRIERS BY RED CELL PARAMETERS OBTAINED FROM THE H2 AUTOMATIC COUNTER USING MATHEMATICAL FORMULAS.

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Background: β thalassemia is considered the most widespread genetic mutation. According to the World Health Organization (WHO) between 1.5-7% of the world population carried this disease and 60,000 to 400,000 new patients are born every year. In Israel, the incidence of carriers for β thalassemia is around 20% among Jews from Kurdish origin and between 5 to 10% among the Arab population. The treatment of β thalassemia requires medical and financial significant resources. Therefore the implementation of a prevention program for identifying β thalassemia carriers is an important goal. β thalassemia carriers show low MCV and MCH. Those parameters and others like the hemoglobin level, RDW and the total number of RBC are easily measured by the automatized blood cell counters. Most of those findings are similar in β thalassemia carriers and patients with iron deficiency anemia, but some of them like the RBC and the RDW are normal in β thalassemia carriers. Automatic detection of β thalassemia carriers can improve significantly the recognition of couples at risk for having an affected offspring and then further genetic counseling can be provided.
Aims: In the past, several researchers, using different formulas, tried to determine cutoff points for the diagnosis of β thalassemia carriers compared to patients with Iron deficiency or to normal individuals, but none of those formulas are currently used in daily practice.
Methods: The hematological laboratory in the Emek Medical Center collect blood samples from all pregnant women in the north of Israel since 1987 for blood count and HPLC. We had 39,483 samples, 22,872 samples had all parameters, 19,763 were healthy or iron deficiency women according to HPLC, another 3109 were identified as β thalassemia carriers. We applied the blood count parameters from this large number of samples to the formulas previously published, and also used the proposed a new algorithm SVM (support vector machine) to find a reliable formula that can differentiate blood count parameters between those groups.
Summary and Conclusions: Only one of the previous described formulas (Shine & Lai) was reliable enough to detect between β thalassemia carriers and healthy people. Our new formula, found by SVM algorithm also had statistical significance ($p<0.05$), but also had only 31 false negative results and shows a negative predictive value of 99.81%, and sensitivity of 98.93, results similar to Shine's formula and better to all the other formulas. This suggested formula can be incorporated to any automatic blood counter and issued as an advice to the physician when suspected carriers are detected, even without having specific previous request for clinical suspicion. In those individuals a further simple HPLC analysis should be done in order to confirm the diagnosis. This formula is for now just validated among women at fertility age.

Infectious diseases, supportive care 1

P567

THE IMPACT OF IMPLEMENTING MATCHING MICHIGAN AND EPIC3 RECOMMENDATIONS TO REAL LIFE HAEMATO-ONCOLOGY PATIENTS: RESULTS FROM A PROSPECTIVE UK BASED STUDY

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Background: Central venous catheters are commonly used in haematology/oncology departments to deliver chemotherapy safely. Large retrospective studies report 1.4-2.2 infections per 1000 catheter days for subcutaneous tunnelled lines (1). Catheter related blood stream infections (CRBSI) are associated with a 25% increase in mortality (2). The Michigan Keystone project implemented specific technical and cultural practices to reduce infections. In England this led to a national patient safety initiative known as Matching Michigan, to minimise CRBSI (3). EPIC3 is a national evidenced based practical guideline published in England to prevent Healthcare associated infections in hospitals (4).
Aims: The aim of this study is to determine whether Matching Michigan and EPIC3 recommendations reduce CRBSI in haematology/oncology patients.
Methods: Prospective data on the insertion and removal dates of central venous catheter devices were collected from January 2013 –December 2013 at New Cross Hospital, Wolverhampton using electronic records and case notes. The ECOG score, comorbidities, episodes and duration of neutropenia and type of line inserted were recorded. Central venous catheter (CVC) tunnelled lines were inserted under radiological guidance. The Visual Infusion Phlebitis (VIP) score was recorded to determine its utility in predicting CRBSI.
Results: 137 patients (average age of 57.7 years; 75 were male) were included in the study. 52 patients had haematological malignancies and 85 patients had oncology cancers. Of the 137 lines inserted, 117 were CVC tunnelled lines (15,101 catheter days) and 20 PICC lines (1,390 catheter days). There were a total of 16491 catheter days with 8 proven CRBSI, giving a CRBSI rate of 0.49 infections per 1000 catheter device days. All 8 infections occurred in the CVC tunnelled lines. Catheter related infections were not related to age >65 (p=0.48), ECOG >1 score (p=0.18), diabetes (p=0.21), inpatient chemotherapy regimens (p=0.16) or neutropenia (p=0.14). The duration of neutropenia >10 days was a significant risk factor (p=0.01). The VIP score was documented in 56 out of 137 patients. The VIP score was positive in 7 patients. Of the 6 patients who had confirmed CRBSI, all 6 had negative VIP scores.
Summary and Conclusions: Duration of neutropenia (>10 days) was the only significant factor to influence line infections. The VIP score has a low sensitivity and does not predict CRBSI. Education of staff, reducing the number of operators inserting CVC lines and having a dedicated line team improves outcomes significantly. Prospectively implementing the measures from EPIC3 and Michigan Keystone project can reduce CRBSI significantly as evidenced by our study in cancer patients.

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P568

VARICELLA-ZOSTER VIRUS INFECTIONS IN IMMUNOCOMPROMISED PATIENTS; COMPARISON OF HEMATOLOGY – ONCOLOGY PATIENTS AND OTHER CHRONIC DISEASES

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Background: Varicella is one of the most common childhood infectious diseases. Acute varicella zoster virus (VZV) infection causes varicella and reactivation of a latent infection of VZV seropositive patients causes zoster. Varicella infections are potentially life threatening in immunocompromised patients especially in those whose immune system has been suppressed by diseases such as acute lymphoblastic leukemia (ALL) or by multiagent chemotherapy and corticosteroid treatment. In pediatric hematology - oncology patients varicella-zoster infections are one of the most important cause of mortality and morbidity. Antiviral treatment with acyclovir and varicella zoster immune globulin decreased the mortality rate of varicella infections in children with immune suppression from 7–55% to <1%.
Aims: In this study, we aimed to determine clinical features of varicella and zona zoster infections in our hematology – oncology patients and to compare complication rates, length of hospital stay and acyclovir use in patients with other underlying conditions such as immunosupression and chronic diseases.
Methods: The medical charts of 144 immunocompromised pediatric patients 0 – 18 years of age who were treated in the Department of Pediatric Infectious Diseases between February 2007 – July 2013 were reviewed.
Details on gender, age, underlying conditions, admission symptoms, nature and type of any varicella-associated complications, were noted. Data indicating the length of hospital stay, intensive care unit stay, administration of acyclovir administration of intravenous immunoglobulin antibiotic therapy, and hospitalization outcome (need for intensiv care unit, or death) were collected.
Results: Out of 144 immunocompromised patients 63 (44%) were hematology – oncology patients (Group A) and 81 (56%) had chronic illnesses (Group B). The median age was 5 years 2 months in group A and 3 years in group B. Boys /Girls ratio was 1,6 in the entire study group, 1,1 in group A and 2,1 in group B. Varicella zoster/Zona zoster ratio was 2.5 in group A and 15,2 in group B. In group A leukemia was the major underlying disease (57%, 30 ALL and 6 AML) followed by solid tumors (24%). In group B neurological diseases were the major underlying disease (37%) followed by nephrological diseases (21%). The most common complication was secondary bacterial infection in Group A (n=26, 41%) and group B (n=24, 30%). Neurological complications were observed in 4 children in group B. In group A, 61 patients (96%) and in group B, 64 patients (79%) were treated with intravenous acyclovir. In the entire group median length of hospital stay was 8 days, in group A 8 days and in group B 7 days. The median length of hospital stay was longer in hematology – oncology patients (p<0,001). One children required treatment in intensive care unit. The patient was in group B. Acyclovir treatment rates and duration of acyclovir treatment was higher in hematology – oncology patients (p<0.001).
Summary and Conclusions: In pediatric oncology patients varicella-zoster infections are one of the important causes of mortality and morbidity. With respect to patients with underlying other chronic diseases, oncology patients need longer treatment period. Early diagnosis and treatment of VZV infections in all chronically ill children would reduce morbidity and mortality rates.

P569

CHANGING OF PLATELET DERIVED MICROPARTICLES, SCUBE-1 AND IL-1B IN CHILDREN WITH DENGUE INFECTION

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Background: Dengue infection is one of the most important acquired platelet disorders in tropical countries. Platelet activation can lead to platelet dysfunction in various medical conditions such as cancers and thrombosis. The role of platelet activation in the pathogenesis of platelet disorder in dengue infection is not well studied yet.
Aims: To evaluate the role of platelet activation in dengue infection using novel molecular markers such as platelet derived microparticles (PDMP), signal peptide-CUB-EGF domain-containing protein 1(SCUBE-1) and interleukin 1 β (IL-1β) and to correlate these markers with severity of disease.
Methods: Prospective cohort study.
Twenty children (age 5-15 years) who had serological confirmation of dengue infection from June 1st to November 30th, 2013 were recruited in this study. Eight patients were diagnosed with dengue fever (DF) and twelve patients were diagnosed with dengue hemorrhagic fever (DHF). Mean age was 10.45±3.94 years. Blood samples were collected during three stages of disease: febrile stage, toxic stage and recovery stage. Then PDMP, SCUBE-1 and IL-1β levels were measured from patient's plasma by enzyme-linked immunoadsorbent assay. Forty healthy age-matched children served as the control group.
Results: The plasma levels of SCUBE-1 and IL-1β were significantly higher in all stages of dengue infection compared to control group (p<0.001). PDMP levels were significantly increased compare to control group (p=0.007) only in toxic stage of dengue infection. However, levels of PDMP, IL-1β and SCUBE-1 were not significant different between DF and DHF group in any stages.
Summary and Conclusions: Platelet activation can be one of the mechanisms that lead to platelet disorder in patients with dengue viral infection which include thrombocytopenia and platelet dysfunction. However, the novel molecular markers associated with platelet activation may not be useful as a biological marker for dengue disease severity.

P570

PURE RED CELL APLASIA IN PAEDIATRIC HIV

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Background: Anaemia is common in the progression of HIV infection. Vertical-

ly acquired HIV infection impairs immunity in the infant and young child. Co-infection with Parvovirus B19 is an important cause of pure red cell aplasia (PRCA) in this cohort of children and must be managed differently from other causes of PRCA.
Aims: The aim was to describe the frequency and characteristics of PRCA in HIV infected children with anaemia.
Methods: A chart review was conducted of 240 paediatric patients with HIV infection investigated for one or more cytopenias in our unit. Fifty seven patients with red cell hypoplasia (7) or aplasia (50) were identified. The aetiology and response to treatment was evaluated.
Results: There were 20 female patients and 37 males. The median age of presentation was 7 years (2months to 13 years). Parvovirus was identified as the aetiology in 40 patients. In the early part of the study the presence of the typical red cell inclusions and/or the presence of parvovirus IgM was used but since 2003 the parvovirus PCR was used. The remaining patients with PRCA were drug related (lamivudine 5, zidovudine 2) or associated with another co-infection like tuberculosis (5), CMV (2), CMV and EBV (1). In 4 patients the parvovirus studies were not done and no cause could be assigned. The presenting Hb was very low in most patients and the diagnosis often only first considered after the need for multiple transfusions. The Hb was <2g/dl in 13 patients and 2<3g/dl in 23 patients. Treatment with parvovirus PRCA was with transfusions, intra-venous immunoglobulin(IvIg) and starting anti-retroviral therapy. In most instances repeated doses of IvIg were necessary. Drug related PRCA warranted a change in the regimen and erythropoeitin was indicated in these patients with a delayed or inadequate response. Patients with tuberculosis have a variety of changes in the bone marrow which respond to appropriate treatment. Bone marrows have been useful in identfying MOTTs and resistant tuberculosis.
Summary and Conclusions: Response in children with parvovirus related PRCA is similar to that described in adults. The large number of patients we have managed reflects the burden of paediatric HIV disease in the province as well as the cumulative exposure to parvovirus in childhood. Severe and life-threatening anaemia is common and needs transfusion support. Erythropoeitin is indcated in the drug related PRCA and was not usually needed when causative infections were treated. Lamivudine is an important cause at our centre.

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ROLE OF PROPHYLAXIS WITH LAMIVUDINE IN MAINTENANCE TREATMENT WITH RITUXIMAB IN NON HODGKIN LYMPHOMA CD20+
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Background: In literature, sporadic Hepatitis B Virus (HBV) reactivation cases are reported not only in patients treated with Rituximab-based regimens but also in patient after maintenance with Rituximab single treatment. The aim of this work is to evaluate how many HBV reactivation occurred among patients with positivity of Hepatitis B core antigen (HBcAB +) and Hepatitis B surface antigen negative (HBsAg-) who underwent to maintenance treatment with Rituximab as single agent.
Aims: We analyzed retrospectively the prevalence of HBV reactivation among patients with diagnosis of Non Hodgkin Lymphoma CD20+ HBcAb +/HBsAg– who underwent to maintenance treatment with Rituximab after standard Rituximab-based chemoterapy schedule.
Methods: From January 2007 to December 2013, in our Unit, 92 patients with diagnosis of Non Hodgkin Lymphoma CD20+ underwent, after standard chemoterapy schedule Rituximab-based, to maintenance treatment with Rituximab as single agent (schedule: 375 mg/mq every 3 months for 2 years). Patients were previously treated with different chemotherapy treatments: 42% (39/92) with R-CHOP; 50% (46/92) with R-FN; 3% (3/92) with R-F; 4% (4/92) with R-Chlorambucil. None of these patients received prophylactic treatment with lamivudine during induction or maintenance. All the patients were given blood tests for HBV (HBsAg; HBsAb; HBeAg; HBeAb; HBcAb) before starting maintenance treatment and liver function tests before each administration of Rituximab.
Results: 19% of the patients (18/92) were HBcAb positive. 65% of the patients (60/92) completed the maintenance treatment and 28% of them were HBcAb positive (7/25): only in one of these patients occurred the HBV reactivation (median follow up: 24 months). 28% of the patients (26/92) are still in therapy with Rituximab and 11% of them are HBcAb positive (3/26): all these patients are at risk for HBV reactivation too.
Summary and Conclusions: In patients HBcAb +/ HBsAg– in maintenance with Rituximab as single agent, is suggested prophylaxis with lamivudine. In our retrospective study, HBcAb +/ HBsAg- patients didn't receive any prophylactic treatment with lamivudine during the maintenance treatment with Rituximab and the HBV reactivation occurred only in one patient HBcAb+/HBsAg-, three months after the end of the maintenance therapy. More ambitious prospective studies are required to establish the clinical utility of prophylactic treatment with lamivudine during Rituximab-based-maintenance.

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THE IMPACT OF HEPATITIS B VIRUS (HBV) INFECTION ON PROGNOSIS OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AND THE OCCURRENCE OF HEPATITIS FLARES AFTER WITHDRAWAL OF PROPHYLACTIC ANTIVIRAL TREATMENT
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Background: Hepatitis B virus (HBV) infection is a worldwide health problem. It is important to understand its impact on outcomes and prognosis among patients with lymphoma receiving treatment. There is also no consensus on the duration of prophylactic antiviral treatment after cessation of chemotherapy.
Aims: We performed a retrospective study to analyze the characteristics and clinical outcomes of diffuse large B-cell lymphoma (DLBCL) patients with hepatitis B virus (HBV) infection and compare with those without HBV infection. We also assessed the occurrence of hepatitis flares after withdrawal of prophylactic antiviral treatment on completion of chemotherapy.
Methods: Patient records were reviewed retrospectively from all patients aged 18 or above, with untreated DLBCL who had received chemotherapy between January 1996 and December 2010 in Tuen Mun Hospital, Hong Kong. Patients with newly diagnosed DLBCL were treated with curative intent. The hepatitis B surface antigen (HBsAg) positive patients were given prophylactic lamivudine until 6 months after finishing chemotherapy. After chemotherapy and withdrawal of antiviral therapy, patients were followed up regularly to examine for hepatitis flare.
Results: 81 patients were recruited with 16 in the HBsAg positive group and 65 in the HBsAg negative group. The prevalence of hepatitis B among DLBCL patients was 20% in our cohort. The clinical characteristics were similar in both groups of patients. There was no significant difference in terms of marrow involvement, lactate dehydrogenase (LDH) levels, extranodal involvement, proportion of patients receiving rituximab (R) and incidence of advanced stage diseases in the two groups. The median follow-up time for the patients was 47.8 (range 1.1 - 188) months. There was no significant difference in complete remission rate between the two groups (63% in HBsAg positive group *versus* 54% in HBsAg negative group, p=0.59). There was also no statistically significant difference in overall survival between the two groups (p=0.23).
Four of the sixteen HBsAg positive patients (25%) had hepatitis after cessation of chemotherapy and prophylactic lamivudine. One patient was given CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) therapy and the other three were given R-CHOP treatment previously. Hepatitis A and hepatitis C virus infection were excluded as causes of the hepatitis flare. The onset of hepatitis ranged from 2 to 4 months (mean 3 months) after stopping lamivudine. These four patients had normal ALT levels before chemotherapy and stopped lamivudine 6 months after completion of chemotherapy. Lamivudine was resumed in these four patients and one patient required addition of adefovir because of the presence of the tyrosine-methionine-aspartate-aspartate (YMDD) mutant. The liver enzymes gradually normalized in all these patients. The patients were all alive after treatment of the hepatitis flares.
Summary and Conclusions: HBV infection did not appear to affect the clinical outcomes and prognosis of DLBCL patients given prophylactic antiviral treatment. Patients should be monitored closely for hepatitis flare after cessation of antiviral agent on completion of chemotherapy. A significant number of hepatitis flares occurred after 6 months of antiviral prophylaxis upon finishing chemotherapy. It is reasonable to consider prophylactic antiviral therapy to extend to at least one year on completion of chemotherapy.

P573

PREVALENCE, CHARACTERISTICS AND MANAGEMENT OF OCCULT HEPATITIS B VIRUS INFECTION (OBI) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS. MONOCENTRIC EXPERIENCE.
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Background: Several reports have emphasized the risk of Hepatitis B virus (HBV) reactivation in patients with lymphoproliferative disorders undergoing cytotoxic treatment. This risk is relevant also in patients with previous HBV infection and serum HBsAg loss or occult B infection (OBI) affected by CLL, especially if they receive treatment with monoclonal antibodies. OBI is defined by the HBV-DNA in the liver with detectable (<200 IU/ml) or undetectable HBV-DNA in the serum and HBsAg negative. OBI may be distinguished as seropositive-OBI (anti-HBc and/or anti-HBs positive) or seronegative-OBI (anti-HBc and anti-HBs negative) on the basis of the HBV antibody profile. The treatment of active HBV in CLL patients requiring chemo and/or immuno-therapy has been well established, while the management of occult B hepatitis infection (OBI) is still debated.
Aims: We report the prevalence and the management of OBI in CLL population.

Methods: We selected, from our CLL database consisting of 510 patients in the care of our Department from January 1997 to December 2011 and followed until December 2013, all patients studied for CLL and HBV serum markers to detect OBI in this population. All the HBsAg-negative patients found to be positive for anti-HBc were enrolled in the study, independently from the anti-HBs status. All patients undergone immunosuppressive therapy were checked at 3-month interval during treatment and for 18 months thereafter for complete HBV serum markers, including anti-HBc IgM and HBV-DNA serum level. In case of HBs-Ag seroreversion (reappearance of serum HBsAg) with detectable HBV-DNA level, we started antiviral therapy with nucleoside/nucleotide analogues. This strategy was followed until June 2010. After this date all OBI patients who underwent chemo-immunotherapy received universal prophylaxis (UP) during treatment and for 12 months after. All patients provided written informed consent. This study was approved by an internal Ethics Committee.
Results: Here we reported 397 CLL patients assessed at diagnosis for HBV serology. At the time of enrolment all patients were checked for co-infection with hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and routine laboratory parameters assessing liver and kidney function. We observed OBI in 34/397 patients, of whom 24 were HBcAb and HBsAb positive and 10 were HBcAb positive and HBsAb negative. When comparing OBI/CLL with B-CLL patients, we did not find any statistical difference among clinical-biological parameters (sex, age, stage of disease, IgVH, FISH, CD38, Zap-70,) and time dependent end-points (PFS/TTT/OS), the only parameter that showed statistical differences was the lower peripheral blood lymphocyte count in OBI/CLL group (median Lymphocyte count 8.033 *vs* 10.500/mmc; p=0.036). Up to 2010 a careful follow-up and TP were adopted: 2 out of 10 patients (20%) with OBI/CLL receiving chemo-immunotherapy showed seroreversion. They were successfully treated with lamivudine without the development of drug-resistance or any kind of toxicity for 37 and 42 months. Thereafter, we adopted UP during and 12 months after immunosuppressive treatment in all CLL patients with OBI: anyone showed evidence of seroreversion.
Summary and Conclusions: In conclusion in all CLL patients with OBI we recommend UP with lamivudine during and 12 months after the chemo and or immunotherapy in order to avoid HBV seroreversion because these patients had high risk of reactivation due to the loss of immunological control over infection.

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SAFE RITUXIMAB ADMINISTRATION TO NHL PATIENTS WITH CONCOMITANT HBV ACTIVE INFECTION: THE RISK OF HBV REACTIVATION SHOULD NOT PREVENT US TO DELIVER OPTIMAL TREATMENT

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Background: HBV reactivation can occur during treatment (tx) of NHL patients (pts). The standard of care for B-NHL pts is Rituximab(R)- based tx, but R administration is associated with an increased risk of HBV reactivation not only in classical HBsAg+ pts, but also in pts who had resolved HBV infection (HBsAg -/anti HBc+). In HBsAg+ NHL pts undergoing chemotherapy (CT), an antiviral prophylaxis strongly reduces the incidence of HBV hepatitis. High baseline HBVDNA levels are a recognized risk factor for HBV reactivation.
Aims: In November 2013 an Italian Regulatory Agency (AIFA) note prohibited the inclusion of R in the tx of NHL pts with active HBV infection. Since the addition of R to classical CT is associated with superior outcomes, the decision to avoid immunotherapy should be strongly justified even in this small cohort of pts. Here we report our experience of R-tx in NHL pts with active HBV infection.
Methods: From January 2008 until November 2013, 675 NHL pts requiring anti-lymphoma tx referred to our Center and were screened at baseline for HBV parameters. Those pts with signs of active HBV infection (HBsAg+and/or HBVDNA+) were selected for this analysis. Retrospective analysis included: date of diagnosis; HBV parameters and ALT/AST values at baseline, during and after antiviral tx; type and duration of the antiviral tx; HBV reactivations occurrence and timing. HBsAg+ and/or HBVDNA+ pts received Entecavir or Tenofovir. HBsAg and HBVDNA levels were determined at baseline, every 15 days until negativity, and then every 3 months; ALT/AST were tested monthly.
Results: Ten pts were HBsAg+ with active HBVDNA replication (mean 72420327 U/ml, range 10 – 981349498), 3 pts had low HBVDNA level with HBsAg-, and 1 pt tested negative for both HBVDNA and HBsAg, but a previous spontaneously resolved HBV reactivation was documented out of any antiNHL tx. Globally those 14 pts (2,1%) were considered with active HBV infection and an antiviral tx was started. 4 out of 10 HBsAg+ pts had clinical hepatitis (ALT median values 300 U/L, range 87-586) at the time of diagnosis. Seven pts were started on Entecavir, 3 received Tenofovir and 4 (3 HBsAg- and 1 HBsAg+ with very low HBVDNA level) received Lamivudine. Pts with low HBVDNA level could receive the planned antiNHL tx immediately. For pts with high HBVDNA level antiNHL R-tx was started when DNA levels dropped to less than 1000 U/ml, while CT, if required, was administered independently from HBVDNA levels and presence of clinical hepatitis. All pts could receive the planned antiNHL tx, including R administration (median interval from antiviral tx to 1st R dose 1 month, range 0-49). During and after R-tx HBVDNA levels were persistently below 100 U/ml and no HBV reactivation was observed. Three pts died of progressive disease, 3 pts could stop antiviral tx with no need for further antiNHL tx, 6 pts still receive antiviral tx (including 1 pt allotransplanted in June 2011) The mean time of antiviral tx was 25.6 months (range 6-70).
Summary and Conclusions: Our experience suggests that NHL pts with active HBV infection can safely receive R in addiction to chemotherapy, provided that close clinical monitoring and appropriate antiviral tx are ensured. The risk of HBV reactivation should not justify R withdrawal, considering the well established efficacy of the drug in this setting. Prospective trials, especially in endemic areas, could confirm this preliminary data and define optimal antiviral tx characteristics.

P575

A RETROSPECTIVE ANALYSIS OF CYTOMEGALOVIRUS DISEASE IN PATIENTS WITH MALIGNANT LYMPHOMAS WHO HAVE NOT RECEIVED HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: With the widespread use of immunosuppressive treatments, such as rituximab, for elderly patients with malignant lymphoma (ML), fatal cases of cytomegalovirus disease (CMVD) have recently been reported even in ML patients who have not received hematopoietic stem cell transplantation (HSCT). It is well known that HSCT is a risk factor (RF) for the development of CMVD in patients with ML. However, the clinical aspects, such as the incidence or risk RFs for CMVD in ML who have not received HSCT have not been fully clarified.
Aims: To access the clinical background and RFs of the CMVD in patients with ML not receiving HSCT, we performed a retrospective clinical analysis.
Methods: We reviewed all of the ML patients treated in our department from April 2005 to March 2013, and recorded 236 patients (B-cell ML: T/NK-cell ML: Hodgkin's lymphoma=195: 25: 16) who did not receive HSCT, and analyzed the patients with CMVD for their clinical characteristics, overall survival (OS), and RFs for CMVD. CMVD was defined as evidence of CMV by CMV antigenemia or in tissue specimens, associated with clinical symptoms and signs. The OS was estimated using the Kaplan-Meier method and the data were compared by a log-rank test. The RFs for CMVD were evaluated either in a univariate analysis by the chi-squared test, or in a multivariate analysis by the Cox proportional hazards model.
Results: CMVD developed in 5.5% (13/236) of all the ML patients, which included eight males and five females with a median age of 64 years (range, 42–85). The patients included seven cases of B-cell ML and five of T-cell ML. About half (54%; 7/13) of the patients received a steroid pretreatment before the primary treatment (PT) for ML, 62% (8/13) received more than two therapeutic regimens for ML. Twelve (92%) of the patients received anti-CMV treatments. The majority (92%; 12/13 cases) of the patients with CMVD were died. The OS curve in patients with CMVD was significantly worse than that of the patients without CMVD (three-year-OS: 10% *versus* 76%, p<0.0001 by logrank test). The RFs associated with CMVD identified by the univariate analysis were the presence of B-symptoms (p=0.0015), a poor performance status (p=0.0228), a high CRP level (p=0.0001), serum albumin <3.5g/dl (p=0.0003), steroid pretreatment before the PT for ML (p<0.0001), more than two therapeutic regimens for ML (p=0.0070), a diagnosis of T-cell lymphoma (p=0.0008), non-complete remission (p=0.0155). The use of rituximab was not a significant RF for CMVD. In the multivariate analysis, two independent RFs for CMVD, steroid pretreatment before PT for ML (hazard ratio; HR=4.801, p=0.0440), and more than two therapeutic regimens for ML (HR=9.957, p=0.0016), were identified.
Summary and Conclusions: The prognosis of the patients with ML not receiving HSCT who developed CMVD was poor. Attention should be paid to the development of CMVD for the ML patients who have not received HSCT pretreated with steroids or with multiple therapeutic regimens.

P576

TANDEM AUTOLOGOUS STEM CELL TRANSPLANTATION DOESN'T INCREASE THE RISK OF SYMPTOMATIC CYTOMEGALOVIRUS REACTIVATION IN MYELOMA PATIENTS IN THE ERA OF NOVEL AGENTS: A SINGLE INSTITUTION STUDY

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Background: The introduction of proteasome inhibitors and/or immunomodulators in the treatment of myeloma patients has led up to an increase of viral infections by Herpesviridae family. However, few data about the risk of Cytomegalovirus (CMV) reactivation after autologous stem cell transplantation (ASCT) in the era of novel chemotherapeutics are so far published, and it is doubtful whether receipt of tandem transplantation correlates with higher rates of reactivation.

Aims: To address these questions we performed a retrospective chart review single-center study on 78 consecutive multiple myeloma (MM) patients who underwent a tandem non CD34+-selected ASCT.
Methods: From January 2003 to December 2013, a total of 78 consecutive adult patients with a diagnosis of MM (median age at diagnosis 56 years, range 37-69) underwent a non CD34+-selected tandem ASCT following a conditioning regimen Melphalan alone-based at Hematology and Stem Cell Transplantation Unit of Regina Elena National Cancer Institute of Rome. Out of these 78 patients, 42 underwent a tandem ASCT after induction treatment with conventional VAD (Vincristine, Doxorubicin and Dexamethasone; from January 2003 to December 2008), whereas the remaining 36 after treatment with novel agents (Bortezomib-based regimes, n=28, Immunomodulators plus Dexamethasone, n=8; from January 2008 to December 2013). Therefore, a total of 156 procedures of ASCT in 78 myeloma adult patients were included in this analysis. CMV DNAemia by PCR was only tested on clinical suspicion of reactivation and no routine monitoring was adopted. CMV symptomatic infection and end-organ disease were defined according to published criteria.
Results: Overall, we observed 13 episodes of symptomatic reactivation (13/156, 8.3%), in 12 patients (12/78, 15.4%), all successfully treated. No case of end-organ disease or primary infection was documented. Eight patients experienced a CMV reactivation after first ASCT (8/78, 10.2%), of which only one resulted symptomatic also after second transplant. On the other hand, we found a CMV reactivation after second transplant in 4 out of 70 patients (5.7%) who didn't experienced a reactivation after first ASCT. No statistically significant difference was observed between first and second ASCT (8/78, 10.2% *vs.* 5/78, 6.4%; P=0.767). Univariate analysis showed that a pre-transplant treatment with novel agents was the only baseline factor significantly associated with the occurrence of post-ASCT CMV symptomatic reactivation after first [Odds Ratio: 9.897 (95%CI: 1.154-84.840); P=0.021] but not second transplant [Odds Ratio: 5.125 (95%CI: 0.546-48.119); P=0.115].
Summary and Conclusions: Our data suggest that second transplantation doesn't increase the risk of CMV reactivation when compared with the first one and confirm the role of a pre-transplant treatment with novel agents as risk factor for CMV symptomatic reactivation.

P577

IMPACT OF NOSOCOMIAL VIRAL INFECTION IN CHILDREN UNDERGOING INTENSIVE CHEMOTHERAPY OR STEM CELL TRANSPLANTATION FOR HEMATOLOGIC OR MALIGNANT DISEASE
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Background: Community-acquired virus infections by pathogens including influenza virus, parainfluenza virus (PIV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), norovirus, and rotavirus are self-limiting in nature. These viruses can be transmitted to children receiving treatment by their parents, careworkers, or medical staff. Outbreaks of respiratory and intestinal viral infections were recently reported among patients undergoing chemotherapy or stem cell transplantation (SCT). The incidence of such infections should differ among institutions, but no such data are currently available in Japan.
Aims: To clarify the clinical outcomes and risk factors for the progression to a severe disease in children with nosocomial infection of a community-acquired virus while undergoing chemotherapy or SCT.
Methods: A total of 126 patients (age, 1 day –17 years) who underwent chemotherapy (n=80) or SCT (n=46) between October 2008 and September 2013 were included in this study. Underlying diseases were hematologic malignancy (n=72) and solid tumor (n=54). A direct immunochromatography assay was used to screen influenza, RSV, hMPV, norovirus, and rotavirus antigens, and the shell vial assay was used to screen the PIV antigen. Severe disease in cases of respiratory viral infection was defined as lower respiratory tract infection or disease duration >14 days, while that of intestinal viral infection was defined as dehydration >10% body weight or disease duration >10 days. Supportive care was provided to all patients. Neuraminidase inhibitors were administered to all patients with influenza infection and ribabirin to 8 of 13 patients with RSV infection.
Results: In total, 37 patients were diagnosed with respiratory viral infection (influenza virus, n=13; RSV, n=13; PIV, n=8; and hMPV, n=3). In 12 of these patients, infection progressed to severe disease, including 4 serious complications after RSV or PIV infection; fungal pneumonia (n=3) and multiorgan failure (n=1). In addition, 31 patients were diagnosed with intestinal viral infection (rotavirus, n=10; norovirus, n=21). In 14 of these patients, infection progressed to severe disease, including a fatal complication; sinusoidal obstruction syndrome. Among patients with respiratory viral infection, median age at the time of severe disease (1.5 years) was significantly lower than that at the time of non-severe disease (3 years) (p=0.05). However, duration of neutropenia (absolute neutrophil count <500/uL) and lymphocytopenia (absolute lymphocyte count <200/uL) were not associated with disease severity. In patients with intestinal viral infection, neither age nor duration of neutropenia or lymphocytopenia was associated with disease severity.
Summary and Conclusions: This case series showed that RSV and PIV infections are likely to progress to severe disease in children undergoing intensive chemotherapy or SCT. A significant risk factor for progression with respiratory viral infection was younger age, but not the duration of lymphocytopenia. Because the symptoms of viral enterocolitis mimic those of acute graft-*versus*-host disease (GVHD), the grade of acute GVHD is likely to be overestimated. Therefore, rapid diagnosis and control of nosocomial infection is more important in cases of high-risk transplantation.

P578

CLINICAL CHARACTERISTICS AND OUTCOMES OF RESPIRATORY SYNCYTIAL VIRUS INFECTION IN RECIPIENTS OF ALLOGENEIC STEM CELL TRANSPLANTS
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Background: Respiratory syncytial virus (RSV) is a common cause of respiratory tract infections in pediatric and immunocompromised patients. RSV infections result in significant morbidity and mortality in patients that have undergone allogeneic stem cell transplantation (allo-SCT). In these patients manifestations include; upper and lower respiratory tract infections (URI/LRI), hyperreactive airway disease, acute lung injury (ALI), acute respiratory distress syndrome (ARDS) and in severe cases obliterative bronchiolitis (OB) and chronic oxygen dependancy.
Aims: We carried out this study to document the clinical characteristics, subsequent events and outcomes associated with RSV infections in patients that have undergone allo-SCT.
Methods: After IRB approval, from the years 2006-2013 we identified 47 allo-SCT recipients at the Mayo Clinic, Minnesota that had been diagnosed to have a RSV infection. All demographic, clinical and transplant related data were retrospectively abstracted. RSV infections were diagnosed based on PCR testing of nasopharyngeal swabs. Both pulmonary and transplant related outcomes were analyzed.
Results: Of the 47 study patients, 23 (49%) were male and the median age was 55 years (range, 15-75 years). The median duration between transplant and RSV infection was 230 days (range, 2-1443 days), with 21 (45%) patients being infected at <6 months, 7 (15%) between 6-12 months and 19 (40%) at >12 months after allo-SCT. Seventeen (36%) patients presented with an URI, 16 (34%) with a LRI and 14 (30%) with acute respiratory failure (ALI/ARDS). Three (6%) patients had recurrence of RSV infection and 1 patient had concurrent influenza A infection. Six (13%) patients had experienced influenza A infection at variable time points before their current RSV infection. Of the 47 patients, 28 (60%) required hospitalization and 15 (32%) of these required ICU level care. The median length of hospitalization was 7.5 days (range, 3-46 days). Five (11%) patients did not receive treatment, 20 (43%) were treated with ribavirin only and 21 (45%) received both ribavirin and IVIG. 9 (19%) patients received steroids or an augmentation in their baseline steroid dosage to prevent RSV related sequelae. 17 (36%) patients required some form of respiratory support (7 nasal cannula, 2 BIPAP, 1 CPAP and 7 intubation with mechanical ventilation). DLCO and FEV1 measurements available from pretransplant testing, or at least 4 weeks before RSV infection were compared to values measured at least 4 weeks after resolution of infection. The median change in DLCO was -8% (range, -34 to 14%). The median change in FEV1 was -8% (range, -49 to 12%). 13 patients had no reduction in DLCO, 13 had a reduction of >5% and 3 a reduction of >25%. Ten patients had no reduction in FEV1, 16 a reduction of >5% and 4 a reduction of >25%. Of those with at least a 5% drop in FEV1, 40% were clinically attributed to the development of OB. Ten (21%) patients were diagnosed to have OB, with 7 of these also having concurrent systemic GVHD. At last follow up, 26 (55%) patients had developed chronic GVHD (8 mild, 9 moderate and 9 severe) and 11 (24%) patients had relapsed at some point after transplant. At last follow up, 13 (28%) patients had died.
Summary and Conclusions: RSV infections in allo-SCT recipients are associated with significant morbidity and mortality. In our single center study, approximately a third of patients experienced a chronic drop in FEV1/DLCO after infection and 20% developed obliterative bronchiolitis resulting in chronic oxygen dependancy. Preventive strategies, including the development of a RSV vaccine, are an absolute need.

P579

THE CLINICAL SPECTRUM AND OUTCOMES OF HUMAN HERPESVIRUS 6-RELATED INFECTIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Human herpesvirus-6 (HHV-6) infection/reactivation is an important cause of morbidity and mortality, in patients that have undergone allogeneic hematopoietic stem cell transplantation (HSCT). HHV-6 infection can be associated with fevers, engraftment failure, bone marrow suppression, skin rashes, focal and diffuse neurological manifestations. The interpretation of HHV-6 viremia and its association with disease remains problematic as most

adults are latently infected with the virus. Thus, detection of HHV-6, can represent asymptomatic reactivation, chromosomal integration or a true infection. Latency is usually characterized by a very low copy number only detected by high-sensitivity PCR, while viral loads in chromosomally integrated HHV-6 are usually >1 million copies per mL.
Aims: We carried out this study, to define the incidence, morbidity, mortality and transplant related outcomes associated with HHV-6 infection/reactivation in patients that have undergone allogeneic HSCT.
Methods: After due IRB approval, we retrospectively identified 119 allogeneic HSCT patients that had HHV-6 testing (Q-PCR) done at the Mayo Clinic, Minnesota, from 2008-2013. Data collected included baseline demographics, clinical, radiologic and pathologic features and related outcomes.
Results: Of 750 allogeneic HSCT performed, 13 (2%) patients were detected to have HHV-6 viremia.. Median age at time of transplant was 54 years (range, 25 to 65 yr) and 7 (54%) were male.The most common indications for HHV-6 testing were cytopenias (27%), neurological alterations (22%), and neutropenic fevers (17%). In these patients, median time to first detection of HHV-6 was 24 days (range, 11 to 433 days) post-transplant and plasma or cerebrospinal fluid (CSF) levels ranged from <500 to 147,000 copies/mL. One patient had suspected chromosomal integration given copy number >2 million. HHV-6 disease included 5 patients with encephalitis (38%) [Table 1], 5 with neutropenic fevers (38%), and 1 with delayed engraftment (8%).. Four of the 5 patients with encephalitis had detectable HHV-6 DNA in the CSF. Eight (62%) patients had concurrent grade 2-4 acute GVHD, while 6 (46%) had concurrent CMV reactivation. All patients had regression of clinical symptoms or decrease in viral load after antiviral therapy (6 with ganciclovir and 1 with foscarnet). The median duration of therapy was 20 days (range, 14 to 46 days). 46% of patients recieved ganciclovir while 8% recieved foscarnet as first line treatment. The median survival post-allogeneic HSCT in patients with HHV-6 viremia was 12 months (range, 2 to 30) compared to 55 months in those that tested negative (range, 1 to 124, log rank test p=0.02).

Table 1.

Summary and Conclusions: The present study further elucidates the natural history of HHV-6 infection after allogeneic HSCT. Neurological manifestations, especially encephalitis, neutropenic fevers and unexplained cytopenias are common manifestations. Ganciclovir and Foscarnet are relatively effective therapies for symptomatic patients.

P580

ANALYSIS OF PNEUMONIA IN ACUTE LEUKEMIA: A PROSPECTIVE STUDY BY THE "RETE EMATOLOGICA LOMBARDA" (REL)

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Background: Pneumonia is a major cause of morbidity and mortality among patients (pts) with hematological malignancies who receive cytotoxic chemotherapy.
Aims: In order to develop a rationale policy for the prevention and management of this significant complication, we analyzed characteristics of pneumonia in acute leukaemia (AL) pts referring to 9 haematological institutions participating to REL.
Methods: We prospectively recorded 515 febrile/infectious episodes encountered during hospitalization of 299 adult AL pts, median age 56 years (18-89 y), admitted in 9 haematological centres of Lombardia (period Dec. 2012 – Jan. 2014). We analyzed etiology, microbiologic spectrum, clinical characteristics and outcome in pts with reported pulmonary infection.
Results: Clinical/radiological findings of pneumonia were found in 128/515(24%) febrile cases: 21(16%) in acute lymphoblastic leukemia and 107(84%) in acute myeloid leukemia pts, mainly with active disease (58 *de novo*/33 resistant AL); in 27 cases pneumonia occurred during the consolidation phase. The majority of pts (107) were neutropenic (median duration 22 days range 2-170): a prophylaxis regimen with fluoroquinolone and antifungals was adopted, in 55/128(42%) and 84/128(65%) of cases respectively. In 70/128(54%) episodes of pneumonia, one/more identifiable microbiological agents were detected, isolated from different sites (62 blood, 20 lung, 5 nasal secretion), by cultures, histology or serological methods. Isolates were mainly bacterial (70/88), while a fungal etiology was microbiologically established in 13/88 cases, so divided: 40 gram+ve (45%), 30 gram-ve (34%), 13 moulds (15%>12 Aspergillus, 1 Fusarium); viral agents were 5 (6%); 23 episodes were polymicrobic (8 cases of fungal and bacterial infection together). Coagulase-negative Staphylococci were the most frequent gram+ve (17/40); S. aureus, Streptococci and Enterococci accounted for 4/40, 5/40, 9/40; Pneumococcus was isolated in 2/40 episodes; methicillin-resistant strains accounted for 14/21 of all staphylococci; vancomicin-resistant for 1/9 of Enterococci. Among gram-ve, 15/30 were Enterobacteriaceae (2 ESBL+ strains, 1 multi-resistance Klebsiella); 10/30 were Xanthomonadales and Pseudomonadales (4 multiresistant strains). According to EORTC guidelines pulmonary infiltrates were classified as specific (42%) *vs* aspecific (88/128,68%), depending on the presence of radiological evidence of fungal infections (IFI). We documented 36 possible/14 probable/2 proven IFI. Thirty-day overall mortality was 19/128 (15%) of cases: deaths were more frequent in patients with pneumonia and microbiological documentation of infection (14/19) but, at univariate analysis, etiology (fungal *vs* bacterial) did not retain a prognostic significance. Anyway in the entire population a febrile episode concomitant with pneumonia significantly correlates with the risk of death (OR 8.85 p<0.00001). Among IFI, deaths were more frequent if "probable" (6/19 - p 0.02); at multivariate analisys resistant leukaemia is the only variable that correlates with the risk of death (OR 3.6, p 0.018).
Summary and Conclusions: Pneumonia remains an important determinant of in AL pts, mainly in those with active disease. Up-to-date check of epidemiology and antibacterial resistance patterns in each institution are needed in order to treat these lifethreatening complications effectively.

P581

INFECTIONS IN ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME ON TREATMENT WITH AZACYTIDINE: EXPERIENCE OF A SINGLE CENTER

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Background: Infection is an important cause of morbidity and mortality in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). In recent years, the hypomethilating agents (HMA) have become the main therapeutic alternative to intensive chemotherapy (IC) in these patients, even in those with relapsed or refractory disease. The characteristics of infection when HMA are used have not been widely described.
Aims: To describe the incidence and pattern of infectious episodes (IE) and analyze what features of the patient or the disease may be associated with a higher incidence of infections in AML and MDS patients treated with HMA.
Methods: We retrospectively studied 50 patients with MDS or AML who have received at least 2 cycles of 5-azacytidine (AZA) at our institution. Disease, treatment and IE characteristics were recorded and subsequently we analyzed which variables may be associated with them.
Results: We studied 50 patients (mean age 69-years old, range 29-84, male 52%) of which 19 patients (38%) were affected of AML and 31 (62%) of MDS (23 high-risk MDS and 8 low-risk MDS). Average Sorror comorbidity index (SCI) score was 2.8 and 13 patient (12 AML and one high-risk MDS) had previously received IC. Severe neutropenia (<500/mm^3) was present in 43 patients (86%) before or during AZA treatment and 31 of these patients (72%) received antimicrobial prophylaxis during the period of neutropenia. We recorded a total of 85 IE in 36 patients (72%) and the main features of this group were as follows: 1) IE mainly occurred during the first 4 courses of AZA (51%, n=43), being less frequent after them (see Figure 1); 2) Hospitalization was necessary in 34 patients (94%) for one or more episodes; 3) They had received a median of 9 cycles of AZA, and suffered an average of one IE every four courses of AZA; 4) Microbiological isolation was achieved in 45% of IE, involving 25 patients, and in most cases by blood culture; and 5) Severe IE (associated with respiratory failure or hemodynamic instability) were observed in 19 patients (53%). Of the total 50 patients analyzed, 35 were deceased at the time of closing this study, and 18 of them (51%) have died from infectious causes. In our statistical analysis we have found that the risk of infection is associated with a higher score for SCI (p=0,023), and the risk of severe infection with higher scores for SCI (p=0,029), IPSS (p=0,039) and IPSS-R (p=0,034). Both IPSS and IPSS-R are also associated with active infection at the time of dying (p=0,042 and p=0,047 respectively). Severe neutropenia, age or previous IC, were not associated with the risk of infection. Antimicrobial prophylaxis in neutropenic patients is significantly associated with less number of IE (p=0,036), but not with the severity of the episode or death of infectious origin. Finally, we have found that patients who develop severe IE have shorter overall survival (p=0,024).

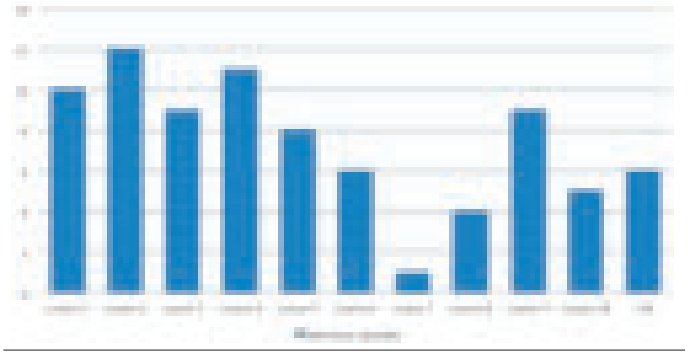

Figure 1. Incidence of infectious episodes on each course of AZA.

Summary and Conclusions: In patients receiving AZA, severe infections (those with respiratory failure or hemodynamic instability) are associated with shorter overall survival. Greater comorbidity and a worse prognostic index were also associated with a higher risk of severe infection. Although antimicrobial prophylaxis seems to decrease the number of total IE, it has not effect on either the severity or the possibility of dying. Studies including a larger number of patients, preferably prospective, are needed to confirm these findings.

P582

ASSESSMENT OF INFECTION EVENTS IN AZACITIDINE (AZA) TREATMENT FOR MYELODYSPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML). A SINGLE CENTRE EXPERIENCE.

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Background: Infection is a major source of morbidity and mortality in patients with hematological diagnosis of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). 5-azacytidine (AZA), is an alternative treatment in patients not candidates for intensive chemotherapy. Clinical and microbiological features of infections in patients AZA treated are not well known.
Aims: To investigate the impact and features of infective episodes on patients who received AZA in a daily clinical practice at our institution.
Methods: We conducted this analysis on patients who received AZA (75mg/m^{2}x7days subcutaneous/ intravenous/28days as induction and 75mg/m^{2}x5days/28days as maintenance) between January 2007 and December 2013 at our institution for MDS or AML. We used SPSS v.19 © statistics for descriptive statistical analysis(c^{2}test to determine statistical relationships between main variables) and Kaplan-Meier for survival analysis. Patients were included if they had received at least one cycle of AZA.

Table 1.

		Infectious event		
		1st(N= 34)	2nd (N= 19)	3rd (N= 12)
Neutrophils median (x10^9/L)*		0.400 (0-46.9)	0.200 (0-14.0)	0.55 (0-4.6)
Hospital admission	Yes	29 (85.3%)	18 (90%)	9 (75%)
	No	5 (14.7%)	2 (10%)	3 (25%)
Hospitalization, median	(days)	8 (1-97)	7 (4-97)	10 (4-97)
Death by infection		4 (11.8%)	2 (10%)	3 (27.3%)
Disease status		1 DP,3 NA	1 DP, 1 NA	1R, 1 DP, 1 NA
Infectious source	Sepsis	2 (5.9%)	4 (20%)	3 (25%)
	Pneumonia	12 (35.3%)	7 (35%)	3 (25%)
	Urinary	3 (8.8%)	2 (10%)	2 (16.7%)
	Gastrointestinal	1 (2.9%)	1 (5%)	1 (8.3%)
	Febrile syndrome	16 (47.1%)	6 (30%)	3 (25%)
Infectious type	Bacterial	10 (30.3%)	9 (45%)	7 (58.3%)
	Viral	2 (6.1%)	2 (10%)	1 (8.3%)
	Fungal	2 (6.1%)	-	-
	Not document	19 (57.6%)	9 (45%)	4 (33.3%)
Disease status at infection time	CR	3 (9.4%)	2 (10.5%)	1 (9.1%)
	PR	1 (3.1%)	3 (15.8%)	1 (9.1%)
	SD	1 (3.1%)	3 (15.8%)	2 (18.2%)
	NA	23 (71.9%)	5 (26.3)	1 (9.1%)
	R	1 (3.1%)	1 (5.3%)	3 (27.3%)
	DP	3 (9.4%)	5 (26.3)	3 (27.3%)

**Neutrophil baseline median value: 0,9 (0.0-47);Complete response (CR);Partial response (PR);Stable Disease (SD);Not available (NA);Relapse (R);Disease progression (DP).*

Results: We studied 59 patients with a median age of 73 years (33-86 y); 36 were male (61%) and 23 female (39%). 24 MDS (39%) and 35 AML (61%);IPSS:1 low risk (1.7%),6 intermediate-1(10.2%),7 intermediate-2(11.9%) and 10 high risk(16.9%). SORROR (HCT-CI): 44 <3 (76.4%), 15 ≥3 (25.4%). Total AZA cycles were 537 (1-34. Median: 6 cycles).At the time of analysis, 49 (83.1%) patients were dead. Overall Survival (OS) was 17.1 months (IC95%: 12.58-21.62) with a median follow up of 14.5 months. Patient with infectious events were 34 (57.6%) and total infections events 73 (13 patients had 1 infection; 8 patients had 2 infections; 8 patients 3 infections; 5 patients ≥4 infections). Among patients with 3 infectious events, 51(80.95%) infections took place before cycle 6 and 14(19.05%) after cycle 6. Time to the first infection was 50.5 days.Infections were found in 12 MDS patients (35.3%) and 22 AML patients (64.7%). OS no Infection *vs.* infection was 19.47 months (IC 95%: 9.17-29.77) *vs.* 15.78 months(IC 95%:5.95-25.62)(p=0.148);The infection rate per 1000 AZA cycles was 135.94‰ and death rate due to infection per 1000 AZA cycles was 13.03 ‰. There was heterogeneity of isolated microorganisms at infection (1 catheter infection by *Enterobacter faecalis)* (Table 1).
Summary and Conclusions: Results show a high infectious rate, probably related to baseline neutropenia more than related to AZA treatment because there are no statistical differences in terms of OS(p=0.148). None of the patients who died by infection were at CR, PR or SD. Most patients were infected during the first 6 cycles of AZA regardless hematologic disease (*p=0.499*).

P583

PREDICTORS OF G-CSF PROPHYLAXIS IN THE FIRST CYCLE OF CHEMOTHERAPY FOR FEBRILE NEUTROPENIA IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background: International guidelines recommend primary prophylaxis with granulocyte-colony stimulating factors (G-CSFs) for patients who are assessed to be at high (≥20%) risk of experiencing febrile neutropenia (FN), to support treatment with myelosuppressive chemotherapy. However, G-CSF use varies widely in clinical practice even in high risk patients. There have been several publications investigating predictors of FN but limited study of predictors of G-CSF prophylaxis.
Aims: The main objective of this analysis was to identify predictors of G-CSF prophylaxis in the first cycle of chemotherapy in order to improve our understanding of the relationship between G-CSF use and FN in Non-Hodgkin's Lymphoma (NHL) patients.
Methods: This is a post-hoc analysis of data from IMPACT NHL, an observational, cohort study. The analysis was performed on the subset of prospectively enrolled adults with any histological type of NHL treated with (R)-CHOP. The relationship between baseline patient characteristics and G-CSF prophylaxis in the first cycle of chemotherapy was examined using bivariable and multivariable logistic regression models. The multivariable models were adjusted for age, gender, country, performance status, histology and regimen type.
Results: A total of 1187 patients were included in the analysis, of whom 49% received G-CSF prophylaxis in the first cycle. In bivariable regression, factors significantly associated with G-CSF prophylaxis included older age, country, no bone marrow involvement, dose-dense regimen, investigator assessed high FN risk, DLBCL histology, poor ECOG performance status, higher number of planned cycles and chemotherapy dose, lower serum albumin, lower haemoglobin and lower glucose, higher AST, higher CRP and higher LDH. In adjusted multivariable regression, investigator-assessed high FN risk (OR 2.9, 95% CI 2.14-3.96), lower glucose (<8.8mmol/L) (OR 1.7, 95%CI 1.02-2.75), lower ANC (<3x10^9/L) (OR 1.3, 95%CI 1.10-1.99), and increased LDH (>400 U/L) (OR 1.4, 95% CI 1.01-1.91) were associated with use of G-CSF prophylaxis.
Summary and Conclusions: In addition to well known risk factors for FN, the analysis provides evidence for less considered factors such as country, glucose and LDH to be associated with G-CSF prophylaxis in the first cycle of NHL patients receiving (R)-CHOP. These factors should be considered for future studies and when interpreting studies of G-CSF prophylaxis and patient selection in clinical practice.

P584

ADMISSION OF PATIENTS WITH HEMATOLOGIC MALIGNANCES TO INTENSIVE CARE UNITS

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Background: Over the past years, improvements in supportive care measures, as well as new diagnostic and therapeutic tools, have improved the survival of patients with hematologic malignancies. Despite all improvements, this group of

patients is at a high risk of becoming critically ill during chemotherapy treatment and may need admission in an Intensive Care Unit (ICU). It is nowadays clear that these patients will benefit from ICU admission, however there is still some discussion about which are the patients that will really benefit from this admission.
Aims: To evaluate the outcome of critically ill patients with hematologic malignancies admitted to ICU and try to correlate prognostic factors with survival.
Methods: Retrospective analysis of all critically ill patients with hematologic malignancies admitted to ICU between 01 of July 2009 to 30 of June 2012. We then evaluated clinical and demographic data and tried to identify clinically useful prognostic factors.
Results: We evaluated 65 admissions in ICU. 43.1% were males with a median age of 56 years (22-78). The most frequent hematologic diagnosis was AML/high risk MDS in 63.1%, indolent lymphoma in 13.8%, aggressive lymphoma in 10.8%, multiple myeloma in 7.7% and chronic leukemia in 4.6%. At the time of admission in ICU, 55.4% were at their first approach to the hematologic disease, 35.4% were with progressive disease or relapse, 4.6% in complete remission and 4.6% after autologous stem cell transplant. The most frequent reason for ICU admission was acute respiratory failure (61.5%), septic shock with multiple organ failure (23.1%) and neurologic dysfunction (15.4%). 16.9% were admitted directly to ICU from the Emergency Department; 49.2% were neutropenic at time of admission to ICU. Only 57.4% of the patients that were referred from an hematologic unit had microbiology isolations; 91% were receiving broad spectrum antibiotics and 24% antifungal therapy. At ICU 84.6% needed invasive mechanical ventilation, 76.9% vasoactive support and 36.9% renal replacement therapy. The median number of days in ICU was 9. The survival to ICU was 41.5% and from these, 81.5% were discharged from the hospital. In univariate analysis only the need for invasive mechanical ventilation and vasoactive support had a negative influence in survival, and this keeps statistically significance in multivariate analysis. In our cohort, the days of neutropenia, the hematologic diagnosis and the state of the disease at ICU admission have not influenced the outcome of the patients.
Summary and Conclusions: In our cohort of patients we could not find any variable related to the disease with influence in the outcome, but only variables related to the severity of the acute episode. There was no clinical feature or combination of features that could reliably predict a better outcome or even treatment futility. We were lead to assume that the most important strategy to try to improve the outcome of these patients is early admission to ICU in a stage in which organ failure is still minimal or restricted.

P585

PROSPECTIVE OBSERVATIONAL STUDY ON CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING (CINV) FOR HEMATOLOGIC MALIGNANCY PATIENTS AND PRIMARY CARE MEDICAL STAFF'S PERCEPTION BY THE CINV STUDY GROUP OF JAPAN

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Background: There has been to no nationwide survey on chemotherapy-induced nausea and vomiting (CINV) and validation of the guideline made after introduction of NK-1 receptor antagonist to the Japanese market.
Aims: The aim of the study is to investigate occurrence of CINV in hematologic malignancy patients(pts) who are to receive chemotherapy for the first time, and primary care medical staff's perception on CINV for their pts.
Methods: A nationwide survey on CINV was conducted by the CINV Study Group of Japan. Sixteen institutions participated in the study which was approved by the review board. The written consent was obtained from the pts. A 7-day diary for CINV was provided to the patient prior to moderately emetic chemotherapy (MEC) and highly emetic chemotherapy (HEC) to record daily occurrence and severity of CINV and an amount of food intake. Acute and delayed CINV was defined as nausea and vomiting which developed within or after 24 hours after the start of chemotherapy, respectively. The medical staff's also filled out questionnaires of estimating their pts' CINV.
Results: A total of 203 pts were registered from May 2011 to November 2012. The number of patient's diary paired with their staff's report was 196 after pts received HEC or MEC. Underlying diseases were leukemia (38pts), multiple myeloma (11pts), Hodgkin's lymphoma (8pts), and Non Hodgkin's lymphoma(139pts). There were 108 males with a median age of 61 (range: 22-87) and 88 females with a median age of 62.5 (range: 22-87). MEC was given to 19 pts, HEC was given to 177 pts. Acute vomiting was noted in 0 pts (0.0%) with MEC as was in 8 pts (4.5%) with HEC, while delayed vomiting was experienced in 2 pts (10.5%) with MEC and 16 pts (9.0%) with HEC, respectively. Acute nausea was noted in 2 pts (10.5%) with MEC and in 34 pts (19.2%) with HEC, while delayed nausea was experienced in 4 pts (21.1%) with MEC and in 70 pts (39.5%) with HEC, respectively.
The staff estimations' positive predictive value were 14.3% in acute CINV, 25.0% in delayed CINV, respectively. Their negative predictive value were 19.9% in acute CINV, 19.4% in delayed CINV, respectively. Risk factors for acute nausea, acute vomiting, delayed nausea, and delayed vomiting were assessed separately. In acute nausea, multivariate analysis showed risk factors as female gender (Odds ratio 3.178; 95%CI 1.410-7.164; p=0.0053), younger age (Odds ratio 0.6244 (every ten years); 95%CI 0.4737-0.8339; p=0.0013), and increased concentration of hemoglobin (Odds ratio 1.226 (every 1.0 g/dl); 95%CI 1.005-1.496; p=0.0449). In delayed nausea, multivariate analysis showed a risk factor as female gender (Odds ratio 2.209; 95%CI 1.222-3.991; p=0.0087). A combination of 3 anti-emetics (5HT3 receptor antagonist, steroid (prednisolone or dexamethasone), and NK-1 receptor antagonist) was given along the guideline to 26.3% of the pts with MEC and 22.6% of those with HEC. However, 147 pts were received an intermediate- or high-dose steroid for 5 days or more. Two anti-emetics (5HT3 receptor antagonist and steroid) was not risk factor *versus* 3 anti-emetics in multivariate analysis (Table 1).

Table 1.

Summary and Conclusions: Antiemetic use by the Japanese hematologists for hematologic malignancies was not in accordance with the Japanese guideline. It appears that nausea was not still well under control, although vomiting was rarely experienced even in HEC. Further study is especially needed to prevent delayed nausea. It is of interest that Japanese medical staff has overestimated the incidence of CINV.

P586

PROPHYLAXIS WITH FEBUXOSTAT IN COMPARISON TO ALLOPURINOL REDUCES EXPOSURE TO URIC ACID IN PATIENTS AT INTERMEDIATE TO HIGH RISK OF TUMOR LYSIS SYNDROME: RESULTS OF THE FLORENCE STUDY

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Background: The risk of tumor lysis syndrome (TLS) and renal events increases by 1.75 and 2.21 fold respectively for every mg/dl increase of serum uric acid (sUA)[1], thus a better control of sUA exposure during chemotherapy (CT) is beneficial for patients.

Aims: To compare Febuxostat with Allopurinol in terms of sUA exposure control and renal function impairment prevention in patients undergoing CT for hematologic malignancies at intermediate to high risk of TLS (as per TLS expert panel risk stratification[2])
Methods: This was a double-blind randomized controlled trial. 346 patients providing written informed consent were stratified according to TLS risk (intermediate *vs* high) and sUA level (≤7.5 mg/dl *vs* >7.5 mg/dl) and randomized in a 1:1 ratio to receive either Febuxostat or Allopurinol for 7-9 days starting from 2 days prior CT. Dose level was upon investigator's choice among low, standard and high containing either Allopurinol 200, 300 and 600 mg daily or fixed Febuxostat 120 mg daily. Primary endpoints were sUA area under the curve (AUC sUA_{1-8}) and change in serum creatinine level from baseline to Day 8. Secondary endpoints were response rate (sUA ≤7.5 mg/dL from the start of CT to Day 8), incidence of laboratory and clinical TLS and safety. The study was performed in 79 sites in Europe and Brazil (NCT01724528).
Results: Baseline demography was similar in both groups. In Febuxostat arm there was a slightly higher number of acute leukemias (n=34 *vs* 25) and lymphomas (n=59 *vs* 54) and a slightly lower number of chronic lymphoid leukemias (n=80 *vs* 94). The majority of patients were at intermediate risk of TLS (82.1%, n=284), had a baseline sUA ≤7.5 mg/dl (87.6%, n=303) and received standard dose therapy (82.7%, n=286). Mean AUC sUA_{1-8} was significantly lower in the Febuxostat arm (514.0±225.71 mgxh/dl *vs* 708.0±234.42 mgxh/dl, p <.0001). There were no significant differences between the Febuxostat and Allopurinol arms in mean change in serum creatinine (-0.83±26.98% *vs* -4.92±16.70%, p=0.0903) nor in secondary efficacy endpoints. Incidence of all adverse events (AEs) and related AEs was 67.6% and 6.4% *vs* 64.7% and 6.4% in the Febuxostat and Allopurinol arms respectively.
Summary and Conclusions: This is the largest trial every performed comparing different TLS prevention methods. Febuxostat showed a significant 28% higher reduction of exposure to sUA during CT over Allopurinol with comparable renal function impairment prevention and safety profile. These results suggest that prophylaxis with Febuxostat may further reduce the risk of TLS development in patients with hematological malignancies at intermediate to high risk of TLS in comparison to Allopurinol.

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Transfusion medicine

P587

BUFFY COAT POOLED PLATELETS CRYOPRESERVED IN DIMETHYL-SULPHOXIDE WITH A NEW SYSTEM PRESERVE THEIR *IN VITRO* FUNCTION UP TO 9 MONTHS

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Background: Cryopreservation of platelets (PLTs) represents a clinical useful method for avoiding platelet shortage.Many studies have tried to define, *in vitro* and *in vivo*, the entity and weight of storage-related PLTs lesions with discordant results related to different methods.
Aims: We have performed an *in vitro* prospective study to evaluate PLTs count, viability and function of buffy coat derived pooled platelet concentrates (BC-PLTs) treated with dimethyl-sulphoxide (DMSO) and cryopreserved at -80°C up to 9 months with an innovative patented system that avoids external manipulations.
Methods: Each BC-PLTs was obtained from 5 buffy coats and pooled according to standard procedures. The final PLTs concentrates were leukoreduced by filtration and transferred to a 650 mL cryopreservation kit (Promedical ®) which allowed mixing with DMSO 25% in a closed system and following removal of supernatant without further manipulations. BC-PLTs were washed prior freezing with removal of at least 84% supernatant solution, suspended in homologous plasma from 1 of the 5 donors to a final concentration of 200 mL and frozen at –80°. BC-PLTs were analyzed immediately pre-freezing (T0) and 3, 6 and 9 months after cryopreservation (CRY BC-PLTs).The following parameters were assayed: PLTs count (PC), mean platelet volume (MPV), pH, flow cytometry (FACS) expression (%) of CD41a, CD42b, CD61a, CD62p, PAC-1, Annexin V PLTs surface antigens and thromboelastography (TEG). CRY BC-PLTs samples were thawed in a bath at 37°C for 5 minutes and evaluated promptly. All the tests were performed according to current European recommendations. PLTs swirl was furthermore visually assessed. Results were expressed as mean +/- standard deviation (SD). Results obtained at T0 and at 3, 6 and 9 months after cryopreservation respectively were analyzed with "Two factor study repeated measure analysis of variance" (ANOVA).
Results: *In vitro* cell parameters were measured on 49 BC-PLTs, 49 CRY BC-PLTs at 3 (T3) and 6 (T6) months and 34 CRY BC-PLT at 9 (T9) months. All the analyzed parameters showed stable values during the cryopreservation period. There were no differences between the groups in PC (1427 +/- 150x109/L at T0 *vs* 1400+/- 170 at T9),CD41a (98.5 +/- 1.94 at T0 *vs* 98.1 +/- 3.07 at T3,98.3 +/- 1.24 at T6 and 97.96 +/- 3.1 at T9), CD62p (59.0 +/- 11.02 at T0,71.1 +/- 14.6 at T3,76.89 +/- 8.65 at T6 and 70.9 +/- 7.4 at T9) and Annexin V expression while a significant reduction in CD 42b (92.7 +/- 4.29 at TO *vs* 23.6 +/- 27.5 at T3, 16.38+/- 12.54 at T6 and 17.3 +/- 9.6 at T9), PAC-1(1.9 +/- 1.34 at T0 *vs* 0.62 +/- 0.4 at T3, 0.63 +/- 0.83 at T6, 0.49 +/- 0.48 at T9) for CRY BC-PLTs was observed after cryoprserevation. TEG parameters were all significantly reduced in CRY BC-PLTs samples(TEG R: 8.3 +/- 2.55 min at T0 *vs* 10.85 +/- 1.6 at T3,11.03+/- 2.69 at T6 and 9.5+/- 1.89 at T9; TEG K:1.6 +/- 0.3 min at T0 *vs* 2.96+/- 1.04 at T3,2.9 +/- 1.3 at T6 and 2.8 +/- 0.6 at T9;TEGα:71.9 +/- 10.7 deg at T0 *vs* 53.4+/- 9.5 at T3,52.11 +/- 13.9 at T6 and 57.6 +/- 11.0 at T9; TEG-MA:70.61 +/- 10 mm at T0 *vs* 62.3+/- 8.79 at T3,62.4 +/- 7.86 at T6 and 60.9 +/- 7.71 at T9) without affecting hemostasis.
Summary and Conclusions: The results of our investigation confirm the potential of a new system to overcome limits to PLTs storage. This method allows cryopreservation of PLTs up to 9 months, as shown by stable *in vitro* function during this period, and avoids apoptosis. Adequate hemostasis achieved at TEG supports the hypothesis that *in vitro* PLTs activation/deterioration doesn't necessarily mirror an impaired hemostatic *in vivo* function of CRY BC-PLTs. The next step of current study will be to evaluate CRY BC- PLTs efficay, recovery and survival *in vivo.*

P588

HEMOSTATIC FUNCTION AND BIOMARKERS OF ENDOTHELIAL DAMAGE BEFORE AND AFTER PLATELET TRANSFUSION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Platelet transfusion is a cornerstone of the care in hematologic patients. Though the beneficial effect of platelet transfusion on hemostasis is

well established, there is emerging evidence that platelet transfusion induces an inflammatory response in vascular endothelial cells.
Aims: To investigate functional whole blood hemostasis and circulating levels of endothelial biomarkers before and after platelet transfusion in patients with acute myeloid leukemia (AML), hypothesizing that platelet transfusion would improve hemostasis but also cause a measurable change in endothelial and inflammatory biomarkers.
Methods: Blood were sampled from AML patients immediately before and 1- and 24 hours after platelet transfusion. Remnant samples from the transfused platelet products were also collected. Blood samples from 31 healthy blood donors served as controls. Primary and secondary hemostasis was evaluated by whole blood impedance aggregometry (Multiplate) and thromboelastography (TEG). Samples were analyzed for biomarkers of endothelial activation (sICAM-1), damage (syndecan-1, sThrombomodulin (sTM)) and integrity (sVE-Cadherin) and of platelet activation (sCD40L, TGF-β) by ELISA. Blood counts, standard coagulation tests and HsCRP were measured as routine. Bleeding symptoms on the day of study inclusion were assessed according to the WHO bleeding score (grade 1-4).
Results: 21 patients with AML and 1 patient with high-risk-MDS were included in the study. Median age was 55 years. Pre-transfusion median platelet count increased from 10 10^9/L to 33 and 26 10^9/L, 1 and 24 hours after transfusion, respectively. Despite continued low platelet counts, platelet transfusion normalized the median values of all investigated TEG parameters. Platelet aggregation before and after transfusion also improved, but did not normalize (all $p<0.05$). Considering circulating biomarkers, sVE-Cadherin, reflecting endothelial junction function, was reduced (2214 *vs.* 2049 ng/mL) whereas the platelet activation marker sCD40L was 3-fold increased 1 hour after transfusion (33 *vs.* 91 pg/mL) (both $p<0.05$). Compared to healthy individuals, AML patients had higher sTM, sICAM-1 and HsCRP and lower sCD40L pre-transfusion levels. The change in platelet count (1h δ-value) correlated negatively with the corresponding change in sVE-Cadherin (Figure 1A), indicating that higher platelet count was associated with decreased sVE-Cadherin shedding *i.e.* improved endothelial junction function. The change in platelet count correlated positively with that of sCD40L (Figure 1B), indicating that higher platelet count was associated with increased sCD40L (both $p<0.05$). Interestingly, the 1h sCD40L level correlated positively with Syndecan-1 (24h δ value) and sTM (1h δ value), biomarkers of endothelial glycocalyx and cell damage, indicating that high sCD40L levels disrupted the endothelium (Figure 1C and D). Six patients (27%) were transfused because of bleeding symptoms (all WHO grade 1) and the level of TGF-β was significantly higher in patients with bleeding both before and 1 hour after transfusion compared to patients with no bleeding (n=16). The level of TGF-β in the transfusion products correlated to the post-transfusion change in the biomarker.

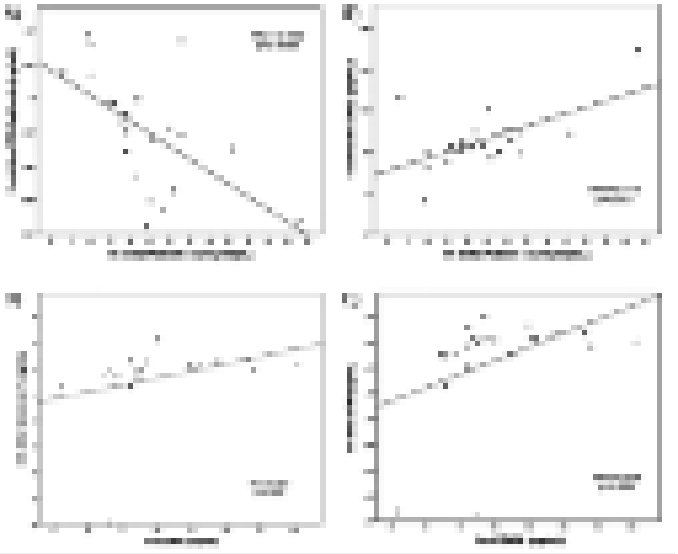

Figure 1.

Summary and Conclusions: Platelet transfusion and the increase in platelet count improved hemostatic function and vascular integrity, the latter assessed by lower sVE-Cadherin. In contrast, the post-transfusion level of sCD40L correlated with biomarkers of endothelial damage. These findings indicate that transfused platelets and platelet-derived pro-inflammatory mediators have opposite directed effects on the endothelium.

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Abstract withdrawn

P590

ASSESSING RISK OF BLEEDING IN THROMBOCYTOPENIC PATIENTS REQUIRING PLATELET TRANSFUSION PRIOR TO INVASIVE PROCEDURES

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Background: Prophylactic platelet transfusion is widely used in thrombocytopenic patients undergoing surgical procedures. Threshold for transfusion in this setting is not well defined and the common practice of maintaining platelet count above $50x10^9$/L is based only on expert opinion and observational studies. Moreover, transfusion outside recommended indications continues to represent a substantial challenge and leads to inappropriate use of blood products and unnecessary transfusions.
Aims: The objective of this study was to assess potential predictors of bleeding in thrombocytopenic patients receiving platelet transfusion prior to invasive procedures.
Methods: We prospectively identified and followed up a total of 58 consecutive thrombocytopenic patients from various medical and surgical units requiring prophylactic preprocedural platelet transfusion. Computer charts and clinical notes were reviewed and patients were interviewed and examined when necessary. Several clinical and laboratory parameters including age, past history of bleeding, raised body temperature, splenomegaly, heparin administration, platelet counts, haematocrit, PT, aPTT and hepatic and renal functions were evaluated. Significant haemorrhage (WHO bleeding grades 2, 3 and 4) during postoperative period was considered as our primary endpoint.
Results: We found out of 58 procedures performed, 17 patients (29%) developed bleeding of WHO grade 2, 3 or 4. Eighty two percent (14/17) of bleeding episodes occurred within the first 72 hours postoperatively. Among factors assessed using multivariate regression analysis, only pretransfusion platelet count and prolonged baseline clotting times were found to significantly influence risk of bleeding. Patients with a pretransfusion platelet count of less than $20x10^9$/ L had more bleeding episodes [P=0.031, 95% confidence interval (CI) 0.03 to 0.67] and a shorter time for the first bleeding event (P=0.035, 95% CI 0.11 to 3.10). A prolongation of PT or aPTT of more than 1.5 times normal value was associated with a shorter time for the first bleeding episode (P=0.017, 95% CI 0.28 to 2.89). However, its correlation with incidence of bleeding was statistically insignificant (P=0.057).
Summary and Conclusions: Our study suggests that thrombocytopenic patients receiving preprocedural platelet transfusion have significant risk of bleeding if their pretransfusion platelet count is lower than $20x10^9$/L or their clotting times are more than 1.5 times normal. Larger randomized controlled trials are required to confirm these findings.

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RED CELL BLOOD GROUP GENOTYPING IN A COHORT OF BLOOD DONORS: FOCUS ON THE DUFFY ANTIGEN SYSTEM

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Background: The Department of Transfusion Medicine of Padua Province supplies nearly 55.000 allogeneic blood units to 12.000 patients. A phenotype matching to AB0, RhD, RhCE and Kell is routinely performed. Nevertheless, the risk of alloimmunization to minor red blood cell (RBC) group antigens is associated with chronic transfusion therapy. In case of alloimmunization there may be long delays in identifying adequate units of RBCs, contributing to the morbidity of diseases. Recently, in our Department, high-throughput molecular techniques of RBC typing have been implemented, in order to obtain mass scale typing of blood donors. Among minor RBC blood group, the Duffy system is clinically significant because implicated in alloimmunization and severe hemolytic transfusion reactions. It is also involved in hemolytic disease of newborn. The FY gene encodes the Duffy antigens. FY has two major codominant alleles, FYA and FYB, that result from a single nucleotide polymorphism (SNP, 125G→A) in exon 2, and the corresponding Fy^a and Fy^b antigens differ by a single aminoacid. Anti-Fy^a and anti-Fy^b, antibodies define 4 main Duffy phenotypes: Fy(a+b-), Fy(a+b+), Fy(a-b+), Fy(a-b-). The rare Fy(a-b-) phenotype in Caucasian population results from a SNP in the promoter region of FY gene (FYGATA) that introduces a premature stop codon into the coding sequence, avoiding Duffy antigen expression on cell surface. Another polymorphism, FYX, is determined by a SNP in the coding sequence of FYB (265C→T). Consequently, the aminoacidic substitution determines instability in the translocation process and reduced amount of Fy^b antigen on cell surface. FYX has been reported in 3.5% of Caucasian donors.
Aims: The aim of this work is to describe the prevalence of FYX and FYGATA in a cohort of blood donors typed in our Department.
Methods: From August 2013 to January 2014 we performed genotyping on DNA samples of 95 blood donors. RBC genotyping was performed with

BLOODchip® (IDCore+, Progenika Grifols, Derio, Spain) using xMAP Luminex® technology. The BloodChip® allows the study of 33 SNPs of 9 erythrocyte system and the deduction of the corresponding phenotype. DNA was extracted from whole blood with automated (EZ1, DNA Blood Kit Qiagen) or manual processing (QIAamp DNA Mini Kit, Qiagen). The medium concentration of DNA was 27,7 ng/uL (optimal range for method 10-80), the medium OD260/OD280 range was 1.9 (optimal range 1.60-1,95). 48/95 donor samples were also typed with serological methods (agglutination on micro-column, Biorad-Diamed).
Results: The genotype was FYA/FYA in 15 donors (16% of total), FYA/FYB in 44 (46%), FYB/FYB in 36 (38%). The genotype FYX has been detected in 8 donors (8.5%). In 4 of them the genotype was FYA/FYB and, in the remaining 4 donors, the genotype was FYB/FYB. 4 of FYX samples have been typed with serological method: in 2 samples the phenotype was Fy(a+b-) (FYA/FYB genotype), in the other 2 samples the phenotype was Fy(a-b+), with FYB/FYB genotype. FYGATA polymorphism has been detected in one donor with FYA/FYB genotype and Fy(a+b-) phenotype.
Summary and Conclusions: It is well known that antibodies used for serological typing cause an "antigen-dose-dependent" agglutination. In case of FYX polymorphism, especially in FYA/FYB genotype, serological methods are not able to identify Fy^b antigen due to Fy^b low-dose on cell surface. The high frequency of FYX polymorphism in our donor cohort and the possible risk of alloimmunization, even with a low dose of antigen, highlight the relevance of RCB group genotyping and the importance of the genotype-phenotype correlation.

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A NEW BLOOD PRODUCT SUPPORT PROTOCOL HAS SIGNIFICANTLY REDUCED MASSIVE BLOOD LOSS IN PATIENTS UNDERGOING SURGERY FOR PSEUDOMYXOMA PERITONEI.

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Background: Hampshire Hospitals NHS Foundation Trust (HHFT) is one of only two hospitals in the United Kingdom that performs complete cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) for pseudomyxoma peritonei (PMP), also called the Sugarbaker technique. Currently our center has the largest case series of patients undergoing PMP surgery in the world. PMP surgery is notorious for being synonymous with massive surgical blood loss due to the complexity of the surgery. This includes extensive surgery to remove the tumour throughout the abdomen with instillation of heated chemotherapy into the abdominal cavity. Historical observations & audits within the haematology department have shown that patients often have a rapid & dramatic decrease in their fibrinogen levels with severe derangement of their coagulation parameters. These abnormalities have repeatedly been shown not to improve with the early aggressive use of fresh frozen plasma (FFP) & have only improved with the additional use of cryoprecipitate. Until recently our surgical protocol for blood product use has continued to follow other international centres that perform this surgery with the early & aggressive transfusion of FFP.
Aims: We introduced a new protocol for blood product support in 2013. The aim was to maintain the fibrinogen level as close to 2 g/l throughout the surgery using cryoprecipitate. Based on previous observation we expected to see a reduction in the use of red cells during surgery.
Methods: We compared blood product use on consecutive patients undergoing PMP surgery following the new protocol & compared it to the retrospective requirements using the old protocol.
Results: The implementation of the current protocol resulted in a dramatic fall in the number of red cells & FFP transfused perioperatively. There was also an increase in the number of patients that did not require any red cell transfusions during PMP surgery.
Summary and Conclusions: Our findings support the earlier observation that the most important clotting factor to maintain during PMP surgery is the fibrinogen. Maintaining a fibrinogen level close to 2 g/l resulted in a significant reduction in red cells & FFP required during the surgery. With the extended role of CRS with HIPEC to surgery involving other peritoneal malignancies we feel that cryoprecipitate has a key role to play in preventing the rapid fall of the fibrinogen & hence blood loss during surgery. This finding raises a further question as to the role of FFP in the early treatment of massive surgical blood loss. We believe that these findings may have important implications in other clinical scenarios.

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RED BLOOD CELL ALLOIMMUNIZATION IN 18-50 YEAR OLD TRANSFUSED WOMEN A 3 YEAR STUDY

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Background: Following a red blood cell (RBC) transfusion, delayed adverse transfusion reactions (AR), such as RBC alloimmunization, may occur. The risks for the 18-50 year old female patient population may be severe. Therefore a strict transfusion policy is applied for that category of patients with the objective of preventing complications during pregnancy and delivery such as the risk for haemolytic disease of the foetus and newborn. According to French regulations, RBC concentrates in the Rhesus and Kell systems (C, E, c, e and K antigens) must be matched to these recipients.
Aims: In order to evaluate the incidence of delayed RBC alloimmunization in these female transfused patients, all AR reports collected during a period of three years were studied.
Methods: The study focused on RBC alloimmunization in female patients between 18 and 50 years of age cared for in all hospitals of the Rhone-Alpes area. Each AR had been included in the national haemovigilance database. The specificity of the RBC antibody (Ab), the blood product involved and the imputability were notified.
Results: From January 1st 2010 to December 31st 2012, 8,953 female patients (18-50 year old women) were transfused. Thirty one delayed RBC alloimmunization reports were notified in 30 female patients. Among them, three (10.0%) had sickle cell disease and 2 (6.7%) had thalassemia. Sixteen women (53.3%) had had at least one prior gestation, 18 (60.0%) had been previously transfused and 6 (20.0%) were already RBC alloimmunized at the time of the transfusion. The blood components involved were RBC concentrates in 28 cases (90.3%), apheresis platelet concentrate in one case and pooled platelets concentrates in 2 cases. In 30 reports, the new acquired RBC Ab had one specificity and in one, two specificities (anti-K and anti-Jka). In the 30 new alloimmunizations with only one specificity, anti-S was observed in 7 cases, anti-Kpa in 7, anti-Fya in 4 and anti-Jka in 4. Nevertheless, despite matching between RBC concentrates and recipients in the Rhesus and Kell systems regulations, two alloimmunizations were detected after transfusion in 2 patients, anti-c (one case) and anti-K (one case); the anti-c alloimmunized woman had been transfused with c-positive RBC concentrates because of a life-threatening pathology (severe anaemia due to bleeding in an ectopic pregnancy). Imputability of the blood component was certain in 13 cases (42.0%), probable in 17 cases (54.8%) and possible in one case (3.2%).
Summary and Conclusions: During the 3 year study, among all AR indicating a delayed RBC alloimmunization in a 18-50 year old transfused women population, only five had haemoglobinopathy. The anti-S and anti-Kpa specificities were the most frequently observed and RBC concentrate was the most involved blood component.

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RED BLOOD CELLS ALLOIMMUNIZATION IN POLYTRANSFUSED PATIENTS

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Background: Polytransfused patients were considered if they received more than 20 packed red blood cells (RBC) for a period of over 150 days.
Aims: To know the frequency of alloimmunization in polytransfused patients at a recently opened centre in 2008 with 350 beds.
Methods: Demographic data, as well as total number of RBC received, alloimmunization incidence, alloantibody specificity and system, number of RBC transfused before the first alloantibody and incidence of posterior alloimmunizations, from polytransfused patients were retrospectively analysed.
Results: We found that 121 patients with a median age of 75 years (41-93) had been polytransfused between 28/02/2008 and 31/12/2013. 73 were male (60.3%) and 48 female (39.7%). The median of transfused RBC was 32 (12-203). The frequency of alloimmunization was 14.9% with a median of RBC received before the appearance of the first alloantibody of 9.5 (0-51). 22 antibodies were detected in 18 patients (11 female and 7 male; p=0,079) and its distribution according to specificity was: E (4), C (4), K (4), Kpa (4), D (1), Cw (1) and autoantibodies (4). Interestingly, over half showed Rhesus (Rh) specificity (55.5%) and the rest Kell specificity. In 4 patients an autoantibody was detected with the first alloantibody. 3/18 patients (16.7%) developed a second alloantibody was detected with a median of 23 RBC received (16-32). Among the group of alloimmunized patients, the distribution according to their diagnosis was: hematologic disease 44.6% (5 patients with myelodysplastic syndrome [MDS], 2 patients with acute myeloid leukaemia and 1 patient with Non-Hodgkin's lymphoma), solid neoplasms 22.4%, multifactorial anaemia (renal failure, liver disease, surgery, etc,) 27.4% and HIV 5.6%.
Summary and Conclusions: In our centre, alloimmunization incidence in polytransfused patients was 14.9%. A greater tendency was observed in women than in men. Usually, the alloantibodies discovered were clinically significant which entailed problems in pre-transfusion tests and a great effort was required in the selection of the blood components. In our series, Rh and Kell specificity represented 100% of the alloantibodies system. In view to our data, and in order to prevent alloimmunization in polytransfused patients, we suggest to consider the transfusion of phenotypically identical units for Rh and Kell for patients who are expected to receive high transfusion requirements.

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IDENTIFICATION OF ABCB6 GENETIC VARIANTS WITH LOW OR ABSENT PROTEIN EXPRESSION AS THE GENETIC BASIS OF HIGH FREQUENCY BLOOD GROUP ANTIGEN LANGEREIS

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Background: Blood group antigen variants may trigger severe diseases during pregnancy and blood transfusion, such as hemolytic disease of the fetus and newborn (HDFN) or adverse hemolytic transfusion reactions. Human erythrocytes express about 350 different integral membrane proteins, including transporters, receptors, which may serve as blood group antigens. The molecular basis of the high frequency red-blood-cell (RBC) blood group antigen Langereis (Lan) has recently been ascribed to the *ABCB6* gene. The frequency of Lan-negative blood type is believed to be extremely low (0.02%).
Aims: To identify the genetic background of low RBC ABCB6 protein expression level in healthy individuals.
Methods: 239 healthy volunteers (235 unrelated individuals and 4 family members of one proband selected from the volunteers with low ABCB6 transporter expression) and 2 Lan-negative sisters were enrolled. RBC ABCB6 protein expression level was measured in 51/239 volunteers by flow cytometry (FACS) using anticoagulated blood sample, stained with OSK43 monoclonal antibody specifically recognizing an intracellular epitope of the ABCB6 protein. Screening for *ABCB6* mutations was performed by direct sequencing of all exons and flanking intronic regions. *ABCB6* SNPs R192Q (rs150221689) and G588S (rs145526996) were genotyped by RFLP and LightCycler allelic discrimination system.
Results: Sequencing of two Lan-negative sisters without detectable ABCB6 protein expression revealed a previously described mutation in homozygous form (ABCB6 R192W, rs149202834). FACS analysis of 51 healthy volunteers identified 4 individuals with significantly lower RBC ABCB6 protein expression levels, as they were below 10th percentile of the average ABCB6 expression level in this group and there was a clear cut-off between these 4 individuals and the rest of the group, observed on a histogram set up according to the expression values. Sequencing of the *ABCB6* gene of these 4 individuals revealed three heterozygous mutations: R192Q (affecting the same codon as R192W); IVS9+1G>A (a putative splice variant at the boundary of exon 9); and G588S, present in two unrelated individuals. Analysis of the family members of the individual in whom we originally detected the R192Q missense mutation revealed that low ABCB6 expression segregated with the R192Q mutation. Genotyping of 235 unrelated volunteers for R192Q and G599S variants, identified no further R192Q carriers (allele frequency: 0.2±0.4%). For G588S, 3 additional heterozygous individuals were identified (5/235), resulting in allele frequency of 1.1±0.9% for this variant.
Summary and Conclusions: By screening healthy individuals we identified a new mutation causing the Lan- phenotype. We found significant differences between the expression levels of the wild-type ABCB6 protein and the R192W, R192Q, IVS9+1G>A and G588S polymorphic variants. Our results suggest that the ABCB6 genetic variants linked to lower or absent expression of Langereis may be more common than previously thought.

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ANALYSIS OF ADVERSE REACTIONS IN VOLUNTARY WHOLE BLOOD DONORS: EXPERIENCE FROM A GREEK BLOOD CENTRE

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Background: Adverse reactions (ARs) occasionally occur during or after blood donation and may discourage the donors from further donating.
Aims: The aim of the present study was to assess the frequency, type and cause of ARs and suggest additional preventive measures.
Methods: The study involved all donations (25.774) throughout the year 2012. Each AR, occurring during or after a donation, was recorded by the staff on a new specifically designed individual report form. ARs were classified as local or general. Local ARs include haematoma (due to difficult venous access, vein injury or arterial puncture), delayed bleeding, nerve irritation or injury, local thrombophlebitis, tendon injury, allergy to the needle metal or disinfectant. Systemic ARs are classified as mild, moderate or severe and diagnosed as vasovagal, hypocalcemic, or "other". We registered donors' age, donation status (first time or repeat donor), the time from last meal, insufficient sleep, known postural hypotension, fatigue, fear of the needle stick and/or the sight of blood, seeing someone else fainting, etc.
Results: 91 ARs were recorded (overall incidence 0,35%): 87 general and 4 local. 65 occurred in male donors (71,42%) and 26 in females (28.58%). Vasovagal reactions (87/91) –immediate or delayed- were characterized by discomfort, weakness, pallor, sweating, dizziness, nausea, hypotension, bradycardia. In one case, tetany due to hyperpnoea developed after recovery from a vasovagal reaction. The majority of the vasovagal reactions (57/87) were mild (subjective symptoms only) while 26/87 were moderate (28,57%). In 4 (4,39%). loss of consciousness occurred along with convulsions and incontinence(1), fall causing injury of the donor(1), fall without injury(1). The local ARs included: haematoma (2), local pain (1), possible trauma of the nerve (1). Predisposing factors identified: insufficient sleep in 14(16,09%), fatigue in 11(12,64%), fear of the needle in 11(12,64%), postural hypotension in 10(11,49%), fear of the sight of blood in 8 (9,19%), omission of breakfast or long interval in 4(4,59%), donation too soon after last meal in 3(3,44%), seeing another donor fainting in 3(3,44%), anxiety in 1(1,15%). No risk factor was identified in 17 (19.54%). First time donors were implicated in 25 ARs (28,73%), repeat donors without reactions in the past in 51 (58.62%) and repeat donors with a history of previous ARs in 11 (12,64%). Most ARs (79/87) occurred early (during or immediately after the donation (86,81%). Delayed ARs appeared in 12 donors (13,18%) after leaving the site. In most ARs (78/91) donors <40 years of age were involved (85,72%).
Summary and Conclusions: Our results show insufficient sleep as the commonest risk factor, followed by fatigue and fear of the needle, postural hypotension, fear of the sight of blood, omission of breakfast or lunch. ARs predominated in repeat donors possibly due to less attention by the staff to repeat donors and/or these donors overestimating their physical status and omitting to report predisposing factors. Good interview practices and consistent auditing and reporting ARs help to identify predisposing factors and adopt preventive strategies. Staff should be experienced and adequate at all times for efficient surveillance of the donors.

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LEUKOREDUCTION FILTER OBSTRUCTION - THE OPPORTUNITY TO DETECT BLOOD DONORS WITH SICKLE CELL TRAIT

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Background: The prevalence of hemoglobinopathies in Greece is important. Sickle cell trait (SCT) affects 1% of the general population. It is not widely known that blood from SCT donors usually leads to obstruction of leukoreduction filters or to inefficient leukoreduction. Factors that cause hemoglobin S polymerization and ultimately filter obstruction, include: the relative insolubility of deoxygenated hemoglobin S (HbS), decreased deformability of red blood cells (RBCs), the type of anticoagulant and preservative, the temperature and length of storage, pH changes during storage, the material of the filter itself. RBCs from SCT donors should not be transfused, especially to newborns, patients undergoing general anesthesia or having hypoxemia or increased oxygen demands.
Aims: In this study, we planned to examine the blood donor for sickle cell trait (SCT), each time complete or partial obstruction of a post storage leukoreduction filter occurred, in order to exclude donors with SCT from further blood donation.
Methods: According to our protocol, each time very slow flow and eventual obstruction of a poststorage leukoreduction filter occurs during filtration of packed RBCs, a blood sample from the bag has to be examined for hemoglobin fractions by HPLC (High-performance liquid chromatography). In contrast to traditional liquid chromatography which relies on gravity forces, HPLC relies on pumps to pass a pressurized liquid solvent containing the sample through a column filled with a solid adsorbent material. The components are separated because each component in the sample interacts slightly differently with the adsorbent material and flows out the column at a different flow rate. In case of SCT, the hemoglobin fractions include HB S (less than 45%), while normal blood contains only the A (more than 95%), A2 (less than 4,2%) and F (around 1%) hemoglobin fractions.
Results: During one year (2013), complete or partial obstruction of post storage leukoreduction filters was observed 30 times during filtration of units of RBCs. From the 30 blood samples examined, 5 (16,6%) showed HbS fractions (34,3%>37.4% of total hemoglobin) thus identifying donors with SCT. None of them was aware of having SCT. Two had donated in the past in blood services that do not filter blood units. Although, the remaining blood donors had several donations in our Center, filter obstruction was previously reported only once. The donors were notified and asked to refrain from further donating blood.
Summary and Conclusions: Donors with SCT should be identified and excluded from further donation. Our data underestimate the incidence of SCT among blood donors because not all units of RBCs are leukodepleted with poststorage filters. More blood donors with SCT would be identified if clinical departments also reported all cases of obstruction of bedside filters.

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ADMINISTRATION OF FIBRINOGEN CONCENTRATE IN TRAUMA PATIENTS IS ASSOCIATED WITH IMPROVED SURVIVAL AT 6 HOURS BUT NOT AT DISCHARGE

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Background: Despite poor evidence and high costs, fibrinogen concentrate (FC) represents one of the most frequently used haemostatic agents in trauma.
Aims: The aim was to assess, whether the administration of FC in severely injured patients was associated with improved outcomes.
Methods: Patients (Injury Severity Score [ISS] ≥14) had received FC during initial care between emergency department (ED) arrival and intensive care unit admission or operation room (FC+) were matched with patients who had not received FC (FC-).
Results: The matched-pairs analysis yielded two comparable cohorts (n=14 in each group) with a mean ISS of 34.6±13.6 (FC+) and 34.1±13.2 (FC-) (p=0.73); the mean age was 46±17 *versus* 46±18 (p=0.72), respectively. Patients were predominantly male (73.1% in both groups, p=1.0). On emergency department arrival, hypotension (systolic blood pressure, ≤85 mm Hg) occurred in 52,3% (FC+) and 47,1% (FC-) (p=0.41) and base excess was -7.35±5.1 mmol/L for FC+ and was -7.45±6.3 mmol/L for FC- (p=0.96). Patients were administered 12.7±13.3 (FC+) *versus* 10.7±11.1(FC-) packed red blood cell units (p=0.20). Thromboembolism occurred in 5.9%(FC+) *versus* 3.5% (FC-) (p=0.06) and multiple organ failure occurred in 62.3% *versus* 48.1% (p=0.003), respectively. Whereas 6-hour mortality was 11.6% for FC+, *versus* 15.5% for FC- (p=0.03), the mean time to death was 6.5±13.4 days, *versus* 5.6±7.9 days (p=0.006). The overall hospital mortality rate was 29.5%, *versus* 24.6% (p=0.40), respectively.
Summary and Conclusions: This study investigates the effect of FC administration in bleeding trauma. In our population of severely injured patients, the early use of FC was associated with a significantly lower 6-hour mortality and an increased time to death, but also an increased rate of multiple organ failure. A reduction of overall hospital mortality was not observed in patients receiving FC.

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EVALUATION OF BILIARY INJURY IN NEWBORNS WITH ABO INCOMPATIBILITY

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Background: The ABO blood group related antigens are known to be expressed not only on the surface of erythrocytes, but also on a variety of cells in the human body. In normal liver, these blood groups related antigens are expressed mainly in the epithelial cells of large bile ducts and virtually absent in hepatocytes. Biliary injury mediated by alloimmune responses to major ABH antigens was described in, liver transplantation using ABO incompatible graft.
Aims: To test the validity of a hypothesis linking IgG antibody-mediated hemolysis of fetal erythrocyte and a similar mechanism for biliary injury, we investigated the structural integrity of the biliary tract in ABO hemolytic disease of the newborn (ABO HDN) through measurement of sensitive biliary enzymes.
Methods: Eighty term and near term newborns were studied; 40 newborns with ABO incompatibility and 40 controls (30 of them were healthy of blood group compatible with their mothers and 10 newborns with Rh incompatibility as a hemolytic control). All neonates were studied stressing on blood grouping and markers of hemolysis; indirect bilirubin and lactate dehydrogenase (LDH). Gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) were measured before and after therapy (phototherapy and/or exchange transfusion). Investigations were done to exclude other causes of neonatal hemolytic disease. Patients with ABO HDN were further subdivided according to the age-specific total serum bilirubin nomogram and disease severity into 3 subgroups; mild (n=22), moderate (n=12) and severe (n=6) HDN
Results: Pre-therapy GGT and ALP levels were significantly elevated in all neonates with hemolysis compared with healthy controls (p<0.001). The highest GGT levels observed in ABO-HDN group; however, ALP was consistent among neonates with ABO HDN and Rh HDN. In ABO HDN, pre-therapy GGT and ALP levels were significantly elevated in severe disease compared to mild and moderate disease. GGT and ALP levels were also significantly higher among neonates with ABO HDN with positive direct or indirect antiglobulin test compared to those with negative test (p<0.001). Pre-therapy GGT levels were significantly higher in all ABO HDN subgroups (p<0.05) compared to Rh HDN. However, ALP was significantly higher only in severe hyperbilirubinemia compared to Rh-HDN group. As regards the effect of therapy, both GGT and ALP were significantly decreased post-exchange transfusion among neonates with ABO HDN compared to their baseline levels (p<0.001) while no significant difference was found in Rh HDN group. Moreover, both markers were significantly lower post-exchange transfusion when compared to post-phototherapy (p<0.05). Both pre-therapy GGT and ALP levels were positively correlated in patients with ABO HDN (p=<0.001). Significant positive correlations were found between pre-therapy GGT as well as ALP and each of reticulocyte count, normoblasts, LDH, total and direct bilirubin while GGT and ALP were inversely correlated with hemoglobin among ABO-HDN neonates. Multi regression linear analysis revealed that both LDH and GGT were independently related to ALP levels.
Summary and Conclusions: In ABO-HDN, we assume that binding of anti-A and anti-B antibodies with their corresponding antigens located over the epithelium of the biliary tract resulted in its injury reflected by increased direct bilirubin and both GGT and ALP levels. Early implementation of therapeutic modalities could result in at least partial protection of the biliary structural integrity.

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SENSITIVITY OF INDIVIDUAL AND MINI-POOL NUCLEIC ACID TESTING ASSESSED BY DILUTION OF HEPATITIS B NAT YIELD SAMPLES

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Background: For Nucleic Acid testing (NAT) of blood donations, either the blood samples can be pooled together in a batch of 6 or 8 prior to testing to screen a large number of donations with few tests (MP-NAT), or the tests can be run on every individual sample (ID-NAT). It has been debated in various studies whether pooling of samples results in decreased sensitivity of detection as the volume of individual samples gets lesser in a pool.
Aims: Objective of this study was to investigate the effect of dilution on the sensitivity of tests with low viral load, serology negative, Individual Donor Nucleic Acid Testing (ID-NAT) HBV reactive samples.
Methods: The study was performed on 9 plasma samples which were exclusive Procleix Ultrio Plus HBV yields *i.e.* NAT Ultrio Plus Assay reactive but Serology and Procleix Ultrio non reactive during a Procleix Ultrio Plus pilot study conducted on blood donors for a period of one year, from September 2011 to September 2012, at All India institute of Medical Sciences, New Delhi India. These 9 exclusive Ultrio Plus ID-NAT yield samples were diluted in 1:2, 1:4. 1:6 and 1:8 dilutions using previously tested negative plasma and each dilution of every sample along with archived undiluted sample were retested in three replicates with Procleix Ultrio Plus Assay.
Results: Among NAT yield samples, 88.88% of the samples were detected when retested in ID-NAT in undiluted format.. Samples with higher viral load (sample 5 and 6) were detected by all dilutions. samples with viral load below 20 IU/ml or were not detected by PCR (5 out of 9,55%) when tested in dilutions of 1:6 or 1:8, only 9 out of 27 replicates (33.33%) were detected. This means that more than 76% low viral load samples were missed by MP-NAT of 1:6 or 1:8 dilution out of total NAT yield samples (Figure 1).

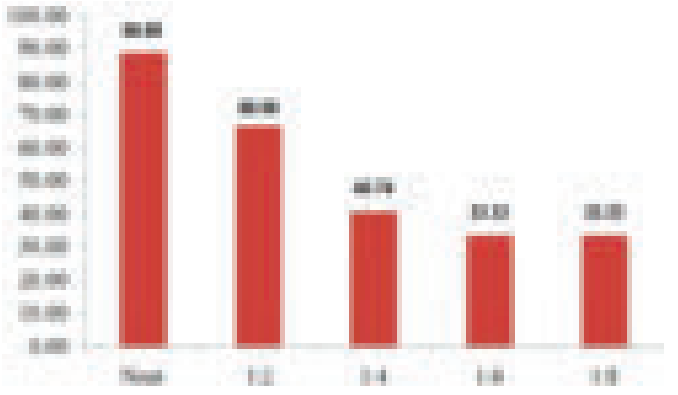

Figure 1.

Summary and Conclusions: Individual Donor NAT is ideal methodology for NAT as dilution due to pooling may miss samples with low viral load as evident in this study.

Immuno-thrombocytopenia

P601

UPDATED RESULTS FROM A LARGE, OBSERVATIONAL STUDY OF PATIENTS (PTS) WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) RECEIVING ROMIPLOSTIM IN EUROPEAN CLINICAL PRACTICE

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Background: ITP is characterized by low platelet counts and increased risk of bleeding. Severe bleeding episodes are uncommon but may be life-threatening and require hospitalization. Romiplostim is a thrombopoietin-mimetic peptibody approved in Europe for second line treatment in splenectomised and non-splenectomised pts where surgery is contraindicated.
Aims: To describe romiplostim use in European clinical practice, and to describe the demographics, clinical outcomes, and resource utilization in romiplostim-treated pts. Results from a planned interim analysis are reported.
Methods: Eligible pts were adults with primary ITP who had received romiplostim and who had provided informed consent. The observation period was for up to 2 years after romiplostim initiation. Data were collected retrospectively on ITP-related hospitalizations for up to 2 years before romiplostim initiation and on bleeding events for up to 6 months before romiplostim initiation.
Results: An interim analysis was conducted in September 2013. In total, 356 pts had enrolled and 339 were included in the Full Analysis Set. The mean (±SD) duration of romiplostim treatment was 21 (±5) months; 25% of pts remained on study, 11% discontinued, and 65% completed the 2-year observation period. Adverse drug reactions (ADRs) led to early romiplostim discontinuation in 5% of pts. At romiplostim initiation, the median age was 62 years (range 18–91) and median duration of ITP was 3 years (range 0–56), with one-third of pts having ITP for less than 1 year. Most pts (55%) had received ≥3 prior ITP therapies and 34% had undergone splenectomy. The median average weekly dose of romiplostim was 2.8 mcg/kg (Q1-Q3: 1.5–4.4) and most pts (70%) started romiplostim at a 1 mcg/kg dose. Some intermittent dosing with romiplostim was observed: 41% of pts had a >3 week period where doses were withheld or missing. Romiplostim was self-administered by 37% of pts. The median baseline platelet count was $20x10^9$/L (Q1-Q3: 9-35), which rose to $69x10^9$/L (Q1-Q3: 28-139) after 2 weeks of romiplostim treatment and remained >$50x10^9$/L thereafter. Thirty pts (9%) ended romiplostim treatment due to achieving a haemostatic platelet count range, with median platelet counts of $172x10^9$/L (Q1-Q3: 90-261). After romiplostim initiation, there was a decrease in rates of grade ≥3 bleeding events (from 12 to 2 per 100 pt-years) and ITP-related hospitalizations (from 87 to 35 per 100 pt-years). There were some differences in baseline demographics between splenectomised and nonsplenectomised pts, but romiplostim doses and bleeding and hospitalization rates were similar between the two groups (Table 1). Thirteen pts reported a serious ADR. Seven pts experienced 8 thrombotic ADRs (2 each of deep vein thrombosis and pulmonary embolism; 1 each of embolism, myocardial infarction, retinal vein thrombosis, and transient ischaemic attack). Bone marrow fibrosis ADRs occurred in two pts as reported in previous interim analyses (myelofibrosis in pts who were subsequently found to have a diagnosis inconsistent with ITP [MDS and metastases to bone marrow]). No fatal ADRs were reported.

Table 1.

Summary and Conclusions: The dosing, effectiveness, and safety of romiplostim in a real-world ITP pt population appeared comparable with that observed in clinical studies (*e.g.* Kuter BrJH 2013). Hospitalization and bleeding were lower after romiplostim treatment, and outcomes were similar between splenectomised and nonsplenectomised pts.

P602

FEASIBILITY OF ROMIPLOSTIM DISCONTINUATION IN ADULT TPO-MIMETIC RESPONSIVE PATIENT WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP): AN OBSERVATIONAL RETROSPECTIVE REPORT IN REAL LIFE CLINICAL PRACTICE

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Background: Thrombopoietin mimetic agents (TPO mimetics) improve platelet (plt) counts in ITP patients (pts) acting on megakariocyte survival and production of plt. They were thought to be a life-long therapy. However, clinical evidence is accumulating showing that this may not be the case
Aims: We report on ITP pts unresponsive to previous therapies who were treated with romiplostim (R) and who were able to discontinue it after achieving response, without relapsing at follow-up
Methods: 30 pts (11 M;mean age at R treatment 50.7 yrs, range 18-82) were treated between Sept 2009-Feb 2014. ITP disease status at R administration: 7 newly, 8 persistent, 15 chronic disease. Lines of previous therapies: mean 2.8, range 2-5. Time between start of any treatment and R: mean 52.7 mos, range 1-264 mos. All pts are enrolled in a local Italian registry (REL-ITP registry), and gave informed consent to demographic and clinical data use at enrollment
Results: 27/30 pts (90%) responded to R according to International Working Party criteria (1).Of the 27 responders: 1 pt discontinued R due to acute myocardial infarction (AMI); 1 pt was lost to follow-up; 5/27 pts R used as bridge to splenectomy; 4/27 pts lost response (12, 6, 11, and 12 mos); in 3/27 pts R administration is ongoing (52, 8 and 7 mos); 13/27 achieved stable, safe plt counts and were able to stop R (Table 1). Pts discontinued R after a mean of 42 weeks (range 12-122) with sustained response maintained at a mean of 26 mos (range 9-51). Mean R dose was 2.43 µg/kg/week (range 0.5-3.8 µg/kg/week). Mean plt count at the time of R discontinuation was $168x10^9$/L (range 48-$500x10^9$/L); mean nadir and peak plt counts off therapy were $128x10^9$/L (range 52-$243x10^9$/L) and $279x10^9$/L (range 120-$411x10^9$/L) respectively. No bleeding events occurred during the observational period; 2 thrombotic events (lower limb superficial phlebitis; AMI) were recorded.

Table 1. Characteristics of patients with sustained response to Romiplostim.

Summary and Conclusions: Sporadic pts have been reported as being able to discontinue treatment with R [2-6] without relapsing off-therapy, however prevalence of long-term remission is currently unknown. In our experience, the percentage of pts able to discontinue R is 48%, if we consider R responders (13/27 responders) and 65% if we consider only pts who received long-term treatment (13/20 pts; *i.e.* not considering pts receiving R as bridge to splenectomy, lost to follow-up, or who discontinued R because of SAE). In 7/13 pts R seemed to be the only treatment responsible for sustained response. In the remaining pts, response might have been either spontaneous (3 newly diagnosed pts) or secondary to rituximab/splenectomy. Nevertheless, use of R may be a treatment option awaiting for plt recovery. No specific pattern of early response (*e.g.*: dose requirements, time to response) seemed to predict for long-term response. However, it seems that pts achieving safe plt counts at lower dosages are probably worth a try of therapy tapering and discontinuation. Manufacturer dose adjustment rules allow R to be discontinued when appropriate and clinical judgment was used to manage pts. If our preliminary retrospective observations were to be confirmed, the proposal of using R as "bridge to recovery" rather than long-term treatment would have relevant clinical impli-

cations: long-term sides effects (*e.g.* marrow fibrosis, thrombotic risk) and treatment costs would be less of an issue.

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P603

TREATMENT OF ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) WITH ROMIPLOSTIM IN ROUTINE CLINICAL PRACTICE IN GERMANY-INTERIM RESULTS FROM AN OBSERVATIONAL STUDY (PLATEAU)

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Background: In the EU, the thrombopoietin-receptor agonist romiplostim (Nplate®) is recommended for second-line treatment of ITP in adult patients who failed splenectomy or are ineligible for splenectomy to achieve sustained increases in platelet counts (Provan *et al.*, 2010).
Aims: The aim of this ongoing study is to assess the use of romiplostim in clinical practice in Germany.
Methods: This multicentre, prospective and retrospective observational study (data collected before and after initiation of romiplostim) is enrolling ITP patients ≥18 years who received at least one dose of romiplostim in routine clinical practice, with an observation period of 2 years following romiplostim initiation. Endpoints included patient demographics, romiplostim use, platelet counts, adverse drug reactions (ADRs), and other clinically relevant parameters. We report data from an interim analysis conducted in 6/2013.
Results: 125 patients (pts) were enrolled (50% male; median [range] age: 63 [18-89] years). Of these, 122 had received romiplostim, 53 (42%) had completed the 2 year observational period and 21 (17%) had withdrawn (n=3 AE, n=4 death, n=14 other reasons). Median (range) time from ITP diagnosis to romiplostim initiation was 25 (0-436) months.111 pts (92% of 121 evaluable) had received prior ITP therapies (median [range] therapies: 2 [1-20]), 102 pts (83%) were non-splenectomized. 45 pts (37%) had experienced bleeding events within 6 months before romiplostim initiation (grade III/IV: n=3 (2.5%)). Median (range) platelet count at initial romiplostim injection was 26 (2-801) x10^9/L and increased during therapy (Figure 1). Over the observation period, romiplostim was injected at a median (range) dose of 3 (1-10) µg/kg/week. Patients received a median [range] of 39 (1-104) injections over a median (range) observation period of 62 (1-104) wks. The most common reported ADRs (≥5%) were fatigue (8 pts [10%], dizziness (7 pts [9%], nausea (7 pts [9%], vomiting (5 pts [6%]) and diarrhea (4 pts [5%]). One patient reported a vascular access related thromboembolic event. There was no increase in bone marrow reticulin. 6 serious ADRs were reported. Exposure-adjusted bleeding rate (grade 3 or 4) before romiplostim initiation was 6.1 per 100 patient-years *vs.* 3.7 per 100 patient-years after romiplostim initiation. Exposure-adjusted ITP related hospitalization rate before romiplostim initiation was 24 per 100 patient-years *vs.* 14.9 per 100 patient-years after romiplostim initiation.

Figure 1.

Summary and Conclusions: These interim data show that platelet counts in patients treated with romiplostim was increased and maintained at the desired range of 50-250x10^9/L and generally well tolerated in unselected ITP patients treated in routine clinical practice in Germany. Bleeding events and ITP related hospitalization rates were decreased after receiving romiplostim.

P604

A PROSPECTIVE, MULTICENTER, OPEN-LABEL STUDY OF RECOMBINANT HUMAN THROMBOPOIETIN ON PLATELET RECOVERY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Delayed platelet recovery is a significant complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and a significant independent risk factor for increased transplantation-related mortality (TRM) and poorer overall survival (OS). But until now, effective treatment for delayed platelet recovery is limited
Aims: This study was designed to assess the efficacy and safety of recombinant human thrombopoietin (rHuTPO) on platelet recovery after allo-HSCT.
Methods: A prospective, multicenter, non-randomized, open-label study (NCT01379391) was conducted in China. The protocol was approved by ethics committee of Nanfang hospital of southern medical university and informed consent was obtained from each subject. The patient whose platelet counts below 20x10*9/L on +14days after transplantation was enrolled and assigned into TPO arm or TPO-free arm, according to protocol TPO-HSCT-2011.
Results: From JUN 2011 to NOV 2013, a total of 58 patients were enrolled in this study in 10 clinical centers, 57 of them were evaluable. Among them, 30 cases were enrolled into TPO arm and 27 cases in TPO-free arm. TPO arm consisted of 16 patients with precursor cell lymphoblastic leukemia/lymphoma (LBL/ALL, 6 of 15 were refractory or relapsed), 9 patients with acute myeloid leukemia (AML), 2 patients relapsed peripheral T-Cell non-Hodgkin's lymphoma (PTCL-NHL), 2 patients with myelodysplastic syndromes (MDS), 1 patient with severe aplastic anemia (SAA). Among TPO arm, 16 patients with LBL/ALL received intensified HDE-ALL-2011 (NCT01457040) as conditioning regimen, 9 high-risk AML received BU/CY2, 2 received GIAC and 2 received BU/FLU, 1 SAA patient received ATG/CTX/FLU. Eleven of 30 cases in TPO arm underwent HLA-matched sibling HSCT, 9 received haploidentical HSCT, 8 received matched-unrelated donor HSCT, and 2 received mismatched HSCT. The median ANC and CD34+ cells infused in TPO arm was 8.0x10*8/kg (range 2.7-11.9), 3.5 x10*6/kg (range 1.1-10.6), respectively. TPO-free arm consisted of 14 LBL/ALL cases, 5 AML cases, 5 cases of CML and 3 cases of SAA. Among TPO-free arm, 11 patients underwent HDE-ALL-2011 as conditioning regimen, 4 patients with BU/CY2, 3 cases with TBI/CY2 and 3 cases with ATG/CTX/FLU. Ten out of 27 cases in TPO-free arm received HLA-matched sibling donor HSCT, 9 cases received mismatched HSCT, 4 cases with MUD HSCT and 4 with haploidentical HSCT. The median ANC and CD34+ cells infused in TPO-free arm was 7.0x10*8/kg (range 3.5-11.1), 3.8 x10*6/kg (range 2.8-9.7), respectively. No NCI-CTCAE grade III/IV TPO-related adverse events were observed in TPO arm. Incidence of CMV/EBV viremia in TPO, TPO-free arm was 43.3% *vs* 59.3%. The median platelet count significantly increased from 14.5x10*9/L to 44.0x10*9/L in TPO arm after 14 days' TPO treatment, compared to 15.0x10*9/L to 32.0x10*9/L in TPO-free arm. The median time (days) of platelet count above 20x10*9/L, 50x10*9/L, 100x10*9/L in TPO and TPO-free arm were 18.0 *vs* 21.0 (p>0.05), 28.5 *vs* 31.0 (p>0.05) and 36.0 *vs* 46.0 (p<0.05). CFU-MK and flow analysis for megakaryocytic progenitor cells were performed at +14d (prior to TPO), +28d (completion of TPO) after transplantation in 10 cases in TPO arm. CFU-MK assay demonstrated significant increase from 10 to 29 colonies (p<0.01). Flow analysis also showed significant growth of CD34+CD41+ and increasing tendency of CD34+CD61+ population, but not CD41+CD61+ subsets. After a median of 12 months of follow-up, TRMs in TPO and TPO-free arm were 13.3% *vs* 33.3% (p<0.01). Estimated 2-year OS in TPO and TPO-free arm were 70.0% *vs* 33.3% (p<0.01).
Summary and Conclusions: TPO administration could promote platelet engraftment, reduce TRM and improve OS for high-risk delayed platelet recovery after allo-HSCT.

P605

SHORT TERM RE-EXPOSURE TO ROMIPLOSTIM IN PREVIOUSLY TPO-MIMETIC RESPONSIVE ITP PATIENTS ALLOWS RECOVERY OF PLATELET COUNT AND LONG TERM SUSTAINED REMISSION: AN OBSERVATIONAL RETROSPECTIVE CASE-SERIES

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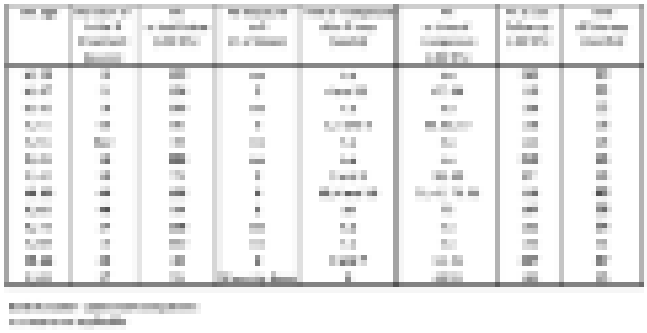
Background: Thrombopoietin mimetic agents such as Romiplostim (R) improve platelet (plt)count in patients (pts) with Primary Immune Thrombocytopenia (ITP) during its use, but most patients (nearly 90% in phase III studies [1,2]) lose response after last dose if drug is discontinued. Efficacy of re-exposure to R in previously responsive patients after a decrease of plt count has not yet been described in real life clinical practice.
Aims: We report on ITP patients who achieved a complete response during R treatment and who were able to discontinue the drug. During follow up some of them experienced an abrupt decrease of plt but a short term re-exposure to R has resulted to a complete long-term response
Methods: 30 pts (11 M; mean age at R treatment 50.7 yrs, range 18-82) were treated with R (ITP disease status at R start: 7 newly diagnosed, 8 persistent, 15 chronic disease) because of unresponsive disease (lines of previous therapies: mean 2.8, range 2-5). All pts are enrolled in a local Italian registry (REL-ITP registry), and gave informed consent to clinical data use
Results: 27/30 pts (90%) responded to R according to International Working Party criteria[3]. 13/27 pts discontinued R after a mean of 42 weeks (range 12-122) because of a complete response (CR) with plt >100x10^9/L (7/13) or response (PR) with plt>50x10^9/L (6/13 pts). 6/13 pts showed stability of plt count after last dose of R (5 pts achieved CR, 1 PR) and didn't require any further treatment (mean time off therapy 28 months, range 11-51). 7/13 pts (2 with previous CR) after last R dose showed a decrease of plt count (mean nadir 45 x10^9/L, range 12-75 x10^9/L) and were re- exposed to R after a mean of 5.7 weeks (range 3-12) since last R dose: one pt needed one single re-exposure after 10 weeks since last R dose to obtain again a stable RC (time off therapy since then 28 months); one pt was treated after 4 weeks since last dose with 10 weekly R doses (now off therapy since 15 months); in five pts a single R shot was given "on demand" for 2 times (3 patients) or 3 times (in 2 patiens) at variable number of weeks since the previous shot (mean 5.6 weeks, range 3-12) and were off therapy since a mean of 25 months (range 11-40). 12 out of 13 pts achieved a CR after the re-exposure, one pt achieved a stable PR and none of them has ever received any treatment. No bleeding events were observed (Table 1).

Table 1.



Summary and Conclusions: Increasing data of stable response after R withdrawal are now available. In our experience patients who achieved a stable CR or in some cases even a stable PR with plt >50x10^9/L deserve an attempt to discontinue R: an occasional drop of platelet count even after weeks since last R dose seems to be easily managed with re-exposure and didn't affect a possible long term response. If these retrospective observational results were to be confirmed, a personalized patient-tailored R administration could lead to a relevant number of definitive suspension sparing chronic treatment.

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P606

THROMBOPOIETIN RECEPTOR AGONISTS IN PRIMARY IMMUNE THROMBOCYTOPENIA: EVALUATION OF EFFICACY (RESPONSE AND SUSTAINED RESPONSE OFF-TREATMENT) AND SAFETY IN A SINGLE CENTER POPULATION

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Background: Romiplostim and Eltrombopag are two thrombopoietin receptor agonists (TPO-RAs) which show clinical efficacy in primary immune thrombocytopenia (pITP) and are changing the management of this autoimmune disorder.
Aims: To retrospectively describe the efficacy and safety of TPO-RAs in a pITP population from a single Center.
Methods: All adult pITP patients treated with TPO-RAs are included. Data were captured from the medical records. Response was defined as platelet (plt) count ≥50x10^9/L and at least a 2-fold increase of the baseline value. Complete response was defined as a plt count ≥100x10^9/L. Period in response during TPO-RAs treatment was expressed as the percentage of weeks in response/weeks on treatment. Phases of pITP were defined in relation to diagnosis as follows: acute, within 3 months; persistent, between 3 and 12 months; chronic, more than 12 months.
Results: Since February 2009, 39 pITP patients (25 F, 14 M) resistant to one or more therapy lines, have been treated with TPO-RAs. The median number of therapy lines before the start of TPO-RAs was 2 (1-5): prednisone, dexamethasone, azathioprine, rituximab, splenectomy, interferon. Nine patients had been previously splenectomized. The median age at TPO-RAs start was 63.4 years (19.2-79.3) and the median period between pITP diagnosis and TPO-RAs start 6.9 years (0.1-38.9). Twenty eight patients were treated with Romiplostim and 11 with Eltrombopag. At the start of TPO-RAs, 32 patients were in a chronic phase, 5 in a persistent and 2 in an acute phase. These last 2 patients were severely symptomatic and resistant to high dose steroids and intravenous immunoglobulins. The median plt count at the start of TPO-RAs was 11x10^9/L (2-52). Steroids were a concomitant treatment in 34/39 patients. The median number of weeks on TPO-RAs treatment was 73 (2-262) and the median TPO-RAs dosage was 4 µg/dose (1-10) for Romiplostim and 75 mg/day (25-75) for Eltrombopag. Thirty two/39 patients (82%) obtained a response at least once (26 on concomitant treatment with steroids) after a median period of 3 weeks (1-5). Twenty four/26 maintained the response after steroids discontinuation. Globally, 30/39 (77%) patients obtained a response without concomitant treatment after a median of 8 weeks (2-37). With regard to the entire population, the median percentage of weeks in response/weeks on treatment was 80% (0-100). Twenty/39 patients stopped the first TPO-RA treatment, the reasons being: response to splenectomy in 1, no response in 6, side effects in 5, poor compliance in 1, CR in 7. Among the latter 7 patients, 5 maintained a sustained response at a median follow-up of 2.3 years (0.8-3.4), 1 relapsed after a period of 6 months, 1 developed a splenic lymphoma after 17 months from the last TPO-RA administration. Seven patients switched to the other TPO-RA, obtaining a response in 5 cases (3 without any concomitant treatment). On the whole, 24/39 patients never stopped TPO-RA treatment. We did not observe adverse events possibly related to the study drug. Five patients experienced side effects: osteo arthritic muscle pain in 4, hepatic toxicity in 1.
Summary and Conclusions: We confirm the efficacy and safety of TPO-RAs in pITP patients refractory or resistant to one or more therapy lines. We did not record severe adverse events, but 5 patients experienced side effects which led to stop treatment. We observed 5/39 (13%) persistent responders off treatment, with a median follow-up of 2.3 years. The use of TPO-RAs is progressively increasing and extending to severe patients also in acute or persistent phase, while other therapies are failing or contraindicated.

P607

PREDICTIVE FACTORS ASSOCIATED WITH LONG-TERM EFFECTS OF SPLENECTOMY FOR IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Splenectomy leads to a good response in 60-80% of adult patients with corticosteroid refractory idiopathic thrombocytopenic purpura (ITP). However, data on the long-term efficacy are controversial. Furthermore, it is not clear whether there are pre or post-operative parameters able to predict the therapy failure.
Aims: To evalute long-term efficacy and to determine the preoperative and postoperative factors that predict a successful splenectomy outcome.
Methods: We retrospectively analyzed the data on 93 patients (median age 39 years, range 19-74; 71/22 female/male ratio; median follow-up from diagnosis 86 months, range 6-182; median follow-up from splenectomy 71 months, range 2-148), who underwent splenectomy (laparotomic 55, laparascopic 38) for ITP between 2002 and 2013. Platelet kinetic study with Indium-111 was performed in 33 patients before splenectomy. Patients with platelet count <30×10^9/L or steroid-dependent were splenectomized. Complete response (CR) and partial response (PR) were defined as platelet count >150×10^9/L and 50×10^9/L one month after surgery. Refractoriness was diagnosed when the platelet count remained <30×109/L. Relapse was defined as a drop in the platelet count <150×10^9/L or 50×10^9/L after CR or PR. Refractory or relapsed patients with a platelet count <30×10^9/L or bleeding were treated with steroids, azathioprine, danazol, vinca alkaloids and TPO-agonist.
Results: The median time from diagnosis to splenectomy was 3 months (range 2-132). The median pre-operative platelet count was 67×10^9/L (range 20-

320×10^9/L). Sixty nine of 93 patients (75%) achieved CR and 7/93 (7%) PR. Remaining 15/93 (17%) were refractory. All refractory patients were treated with good response in 8/15 (53%) cases. Fifteen of the 76 (19.7%) responsive patients relapsed with a median time to relapse of 6 months (range 3-20). Eleven of these 15 patients (73%) were treated with a good response in 9/11 (82%) cases. Patients with good response (CR+PR) to splenectomy were previously treated with only one drug (79% *vs.*43% p=0.003) or had lower median an number of pre-splenectomy therapies (1 (range 1-3) *vs.* 2 (range 1-5), p=0.028), had a higher platelet count at the time of splenectomy (91×10^9/L *vs.* 45×10^9/L, p=0.021) and higher platelet count seven days after splenectomy (387×25^9/L *vs.* 25×10^9/L, p=0.0035) than refractory patients. Mixed (the liver and the spleen) platelet destruction site was frequent in refractory patients (4/4 (100%) *vs.* 4/29 (14%) p=0.0003). By multivariate analysis, only the number of former therapies (p=0.045) and higher peak post-splenectomy platelet count (p=0.0089) were predictive of a favorable response to splenectomy. Relapsed patients had significantly lower platelet count seven days (213×10^9/L *vs* 387×10^9/L, p=0.048) and three months (55×10^9/L *vs* 346×10^9/L, p=0.007) after splenectomy. The same parameters retained statistical significance (p=0.076, p=0.0023) by multivariate analysis.

Summary and Conclusions: Splenectomy is effective in approximately two thirds of patients with ITP. Number of pre-splenectomy treatments and the platelet count seven days after surgery were predictive of refractoriness. Platelet count seven days and three months after surgery was predictive of relapse. Further large population based studies for testing a cut-off platelet count that could predict the refractoriness or relapse are needed.

P608

THROMBOPOIETIN RECEPTOR AGONISTS AFFECTS THE LEVELS OF HEPATOCYTE GROWTH FACTOR AND TRANSFORMING GROWTH FACTORS IN IMMUNE THROMBOCYTOPENIA

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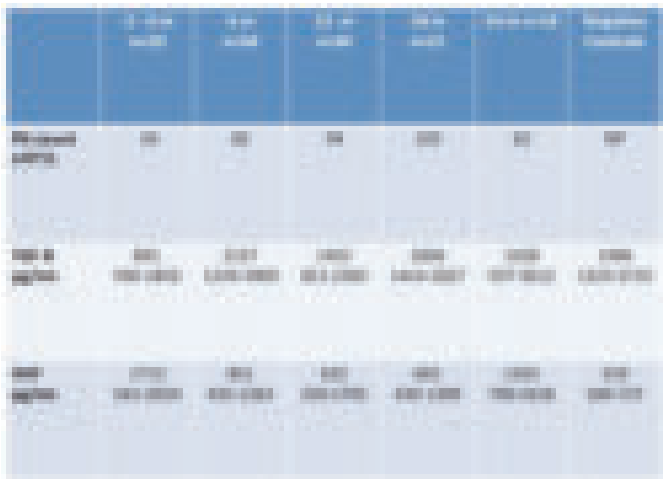
Background: Hepatocyte growth factor (HGF) is a pleotropic cytokine that plays an essential role in differentiation of hematopoietic progenitor cells. It has been shown that the administration of HGF increases platelet counts (PC) and megakaryocytes, probably through enhancement of thrombopoietin (TPO) mRNA expression in hepatocytes. Transgenic mice overexpressing HGF display elevated levels of TPO and develop thrombocytosis. Among other properties, HGF exhibits a potent anti-fibrotic property mediated through the inhibition of α-SMA–positive myofibroblast activation from interstitial fibroblasts, which antagonizes the effect of transforming growth factor B (TGF-B) through inhibition of TGF-B signaling pathway and expression. Romiplostin and eltrombopag are two thrombopoietin receptor agonists (TPO-RA) used in patients with immune thrombocytopenia (ITP) to elevate PC. These agents act by promoting megakaryocyte proliferation and differentiation. The effect of exogenous thrombopoietin (TPO) on the levels of HGF has not been studied previously.
Aims: To determine blood levels of HGF and TGF-B in consecutive samples collected from ITP patients treated with TPO-RA.
Methods: Blood samples from 48 ITP patients receiving treatment with TPO-RA at the Unit of Platelet Disorders at the New York Presbyterian Hospital, New York, USA, were retrospectively retrieved. Pre-treatment (<2 months) with TPO-RA and thereafter sequential on-treatment frozen blood samples were retrieved at 6 months intervals (+/- 1 month), whenever available and up to 24 months. These blood samples were collected during routine hospital visits. Samples were centrifuged at 1400 g for 10 min and aliquoted in appropriate tubes as EDTA plasma and stored at -80°C until assayed. Plasma samples from 16 healthy individuals were analyzed for HGF and TGF-B. These were 9 females/7 males, median age 37 years,(IQR 22-51). TGF-B was analyzed using ELISA and HGF was analyzed by an immuno-bead-based multiplex assay (R&D systems, Minneapolis, MN, USA). The protocol was approved by the Institutional Review Board of the Weill Medical College of Cornell University, NY, USA and consent was obtained from the patients.
Results: Median age of 46 patients was 50 years (IQR 20-69); 28 (58%) females. Median levels (interquartile range) of PC, HGF and TGF-B are shown in the Table 1. PC increased after treatment with TPO-RA and remained elevated over baseline levels in all measurements. Median pre-treatment HGF levels were higher than controls (p=0.0001) and higher than all of the on-treatment levels (p-values at 6, 12, 18, 24 months were 0.07, 0.1, 0.03, 0.002 respectively). Paired pre-treatment values differed significantly from values at 6, 12, 18, and 24 months with p-values of 0.027, 0.005,0.005, and 0.017 respectively. No significant correlations were found between HGF and PC. Median pre-treatment level of TGF-B was significantly lower than in controls (p=0.004), but TGF-B levels increased after initiation of TPO-RA to levels comparable to those of controls. The differences between pre- and on-treatment levels were not significant except at 6 months (p=0.012).
Summary and Conclusions: The study showed that HGF and TGF-B are dysregulated in ITP and that treatment with TPO-RA alters their levels. To our knowledge this is the first study to report on HGF levels in TPO-RA treated ITP patients – a finding that may indicate a regulatory mechanism imposed by low platelet counts to stimulate the production of TPO-RA. Median concentrations of HGF declined after administration of TPO-RA and remained lower than pre-treatment levels. This may indicate a negative regulatory mechanism exerted either by the exogenous TPO or by the platelets and/or the megakaryocytes. However, since there were not significant correlations between HGF and PC, it appears more likely that decreasing levels of HGF are attributable to exogenous TPO. This finding need to be proved and its down stream effects needs to explored using animal models or other relevant methods.

Table 1.



P609

PREOPERATIVE USE OF THROMBOPOIETIN RECEPTOR AGONISTS IN ITP PATIENTS PRIOR TO SPLENECTOMY OR CARDIOVASCULAR SURGERY

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Background: Recently, two thrombopoietin (TPO) receptor agonists (TPO-RA) have been introduced in the treatment of immune thrombocytopenia (ITP): eltrombopag (ETP) and romiplostim (RPL). In Europe they are approved for ITP patients as third-line treatment after splenectomy or second-line treatment when surgery is contraindicated.
Aims: To collect data on the preoperative use of TPO-RA before splenectomy or other surgical procedures in ITP patients.
Methods: A retrospective observational study was conducted by five hematology centers on ITP patients who had received TPO-RA as preoperative procedure prior to splenectomy or other elective surgical interventions.
Twelve patients with chronic ITP (M/F 8/12, median age 39 yrs., range 14-75) were identified. All patients had received previous treatments (one line in 2 patients, two in 4 patients, three in 4 patients, >three in 2 patients). The planned surgical intervention was splenectomy in 9 cases, uterine polipectomy in one, endarterectomy in one, and aortic valve and aortic arch prosthetic substitution in one. The median platelet count prior to starting TPO-RA was 9x10^9/L (range 2-36). Eight patients received RPL and four ETP; one of the latter had first received RPL, which was switched to ETP because of lack of response. Seven patients received concomitant treatment with steroids. The final dose of TPO-RA was between 2 and 8 µgr/kg /week for RPL and 50 or 75 mg /day for ETP.
Results: In 9 patients surgery was carried out after median 3 months from starting treatment (range 1-9); in one remaining patient splenectomy was posposed after 4 years on RPL. The platelet count at the time of surgery was >100x10^9/L (range 102-398) in 8 patients, 75x10^9/L in one, and 49x10^9/L in one. In one patient ETP was withdrawn after 7 weeks because of lack of response and splenectomy was successively carried out using steroids and high-dose immunoglobulins (HD-Ig); no other patient received preoperative HD-Ig. Finally, splenectomy was renounced in a 14-y.o. patient who had underwent 3 previous lines of treatment and after RPL achieved a durable response which was maintained after withdrawal of the drug. Splenectomy induced a complete response in 7 patients (median platelet count after 2 weeks from the intervention 550x10^9/L, range 138-747) and had no effect in one (platelet count 49x10^9/L). No bleeding complication was recorded in any patient. TPO-RA were withdrawn after splenectomy and polipectomy; RPL was prolonged as indefinite treatment in the two patients who underwent cardiovascular surgery

and received anti-vitamin K or antiplatelet agents. One 34-y.o. patient had pulmonary embolism after laparoscopic splenectomy carried out after RPL administration. The platelet count at the time of intervention and one and two weeks after was 75x10^9/L, 117x10^9/L, and 653x10^9/L, respectively; no antithrombic prophylaxis had been given.
Summary and Conclusions: The preoperative use of TPO-RA is effective to achieve a safe platelet count before surgery, and is of special value in those patients undergoing cardiovascular surgery with need of long-term antithrombotic treatment. The occurrence of one venous thromboembolic event in this small series is however cause of concern about safety of TPO-RA in this setting.

P610

A SINGLE CENTRE RETROSPECTIVE ANALYSIS OF 57 A SINGLE CENTRE RETROSPECTIVE ANALYSIS OF 57 CONSECUTIVE LAPAROSCOPIC SPLENECTOMIES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background: Immune thrombocytopenic purpura (ITP) is a common autoimmune disease characterized by autoantibody destruction of platelets, impaired platelet production and altered cellular immunity. It can be primary if it presents alone or secondary, if it takes place in setting of other conditions (HIV, cancer, other autoimmune disorders, hepatitis viruses).
Systemic corticosteroids remain the first line treatment. After failure or dependence to corticosteroids splenectomy has been the best therapeutic option for many years. With the recent emergence of thrombopoietin analogues the role of splenectomy has been questioned, mainly in fragile patients.
Aims: Our primary aim was to analyze the ITP relapse free survival after splenectomy and to describe the early and late complications in the population of study. A secondary objective was to identify variables associated with a higher risk of ITP relapse.
Methods: We retrospectively identified 57 consecutive laparoscopic splenectomies performed in patients with ITP in our center from 1998 to 2013.
Patient's baseline characteristics are resumed in Table 1.

Table 1.

Corticosteroid dependence was defined as the inability to stop corticosteroids without presenting platelets counts inferior to 30x10^9/L. Corticosteroid resistance was defined as the lack of response in the platelets counts. Descriptive and frequencies analyses were performed. We estimated the probability of staging free of relapse after splenectomy (RFS) using the Kaplan Meier analysis. Clinically relevant variables (age, sex, time to splenectomy, resistance to corticosteroids, and platelet count after splenectomy) were analyzed in a univariate analysis. With those variables which showed statistical significance in the univariate analysis we performed a multivariate analysis using Cox's regression.
Results: The laparoscopic splenectomy was successful in 51 cases (89%). The platelet count performed in the following 12 hours after splenectomy was analyzed in 46 cases; it was less than 100x10^9/L in 17% and greater than or equal to 100x10^9/L in 83% of patients. The post surgical complications during admission were mild bleeding in 3 (43%) patients, abscess in 3 (43%) patients and severe bleeding in one patient, the last one (14%) resulting in death. There were 6 complications after 6 months of surgery: 5 (83%)were infectious (3 community acquired pneumonia, 1 herpes zoster and 1 CMV pneumonitis) and 1 (17%) was related to surgery (chronic abdominal pain). The median time to relapse was 47 months (range 0 - 180). The RFS at 180 months of follow up is 68.4% (IC 95% 57.6 – 79.9). When analyzing the RFS by gender, age (less than 60 years old *vs* more than or equal to 60 years old), platelet count after splenectomy (less than 100x10^9/L *vs* greater than or equal to 100x10^9/L), time to splenectomy, and corticosteroids dependence/resistance we found that only platelet count after splenectomy and age showed statistically significant differences in the univariate analysis. The RFS in patients with less than 100x10^9/L platelets was 46.9% *vs* 68.4% in those with more or equal to 100x10^9/L ($p<0.05$). For those patients older than 60 years the RFS was 53.3% and it was 87.7% in the younger group ($p<0.05$). In the multivariate analysis using Cox regression the variables included were age, platelet count after splenectomy and also median time to splenectomy because there are some reports indicating that it may be relevant. We only found statistical significance in platelet count after splenectomy ($p<0.05$) with a hazard ratio of 0.34 (IC 95% 0.08-1.5).
Summary and Conclusions: Laparoscopic splenectomy provides a long lasting remission time in patients with corticosteroid resistance/dependence ITP and remains an excellent option with low rate of complications. Age should be taken into account before splenectomy. The most predictive data of relapse is the platelet count after surgery. We did not find that the time to splenectomy from diagnosis of ITP had an impact in the outcome.

P611

THE TREATMENT WITH ELTROMBOPAG OF THE REFRACTORY ITP

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Background: Refractory ITPs are the cases in which ITP is initially responsive to first line therapy (Prednisone, Dexamethasone – high dose, IVIG) or second line therapy (splenectomy, Danazol, VKR, CVP treatment) but which decay because of the inefficiency of the former therapies.
Aims: The demonstration of the beneficial effect of Eltrombopag 50 mg/day, administered in due course in 18 cases of refractory ITP, diagnosed in the Clinic of Hematology from Craiova, Romania, in between January 2009 – January 2013 (48 months).
Methods: Of a total number of 156 patients diagnosed with ITP in our Clinic, only 18 fulfilled the criteria for refractory ITP. The median age of the patients was of 42 years and the sex ration M/F was of 5 to 13. All of the cases were treated with the same dose of Eltrombopag (50 mg/day, number of thrombocytes ≥90000/mm^3, without the reduction of this dose in the event of obtaining a favourable or complete response). We did not raise the dose to 75 mg/day in the only non-responder patient from the group, taking into account the former therapies (4-6 type of treatment), the complete remission length (in months), the period of time from the initial diagnosis to the point in which the cases were considered refractory ITP, the interval in which complete remission appeared from the start of treatment with Eltrombopag, the type of response (complete, with the normalisation of the number of thrombocytes, or favourable, with a number of thrombocytes ≥90000/mm^3). Only 50% of the patients relapsed after splenectomy (9 patients) and the other half refused the surgical intervention in fear of the comorbidities. The patients were followed-up monthly, observing their clinical state and eventual side effects, and curves which evaluated the evolution of the number of thrombocytes were drawn.
Results: Of the 18 patients with refractory ITP, 33% previously received four types of therapy, whereas 67% five or six types of treatment. The length of complete remission varied between 11 and 34 months. After 11-34 months, the diagnosis was of refractory ITP and the treatment with Eltrombopag was of 50 mg/day, doses which were not adjusted, nor discontinued. Of the 18 patients, 17 achieved complete responses, with the normalisation of the number of thrombocytes (66% - complete remission, with a number of thrombocytes=156000-225000/mm^3, or efficient responses with a number of thrombocytes=90000-133000/mm^3). The period of time until the complete or efficient response was achieved was between one to 24 months. A single patient was a non-responder to Eltrombopag after the continuation of therapy for six months. None of the patients received Eltrombopag 75 mg/day as specified on their informed consent.
Summary and Conclusions: Although expensive, the treatment with Eltrombopag was extremely efficient in 17 of the 18 cases of refractory ITP and had minimal side effects (dizziness – 3 cases, reversible introversion – 7 cases). After the treatment with Eltrombopag, the patients maintain the initial quality of the response: complete (21-47 months of treatment) or efficient (11-39 months of treatment).

P612

THROMBOPOIETIC AGENTS INCREASE PLATELET COUNTS MORE EFFECTIVELY IN ITP PATIENTS WITH HIGHER PLATELET PRODUCTION PRIOR TO TREATMENT

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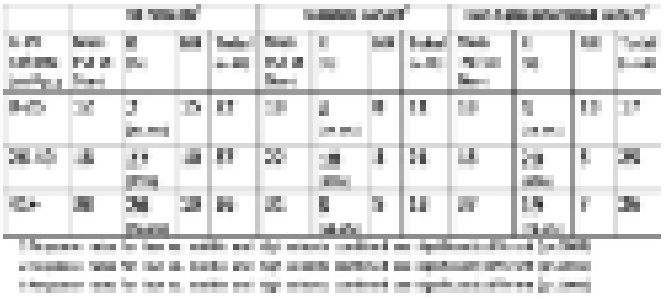
Background: Thrombopoietin Receptor Agonists (TPO Agents) increase platelet counts in ITP patients by stimulating platelet production. Why the majority but not all patients respond to these agents is unknown. The Immature

Platelet Fraction (IPF), a marker of Reticulated, newly-produced Platelets (RPs), is a good indicator of a patient's platelet production abilities.
Aims: The aim of this study was to explore whether a patient's ability to produce new platelets was associated with his/her platelet response to TPO Agents.
Methods: Ninety-five ITP patients treated with a single TPO-A were retrospectively analyzed: 16 were treated with E5501, 21 with romiplostim and 55 with eltrombopag. Forty-four patients were male and 51 female, with a median age of 22 years (range 2-81) and a median duration of ITP before TPO treatment of 4 years (range 0-38). Absolute IPF (A-IPF) values and platelet counts were measured using a Sysmex XE-2100 machine. Platelet and A-IPF counts were collected and organized by month, and the median count from each month was recorded for each of 7 relevant time periods: Pre-Treatment, 1, 2,3,4,5 and 6 months. Patients were deemed platelet count responders if the average of their platelet counts doubled and was higher than $50x10^9$/L. To distinguish patients by their platelet production capabilities, subjects were stratified into three cohorts based on their A-IPF values prior to treatment: 0-25 (low), 26-40 (middle) and 41+ (high). Fisher's exact tests were used to analyze differences in the distribution of variables among patient subsets.
Results: Response rates for the low, medium, and high A-IPF cohorts were, 7/22 (23.5%), 27/37 (73%) and 26/36 (72.2%), respectively (Table). Response rates between the three A-IPF cohorts were significantly different (p=.003). Patients from the middle A-IPF cohort (p=.0028) and patients from the high A-IPF cohort (p=.0056) each had a significantly higher response rate than did patients from the low cohort. Patients from the high and middle cohorts did not have significantly different response rates (p=.794). Among the 68 non-splenectomized patients, response rates between the three A-IPF cohorts were also significantly different (p=.0006); patients with higher A-IPF's had significantly greater response rates (Table). Response rates for the low, medium, and high cohorts were, 4/17 (23.5%), 20/25 (80%) and 19/26 (73.1%), respectively. Similarly in the 45 pediatric patients, response rates between the three A-IPF cohorts were significantly different (p=.0085); patients with higher A-IPF's had significantly greater response rates (Table). Response rates for the low, medium, and high cohorts were, 3/11 (27.3%), 18/21 (86%) and 8/13(61.5%), respectively (Table 1). Age, sex and splenectomy are not significantly related to TPO response. Response rates were almost identical for pediatric (<21yo) *vs.* adult patients, females *vs.* males, and splenectomized *vs.* non-splenectomized patients. Duration of ITP may predict TPO response. Response rates were significantly different (p=.0396) when stratifying patients into three cohorts: 0-1.5, 1.5-3, and 3+ years. Among these groups there were 9/20 (45%), 18/22 (81.8%), 33/53 (62.3%) responders, respectively. Median A-IPF each cohort was between 36 and 38.

Table 1.



Summary and Conclusions: High A-IPF in ITP, the baseline ability to produce platelets, may be useful in predicting the efficacy of TPO-A on an individual basis. Further study of the mechanism of this phenomenon may reveal novel therapeutic approaches for difficult patients with ITP.

P613

ELTROMBOPAG GIVEN 2 TIMES A WEEK AS MAINTENANCE THERAPY IN RESPONDING PATIENTS AFTER AN INDUCTION STANDARD TREATMENT OF 50 MG ORALLY ONCE DAILY IS EQUALLY EFFECTIVE AND SAFE IN ITP PATIENTS.

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Background: Eltrombopag is an oral, non-peptide thrombopoietin receptor agonist that has shown efficacy and safety in chronic immune thrombocytopenia (ITP).) patients not responding to previous therapy.However, when eltrombopag is discontinued, platelet counts usually return to baseline within 2 weeks. As reported in different case series only 15% of patients achieve a durable response after treatment discontinuation.
Aims: To evaluate the efficacy and safety of eltrombopag given at a dose of 25 mg every 3 days as maintenance therapy in responding patients after an initial standard dose of 50 mg orally once daily.
Results: A total of 8 consecutive adult patients, female (50%),median (range) age 50 (24-71) years, median (range) baseline platelet count 19 (3-32)$x10^9$/L.,median of 3 (2-4) prior ITP therapies,received eltrombopag with 50 mg once daily starting dose to maintain platelet counts in the target range of 50-150$x10^9$/L.. The median time since ITP diagnosis was 3 years (range, 1-4 years) and none of the patients had undergone a splenectomy. The efficacy of oral eltrombopag was assessed after 6 weeks administration. All patients achieved a platelet count ≥$50x10^9$/L. and 6 out of 8 pts achieved CR (defined as platelet count ≥100 * $10^{\wedge 9}$/L measured on two occasions >7 days apart and the absence of bleeding). In 4 out of 8 patients occurred more dose adjustments and treatment interruptions to maintain platelet counts in the target range Moreover it was not possible, in this subset, to stop the treatment for a relapse within the first three weeks of discontinuation. For all these reasons a maintenance dose of 25 mg every 3 days starting on the ninth month. The median (range) follow-up of this case series was of 7 months (6-20), during which all patients maintained a safe platelet count without the need to increase the dose or frequency of administration. No adverse events were observed.
Summary and Conclusions: Maintenance therapy was manageable and well-tolerated. Maintenance can allow for improved health care resource allocation providing cost savings and potentially improves patients' quality of life by reducing the time required for laboratory tests, medical examinations togheter with a total adhesion to the treatment and may be an effective alternative for patients who can not discontinue the drug

P614

ROMIPLOSTIM: EFFICACY AND SAFETY IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP). SINGLE CENTRE EXPERIENCE

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Background: Use of thrombopoietin receptor agonists (TPO-RAs) is based on the current knowledge of the ITP pathophysiology. Long-term administration of TPO-RAs results in improved treatment outcomes for patients with chronic ITP (cITP).
Aims: To evaluate efficacy and safety of romiplostim (R) used as a second- and third- line treatment in patients with cITP who are resistant to prior therapy.
Methods: 45 patients (40 female, 5 male) were treated with R. Median age was 48 years (range, 22-74). The mean duration of ITP was 8.5 years (1.5-47). One line of previous therapy (corticosteroids) received 51% patients (23/45), two and more lines of therapy received 49% patients (22/45), 6 of those underwent splenectomy. Second- and subsequent lines of therapy included intravenous immunoglobulin, mycophenolate, rituximab, cyclophosphamide, vincristine and eltrombopag. 14 patients continued to receive concomitant ITP medications at the start of treatment with R. The mean baseline platelet count was 17 ´ 10^9/ L (range,1-48 ´ 10^9/L).The hemorrhagic syndrome mainly presented as cutaneous manifestations with ecchymosis and petechiasis observed in 95% of patients. Additional hemorrhagic events included epistaxis and gingival bleedings (45% of patients),menorrhagia (30%),macrohematuria (2%) and hemoptysis (2%).The initial dose of R was 1 mкg/kg in 34 patients, 2 mkg/kg in 7 and 3 mкg/kg in 4, the maximum dose of R amounting to 10 mкg/kg. The therapy response was defined as platelet count >50 ´ 10^9/L and resolution of hemorrhage.
Results: Mean therapy duration was 47 weeks (range, 5-173).The median dose of R was 4.5 mkg/kg/week. 88% of patients (40/45) achieved sustained response to the therapy. The target platelet count was achieved by 2 week (w2) in 16 (36%) patients, by w3 in 19 (44%) patients, by w4 in 8 (18%) and by w5 in 1 (2%) patient. Five patients did not respond to the therapy despite being administered maximum therapeutic doses of R. One patient was primary refractory (treatment for 10 weeks with no response, dose R 10 mkg/kg was used for 4 weeks of that period). In four patients the achieved response was lost, further escalation dose of R to the maximum dose (its use for 3 weeks) was ineffective and the treatment was discontinued. Concomitant ITP medications could be completely withdrawn in 13/14 of patients. Side effects were minimal and included skin rash, headache and myalgia. A thrombotic complication was seen in one patient 73-year-old female with vascular risk factors experienced a transient ischemic foot attack with local soft tissue necrosis in 59 week of treatment, platelet count was 137 ´ 10^9/L, a dose of R was 7 mкg/kg. The signs of this event were stopped 3 weeks later in the course of conservative therapy with resumption of treatment R 2 mkg/kg for the achievement of the desired clinical benefit (hemostatic effect). Three patients sustain the complete response after discontinue treatment of R without any ITP treatment (24, 5 and 4 months of duration, respectively).
Summary and Conclusions: R is a novel therapeutic option for patients with cITP that was shown to provide long-term control of hemorrhagic syndrome, rapidly increase and effectively sustain platelet counts with acceptable tolerability as well as maitain durable response in some patients after treatment withdrawal.

Bleeding disorders

P615

CORRELATION BETWEEN OXIDATIVE STRESS AND BIOMARKERS OF JOINT DAMAGE IN PATIENTS WITH SEVERE HAEMOPHILIA TREATED BY DIFFERENT PROPHYLAXIS REGIMENS

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Background: Haemophilic arthropathy is the main cause of morbidity in individuals with severe haemophilia and prevention of joint disease is the primary goal of treatment. Recurrent bleeding into joints causes damage to all joint structures, but the precise mechanism by which this induces haemophilic arthropathy is still unclear. Recently, it was shown that one effect of blood in the joint is degradation of cartilage. Biomarkers of cartilage turnover can be measured in physiological fluids, such as plasma and urine.
Aims: The aims of this study were to detect correlation between a marker of oxidative stress -advanced oxidation protein products (AOPP) and serum/urine concentrations of biomarkers of joint cartilage degradation, as well as to estimate the influence of different prophylaxis regimens for severe haemophilia on this process.
Methods: The study included 20 adult patients with severe haemophilia, manifested by plasma factor (F)VIII/IX <1% of normal, without inhibitor. Five patients with haemophilia A received prophylaxis with FVIII concentrate in the standard dose of 20 IU/kg three times per week, while another five patients with haemophilia A were given an intermediate dose of FVIII concentrate as prophylaxis, 10-15 IU/kg thrice weekly. Seven patients with haemophilia A and three with haemophilia B, received FVIII/IX concentrate only on-demand. The following were measured: a) AOPP - a serum marker of oxidative stress and b) biomarkers of joint cartilage degradation - serum cartilage oligomeric matrix protein (COMP) and urinary C-terminal telopeptide of type II collagen (CTX-II). Blood and urine samples were collected initially, before the start of treatment (labelled AOPP-1, COMP-1 and CTX-II-1) and after 3 months follow-up (labelled AOPP-2, COMP-2 and CTX-II-2).
Results: The mean age of the patients was 32 years (range 19-55). In the group of patients given standard dose prophylaxis, the mean values of AOPP-2 (p=0.018), COMP-2 (p=0.043) and CTX-II-2 (p=0.014) were significantly lower than those for AOPP-1, COMP-1 and CTX-II-2. Likewise, the mean values for AOPP-2 (p=0.047) and CTX-II-2 (p=0.028) in the five patients receiving intermediate dose prophylaxis were also decreased when compared to initial values, but COPM level was not significantly changed. In patients treated on demand the mean values for AOPP, COMP and CTX-II did not alter significantly. The results showed marked positive correlations between AOPP and both COMP and CTX-II. Namely, lower values of AOPP were significantly associated with decreased levels of both biomarkers of cartilage degradation: COMP (p=0.008) and CTX-II (p=0.014).
Summary and Conclusions: The precise mechanism of joint disease in patients with severe haemophilia remains unknown but probably involves blood-induced increase of oxidative stress, which leads to higher joint cartilage turnover. The most important clinical strategy for management of these patients and prevention of severe arthropathy is treatment by continuous prophylaxis with intravenously applied FVIII/IX.

P616

BONE MINERAL DENSITY IN MEN AND CHILDREN WITH HAEMOPHILIA A AND B: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Haemophilia is not considered among the classic causes of secondary osteoporosis.
Aims: The aim of this study was to systematically review the literature for case-control trials that have studied bone mass in males with haemophilia and to meta-analyze the best evidence available.
Methods: Electronic databases MEDLINE, EMBASE and CENTRAL were systematically searched for case-control trials that have studied bone mass in men or boys with haemophilia. Standardized mean difference (SMD) for bone mineral density (BMD) in the lumbar spine was the main study outcome and SMD in femoral neck and total hip BMD the secondary ones. Patient and control characteristics, such as age, body mass index (BMI), level of physical activity and blood-borne infections were recorded as possible predictors of the main outcome.
Results: Thirteen studies were included in the systematic review and 10 in the main outcome meta-analysis. Men with haemophilia demonstrated reduced lumbar spine [random effects SMD [95% confidence interval (CI)] -0.56 (-0.84; -0.28), between-study heterogeneity (I^2)=51%] and femoral neck BMD [random effects SMD (95% CI) -0.82 (-1.21; -0.44), I^2=63%] compared with controls, which indicated a large and clinically significant association. Similar results were obtained for children [random effects SMD (95% CI) -0.92 (-1.77; -0.07), I^2=92%]. No evidence of publication bias was detected. There was no evidence that age, BMI, level of physical activity or presence of blood-borne infections predicted lumbar spine BMD. Using the 'Common Language Effect Size' approach, the probability is about 72% that an individual without haemophilia would have higher lumbar spine BMD than an individual with haemophilia, if both individuals were chosen at random from a population.
Summary and Conclusions: This meta-analysis shows that men with haemophilia present a significant reduction in both lumbar spine and hip BMD, which appears to begin in childhood.

P617

CAN SECONDARY PROPHYLAXIS IN HAEMOPHILIA BE A CAUSE OF ISCHEMIA?

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Background: In the treatment of haemophilia, prophylactic factor is used to reduce bleeding frequency, to prevent complications and to improve quality of life. Life expectancy of patients with haemophilia improved with the advent of efficient management of bleeding attacks and widespread use of prophylactic treatment. The incidence of ischemic cardiovascular and cerebrovascular events is known to be lower compared to the general population due to the protective effect of hypocoagulable state associated with factor deficiency in haemophilia patients. The effect of factor administration during bleeding or of prophylactic treatment on ischemia development is not known. SCUBE 1, a new biochemical marker of protein structure, found in endothelial cells and platelets, is stored in α granules of platelets and released when platelets are stimulated. Levels of SCUBE 1 were found elevated in acute coronary syndrome and ischemic cerebrovascular events, making SCUBE 1 an early and potent indicator of ischemia.
Aims: The present study was designed to investigate the effect of factor treatment, SCUBE 1 levels.
Methods: Ten patients with haemophilia A and 3 with haemophilia B receiving prophylactic treatment were included into the study. Bloods samples were collected before and one hour after prophylactic administration. Additionally, during follow-ups, when bleeding episodes were observed blood samples were collected before and one hour after factor administration. In factor-treated cases, blood samples were drawn after administering the first 1000 units. Thirty healthy subjects were used as controls. Sera samples were stored at -80ºC and SCUBE 1 levels were studied using ELISA.
Results: Mean age for the patient and control groups were 35±10 and 34±7 years respectively. SCUBE 1 levels for groups were 235.4±20.3 ng/ml, 258.5±23.4 ng/ml and 235.9±16 in control, patients with bleeding before factor administration and patients before prophylaxis, respectively. SCUBE 1 levels were found to be 233.9±19.6 ng/ml and 280±29.7 ng/ml after factor administration in groups admitting for bleeding and prophylaxis, respectively. Statistically, patients admitting for bleeding had higher SCUBE 1 levels compared to controls, decreasing to that of control after factor administration ($p<0.01$). In prophylaxis group, SCUBE 1 levels of patients before factor administration were found to be comparable to that of controls, increased after factor administration ($p<0.0001$).
Summary and Conclusions: We conclude that, factor administration for prophylaxis may lead to ischemia, while administration in case of bleeding may have anti-ischemic behaviour.

P618

APPLICATION OF THE US NATIONAL INSTITUTE OF HEALTH (NIH) 2008 GUIDELINES FOR VON WILLEBRAND DISEASE IN A NATIONAL PAEDIATRIC COMPREHENSIVE CARE CENTRE.

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Background: Von Willebrand's Disease (VWD) is the most common inherited bleeding condition, affecting males and females in approximately equal proportions, and occurring in up to 1% of the world populations. Clinical and laboratory evaluation for VWD is relatively complex and there isno single laboratory test that can screen for the presence of VWD. The 2008 NHLBI/NIH guidelines on "The Diagnosis, Evaluation and Management of von Willebrand Disease" suggest an algorithmic approach to the clinical and laboratory diagnosis of VWD. These guidelines proposed more stringent diagnostic criteria to replace the 1995 and 2004 UKHCDO guidelines.

Aims: The aim of this project was to reclassify the patient cohort at Our Lady's Children's Hospital Crumlin, Dublin, Ireland (OLCHC) for Von Willebrand disease (VWD). OLCHC is the National Paediatric Comprehensive Care Centre.
Methods: Case records of children under 18 years of age with VWD or possible VWD were retrospectively extracted from the Irish National Bleeding Disorder database. These records were then analysed according to the NHLBI/NIH diagnostic criteria. The algorithm applied was; If VWF levels <30 IU/dl on two separate occasions - VWD; If VWF levels 30-50 IU/dl on two separate occasions - 'Low VWF levels'; If VWF not less than 50 IU/dl on two occasions and multiple testing - not VWD. Where insufficient laboratory data were available patients were recalled to a review clinic, tests for FBC, Coagulation screen, and VW screen were performed. Multimer and gene mutation analysis were performed on patients with possible diagnosis of Type 2 and Type 3 VWD. If patients did not meet the NIH guidelines but still had a significant bleeding history, haemostatic investigations such as factor assays and platelet function tests were performed.
Results: 217 children had been historically diagnosed with VWD or possible VWD. Following the review there was a 72% reduction in the number of patients diagnosed with Type 1 VWD. Predictably, no significant change in numbers diagnosed with Type 2 and Type 3 VWD. 78 (36% of total population) is now classified as Low VWF. 37 (17% of total population) were deemed to have no form of VWD and reclassified as normal. Not all patients (36 (17%)) have returned for reclassification so remain as unspecified or possible VWD (Figure 1).

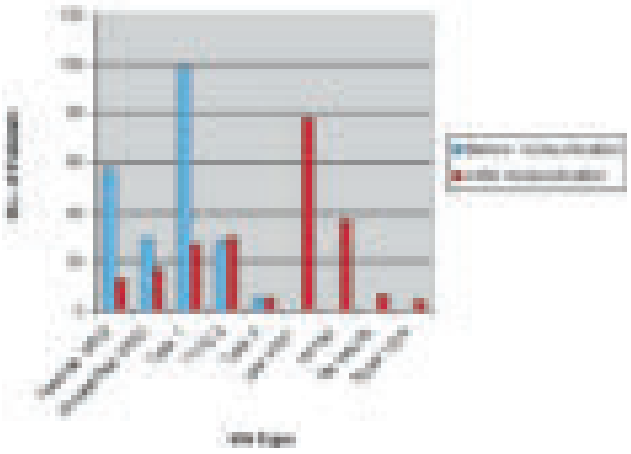

Figure 1. VWD classification.

Summary and Conclusions: Our data strongly suggest overdiagnosis of VWD in this paediatric population using previous guidelines. This review has eliminated possible VWD and unspecified VWD type categories, however a number of patients have not returned for reclassification and remain with the above diagnosis. This has however implications for service resources where newly reclassified patients need to be informed and require counseling. Diagnosis, especially for individuals with mildly decreased VWF (30–50% or IU/dl), requires correlation of clinical assessment (personal and family history of bleeding) and results of laboratory testing. This recommendation does not preclude the diagnosis of VWD in individuals with VWF:RCo of 30–50 IU/dl if there is supporting clinical and/or family evidence for VWD. This recommendation also does not preclude the use of agents to increase VWF levels in those who have VWF:RCo of 30-50 IU/dl and may be at risk for bleeding.

P619

VON WILLEBRAND'S DISEASE AND PLATELET FUNCTION DISORDERS IN WOMEN WITH MENORRHAGIA

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Background: Menstruation disorders are common clinical problems in fertile women and menorrhagia is most common of them. Von Willebrand disease (VWD) is the most common clinical entity among inherited coagulation disorders causing menorrhagia.
Aims: In this study, our aim was to determine the frequency of platelet function disorders and VWD in women referred to gynecology clinics in our province with menorrhagia and no proved local gynecologic pathology.
Methods: Study was performed with 90 voluntary female patients. Menorrhagia was determined with PBAC scoring method. Patients with 100 or more PBAC scores were included in the study. Complete blood count, peripheral smear for evaluating platelet count and morphology, blood group, PT, APTT, fibrinogen, FVIII and FIX levels, VWF: Ag level, ristocetin cofactor activity and platelet aggregation tests with ADP, collagen, ristocetin and epinephrine had been done in study population.
Results: In 90 patients included in the study with menorrhagia; 13.3% (12/90) VWD, 26.7% (24/90) platelet function disorders and 4.4%(4/90) moderate factor VIII deficiency had been determined. No pathology had been detected in 55.6% (50/90). Amongst platelet function disorders, Glanzmann's thrombasthenia in one (4.1%) patients and Bernard–Soulier disease in one (4.1%) patients. In 22 (91.6%) patients, the platelet function defects could not be classified into specific groups. Impaired aggregation response to ristocetine was the most common disorder 40.9% (9/22) among unclassified platelet disorders, whereas decreased response to both ristocetine and ADP was the second most common with a rate of 22.7% (5/22). In the third place, diminished aggregation response to collagen and epinephrine was determined at a rate of 13.6% (3/22). In addition, impaired aggregation responses had been detected in 2 patients (9%) to ADP, in 1 patient (4.5%) to collagen and ADP, in 1 patient (4.5%) to epinephrine and collagen and in 1 patient (4.5%) to ristocetine and epinephrine. In the comparison of menstrual bleeding characteristics and history of bleeding frequency of patients with a diagnosis of VWD and patients without a bleeding disorder, patients with VWD had significantly increased number of used pads during one menstruation cycle (p=0.006), the frequency of changing pads (p=0.031), and PBAC scores (p<0.001). In the comparison of the laboratory data of patients with VWH and platelet function disorder and those without bleeding disorder, there were no significant differences between the groups in terms of Hb, Htc, MCV, PLT, PT and fibrinogen levels, whereas APTT was significantly longer in the VWH group (p=0,01), and FVIII, FIX and VWF:Ag levels were significantly lower in the VWH (respectively; p=0.024, p=0.028, p=0.03).
Summary and Conclusions: In this study, it was emphasized that unrecognized hereditary bleeding disorders could be found in heavy and / or prolonged menstruation and screening of those disorders was absolutely necessary.

P620

MONITORING OF SUBSTITUTION THERAPY WITH RECOMBINANT FACTOR XIII OF A PATIENT WITH SEVERE FACTOR XIII A SUBUNIT DEFICIENCY

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Background: Coagulation factor XIII (FXIII), a tetrameric zymogen (FXIII-A_2B_2), consists of two catalytic A and two inhibitory/carrier B subunits. It is activated by thrombin and Ca^{2+} in the final phase of coagulation cascade. Activated FXIII (FXIIIa) is a transglutaminase which crosslinks peptide chains through ε(γ-glutamyl)lysyl bonds. The main task of FXIIIa is to cross-link fibrin chains and α_2-plasmin inhibitor to fibrin to make fibrin clot more resistant against the shear stress of circulating blood and to provide protection against fibrinolysis. FXIII-A deficiency is a rare (1:1-3 millions) but severe bleeding diathesis and due to the high risk of intracranial bleeding it needs life-long prophylactic substitution, preferably by FXIII concentrate. Beside plasma derived FXIII-A_2B_2 concentrate, most recently recombinant FXIII-A_2 (rFXIII) (Novo Nordisk, Denmark) has also been approved for prophylaxis. rFXIII needs the patient's own FXIII-B to remain in the plasma with a half-life of 8-13 days. A 19-year-old male with long history of bleeding complications was admitted to the clinic with intramuscular hematoma. After diagnosis of FXIII-A deficiency he was administered 35 U/kg rFXIII and later he was put on prophylactic therapy.
Aims: To give a complete laboratory diagnosis of FXIII deficiency and to monitor the changes in FXIII activity, FXIII-A_2B_2, FXIII-A and total plus free FXIII-B antigen in the plasma following the administration of rFXIII. The possible appearance of anti-FXIII-A alloantibody was also investigated.
Methods: FXIII activity was measured by the ammonia release method using blank subtration (REA-Clot FXIII, Reanal-ker, Budapest). FXIII antigens were determined by highly sensitive ELISA methods developed and published by our laboratory. The genetic defect in the FXIII-A gene was detected by fluorescent DNA sequencing. The presence of alloantibody was tested in mixing study.
Results: At admission the patient had normal hemostasis screening tests and platelet count. von Willebrand disease was excluded. FXIII activity was <1%, FXIII-A_2B_2 and FXIII-A antigen were <0.1%. Total FXIII-B was 54%. His platelets were exempt of FXIII activity and FXIII-A antigen. The diagnosis of FXIII-A deficiency was confirmed by genetic testing. The patient was compound heterozygote possessing the missense and nonsense mutations, c.1149G>C, p.Arg382Ser and c.1201C>T, p.Gln400X. One hour following the administration of 35 U/kg rFXIII, FXIII activity, FXIII-A_2B_2 and FXIII-A antigen levels jumped to 70% and did not change significantly in the next 5 hours. In the circulation of our patient the half-life of prophylactic rFXIII was approximately 8 days. After 4 weeks the mean (n=3) pre-dose FXIII activity was 4.5%. FXIII-A_2B_2 and FXIII-A antigen levels were comparable. Free FXIII-B level dropped by 62% one hour after the administration of rFXIII but returned to pre-dose level within three days. Interestingly, total FXIII-B started to raise within one hour after the administration of rFXIII-A and it peaked on days 3-6 and did not decrease to the pretreatment level even on day 28. The patient during 4 months of treatment was exempt of bleeding symptoms in spite of tooth extractions.

Summary and Conclusions: rFXIII treatment resulting in a pre-dose level above 4% provided an efficient safe prophylaxis for a patient with severe FXIII-A deficiency. The patient's own FXIII-B was effectively utilized for complex formation and the administration of rFXIII-A induced the elevation of FXIII-B plasma level.

P621

OPTIMAL MANAGEMENT OF A RARE COAGULATION DISORDER: FACTOR V DEFICIENCY

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Background: Congenital factor V deficiency is a very rare coagulation disorder, with an estimated prevalence of 1/1,000,000. In literature, Most of the reported cases are characterized by a mild to moderate bleeding phenotype.
Aims: To describe the initial presentations, clinical course and management of congenital factor V deficiency cases in Oman.
Methods: This is a prospective case series study.
Results: Two infants presented with intracranial hemorrhage at the ages of 4 weeks and 10 days respectively. The first baby was found to have marked prolongation of both prothrombin time (34 seconds) and partial thromboplastin time (175 seconds), correctable with mixing study. Factor assay revealed severe congenital factor V deficiency. Fresh frozen plasma (FFP) was given repeatedly and he underwent neurosurgical evacuation of his parieto-temporal hematoma. Unfortunately, the second baby was misdiagnosed as late hemorrhagic disease of newborn in a peripheral hospital. She sustained recurrent intracranial bleeds which negatively impacted her neurodevelopment. This baby was referred to our hospital at the age of 3 months and diagnosed with severe factor V deficiency. The sibling of the 1st baby was delivered by an elective cesarean section in our centre, screened at day 3 of life and diagnosed with severe factor V deficiency as well. Our fourth patient has moderate factor V deficiency, manifesting with infrequent musculoskeletal bleeds that require on-demand FFP. All data are displayed in Table 1. Despite the lack of internationally-agreed guidelines, the three babies with severe congenital factor V deficiency were started on regular prophylaxis. To reduce the risk of blood borne infections, we opted to use solvent/detergent (S/D)-treated plasma twice weekly; through a port-a-cath.

Table 1. Clinical characteristics and management of factor V deficiency cases.

Summary and Conclusions: Early recognition and optimal management of rare coagulation disorders are major determinants of prognosis. Compared to on-demand therapy, life-long prophylaxis in severe coagulation disorders is the standard of care. However, many difficulties exist including lack of commercially available purified products, risk of viral transmission and difficult venous access. We recommend establishing a national registry of rare coagulation disorders in collaboration with the international registry to identify the prevalence, genotypes, severity, clinical profile and therapeutic options of such rare conditions.

P622

THE CLINICOLABORATORY CHARACTERISTICS AND TREATMENT OUTCOMES OF 10 KOREAN PATIENTS WITH FACTOR V DEFICIENCY

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Background: Factor V (FV) deficiency is a rare bleeding disorder associated with a spectrum of haemorrhagic symptoms, ranging from mucosa and soft tissue bleedings such as epistaxis and hemarthroses to life-threatening haemorrhages. Until now, more than 200 cases of FV deficiency have been described in literature and occurred approximately 10 times more frequently in Western Asia where consanguineous marriage are common.
Aims: We retrospectively analysed the clinicolaboratory features and treatment outcomes of FV deficiency in 10 Korean patients.
Methods: Between January 1987 and December 2012, nine case reports published in Korean journal or presented at Korea Society on Thrombosis and Hemostasis (KSTH) and one medical record in our institution were reviewed.
Results: The median age of all patients at diagnosis, 6 males and 4 females, was 26 years (range, 1 month - 73 years). Based on population size of 45 million, the estimated incidence of FV deficiency in Korea is 0.01 cases per million person years. Seven of 10 patients (70%) are classified as severe FV deficiency characterized by FV levels below 5% and, 1 (10%) as moderate FV deficiency, showing FV levels 5 ~ 10%. Of all patients with median levels of plasma FV of 3.5% (range, 1% to 16%), 80% was diagnosed as inherited FV deficiency, and 30% had a positive family history. One patient had combined FV and factor VIII deficiency, especially. The most frequent clinical features were mucosal tract bleedings (40%) such as epistaxis, oral cavity haemorrhages, and menorrhagia in female. Hemarthroses and postoperative bleeding occurred in 1 and 4 of affected patients, respectively. In 4 of 7 patients with severe FV deficiency, life-threatening bleeding episodes were occurred in peritoneal cavity (2 cases), central nerve system (1 case), and retroperitoneal space (1 case), respectively. No lethal haemorrhages happened to patients with mild or moderate FV deficiency. Majority of bleeding episodes were controlled with local measures, administration of antifibrinolytic drugs, and fresh-frozen plasma (FFP) replacement, and in 2 cases of severe bleeding not controlled with FFP replacement, the immunosuppressive therapy with corticosteroids and/or cyclophosphamide was done. One of them with acquired FV inhibitors confirmed by the Bethesda method died from postoperative bleeding complications.
Summary and Conclusions: Compared with a few countries in Western Asia, this present study shows that similar bleeding manifestations were noted in all patients with FV deficiency in Korea. However, fatal bleeding events had tendency to occur more frequently in Korean patients with severe FV deficiency.

P623

DETECTION OF ANTI-THROMBOPOIETIN AUTOANTIBODY AMONG IMMUNE THROMBOCYTOPENIC PATIENTS

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder, manifesting isolated thrombocytopenia, caused by autoantibody-mediated platelet destruction and impaired platelet production. Plasma levels of thrombopoitin (TPO) are normal or slightly elevated in ITP despite the low platelet count. However, it has not been cleared what keeps plasma TPO concentration relatively low in ITP.
Aims: In this study, we verified a hypothesis that anti-TPO antibodies are possibly involved in the pathophysiology of ITP by suppressing TPO level.
Methods: Hundred four ITP patients and 69 healthy individuals were enrolled in this study. Plasama samples were examined to detect anti-TPO antibodies by ELISA. Next we refined IgG from 14 samples (6 cases of anti-TPO (+), 4 cases of anti-TPO (-), and 4 healthy controls). We added each IgG fraction to TPO-dependent human leukemia cell line UT-7/TPO to culture in the presence of human recombinant TPO, and examined the effect on the cell growth.
Results: In eight of 104 ITP patients (7.7%) anti-TPO autoantibody was detected, and the plasma TPO levels (32.2±30.2 *vs* 59.9±67.5 pg/mL) and platelet counts (28±22 *vs* 64±47 x10^9/L) seemed to be relatively lower in anti-TPO(+) patients than the others. Next refined plasma IgG fraction from three of six anti-TPO(+) patients significantly inhibited the growth of UT-7/TPO, which is presumably due to neutralization of TPO by anti-TPO antibodies. In contrast, such an inhibitory effect was never observed among either anti-TPO(-) patients or healthy individuals. Additionally we analyzed the clinical features of ITP patients with anti-TPO antibody. Seven of eight anti-TPO(+) cases (87.5%) were positive for anti-nuclear antibodies (ANAs) in contrast to only 44 of 96 anti-TPO(-) cases (45.8%) (Table 1).

Table 1. Clinical features of anti-TPO antibody (+) ITP patients.

Case	1	2	3	4	5	6	7	8
Age	24	77	51	83	42	20	53	39
Male/Female	M	F	F	M	F	M	M	F
PLT (x10^9/L)	4	36	59	9	120	330	71	262
TPO plasma (pg/mL)	25.4	10.3	5.17	186.7	80.5	6.04	95.6	101.5
ANAs	+	+	+	+	+	+	-	+
aPL	nt	nt	-	+	+	+	nt	+
Growth of UT-7/TPO	N	N	I	N	I	nt	nt	I

ANAs, anti-nuclear antibodies; aPL, anti-phospholipid antibodies; nt, not tested; I, inhibited; N, Not inhibited.

Summary and Conclusions: Although some knowledge has been accumulated about pathophysiology of ITP, the diagnosis is still a process of exclusion,

and the etiology is heterogeneous, which can result in a variety of therapeutic responses. Our results demonstrated that anti-TPO antibodies can be detected in a proportion of ITP patients. Although the number is limited, anti-TPO(+) patients seemed to show relatively lower platelet counts and TPO levels, and to be associated with other autoantibodies, such as ANAs and antiphospholipid antibodies. These suggest that anti-TPO antibody could be involved in the pathophysiology of ITP at least in a certain number of patients, and might affect therapeutic response to TPO receptor agonists. Accumulation of cases is required to elucidate the significance of anti-TPO antibody in pathophysiology and clinical features of ITP.

P624

HOMOZYGOUS PROTEIN C DEFICIENCY: A DESCRIPTION OF TWO NEW CASES AND LONG-TERM SURVIVAL OF A THIRD CASE SUCCESSFULLY TREATED WITH ORAL ANTICOAGULANT THERAPY, A REPORT FROM SAUDI ARABIA

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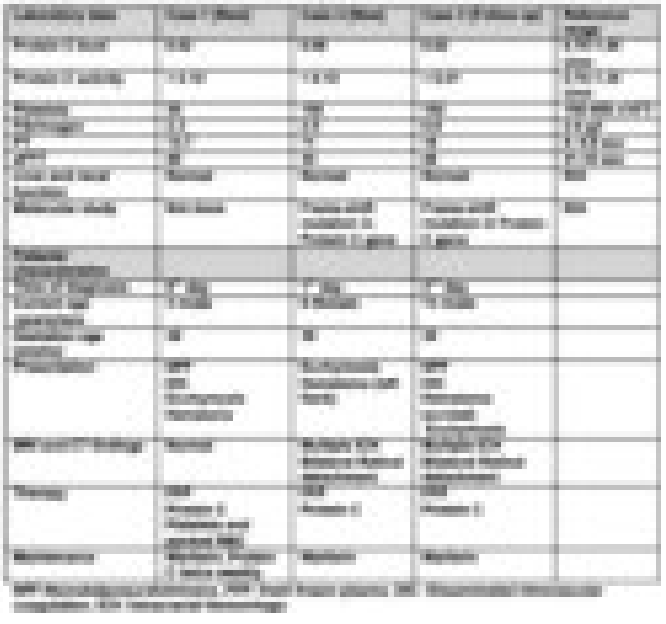
Background: Homozygous protein C deficiency is a rare autosomal recessive disorder that usually presents in the neonatal period with purpura fulminans (PF) and severe disseminated intravascular coagulation (DIC), often with concomitant venous thromboembolism. Mutational analysis of symptomatic patients shows a wide range of genetic mutations.
Aims: We report here a quite rare 3 cases of severe homozygous protein C deficiency, two new cases and a Long-term survival of a third case successfully treated with Warfarin therapy.
Methods: Patients: Three cases from 2 families (case 2and 3 are from same family while case 1 from different family), Informed consent was obtained from parents. Protein C levels and activity: Plasma Protein C antigen level was measured by enzyme immunoassay while the protein C activity was measured by using chromogenic assay. PCR and sequencing: DNA was extracted from the patients and parents blood samples. Protein C gene was amplified by PCR and sequencing was carried out on the amplified PCR products and the samples were run on the DNA analyzer and data were analyzed.
Results: The patients and parents characteristics at diagnosis were summarized on Table 1. Case 1: A 3 year-old boy that developed PF and DIC shortly after birth. He was born after an uneventful pregnancy, no family history of thrombosis but positive for consanguinity. The father and the mother have protein C antigenic levels of 0.56 and 0.36 U/ml respectively and protein C activity levels of 0.57, 0.48 U/ml respectively (Figure 1).

Table 1. Patients characteristics and laboratory data.



Case 2: An 8 year-old female, he was a product of full term unremarkable pregnancy who developed ecchymosis and hematoma of left flank shortly after birth. No family history of thrombosis but positive for consanguinity. The father and the mother have protein C antigenic levels of 0.67 and 0.63 U/ml respectively and protein C activity levels of 0.69, 0.66 U/ml respectively. Case 3: A 13 year-old boy (brother of case 2) that developed PF and DIC shortly after birth. He was born after an uneventful pregnancy at 39 week by ventouse extraction due to fetal distress. Case 1 and 2 were treated during the acute phase by FFP at a dose of 15 ml/kg every 12/hours alternating with protein C concentrate, an initial dose of 100 U/kg followed by 50 U/kg every 12 hours, the treatment was continued until all lesions had resolved. Case 3 was treated by FFP at a dose of 15 ml/kg every 12/hours as a replacement therapy as protein C concentrate was not available. Due to repeated thrombotic attaches in case 1, the patient on protein C replacement therapy as a maintenance therapy (50 U/kg twice weekly) with a small dose of warfarin to maintain INR between 1.5 and 2.5. The maintenance therapy for case 2 and 3 consists of warfarin therapy alone; the dose of Warfarin therapy is adjusted to maintain a target INR between 2.5 and 3.5. Molecular analysis of case 2 and 3 and their parents revealed presence of CCTG nucleotide repeats (effective insertion), homozygous in the case 2 and 3 while heterozygous of parents which resulted in a frame-shift causing a premature stop codon at amino acid 383. Currently our cases are doing well with no neurological deficit but case 2 and 3 are blind with long-term survival on Warfarin therapy alone (8 and 13 years respectively).

Figure 1. Case 1 shows typical skin lesion of neonatal purpurafulminans.

Summary and Conclusions: Homozygous protein C deficiency state is usually not compatible with long-term survival and often fatal unless there is early recognition of the clinical symptoms, prompt diagnosis, and urgent therapy is crucial to avoid further damage after delivery.

P625

AN AUDIT TO DETERMINE THE SAFETY OF THE NOVEL ORAL ANTICOAGULANTS, DABIGATRAN AND RIVAROXABAN, FOR PATIENTS INITIATED ON ANTICOAGULANT THERAPY

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Background: In the UK, dabigatran and rivaroxaban are licensed and approved by National Institute for Health and Care Excellence (NICE) for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF) and rivaroxaban is also licensed for treatment and secondary prevention of deep vein thrombosis (DVT) and pulmonary embolism (PE), approved by NICE in 2012. However, experience amongst clinicians in the UK using novel oral anticoagulants (NOACs) remains fairly limited. Warfarin therapy was considered the first line option for these indications where the experience and knowledge of warfarin use is substantially greater. However, the advantages of these NOAC agents are: no routine monitoring, fixed dosing and no lifestyle/food restrictions. At our hospital, NOACs were prescribed from December 2011 for stroke prevention in AF and VTE treatment where warfarin was not suitable. The aim of this audit is to determine the safety of NOACs in clinical practice, focusing on adverse effects, bleeding outcomes, and management.
Aims: Determine the siginificant risks of using dabigatran and rivaroxaban in clinical practice compared with clinical trial literature and decide whether they are the new safer alternative to warfarin.
Methods: The audit team agreed the aims and standards and reviewed the literature. A pharmacy report identified patients prescribed NOACs from December 2011 to April 2013. Clinical information was accessed using patient medical notes and electronic records. A data template spreadsheet was developed to record audit data.
Results: 165 patients were initiated on NOAC therapy; 33 patients excluded. 132 patients were included in the audit. The majority of patients on rivaroxaban (n=50) were aged 71 to 90years old and on dabigatran (n=43) were aged 61 to 80years old (Table 1).
Summary and Conclusions: The NOACs have many advantages and are generally well tolerated with a good adverse effect profile. However, patients did experience bleeding events and the management is challenging with no specific antidote currently available.

Table 1.

	Rivaroxaban (n=79)	Dabigatran (n=53)
Indication for NOAC		
DVT	23	
PE	15	
DVT and PE	7	
Stroke prevention in AF	34	53
Adverse Effects		
Dyspepsia	1	3
Back Pain	1	0
Chest Pain	2	0
Fatigue	1	0
Diarrhoea	0	1
Arthralgia	1	0
Bleeding Events		
Non-Major Bleeding Event		
Epistaxis	2	0
Haemoptysis	0	1
Haematuria	1	3
Ear	0	0
Skin	1	0
Gastrointestinal related	3	2
Major Bleeding Event		
Decreased in Hamoglobin >4.0 g/dL	1	1
Transfusion of ≥3 units of red blood cells within a 24 hour period	0	0
Requiring intervention e.g. embolisation, superficial vascular repair, nasal packing	0	0
Intraspinal or intramusclar bleed wit compartment syndrome	0	0
Retroperitoneal, pericardial or intraocular bleed	0	0

Management of the major bleeding events involved a seven unit blood transfusion and Novoseven (coagulation factor VIIa recombinant) for a patient on rivaroxaban and a four unit blood transfusion for the patient on dabigatran. Both patients survived.

Quality of life

P626

COMPREHENSIVE SYMPTOM PROFILE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: PRACTICABILITY AND SENSITIVITY OF THE NEW SYMPTOM ASSESSMENT TOOL CSP LEUK-CML

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Background: Symptom profile and severity is one of the important treatment outcomes in patients with chronic myeloid leukemia (CML). Comprehensive symptom assessment before treatment as well as monitoring during treatment and at follow-up is worthwhile. Recently a new tool, Comprehensive Symptom Profile in Chronic Myeloid Leukemia (CSP Leuk-CML), has been developed to assess disease and treatment related symptoms in CML patients.
Aims: We aimed to test practicability and sensitivity of CSP- Leuk-CML in CML patients.
Methods: 75 patients with chronic phase CML resistant or intolerant to imatinib were enrolled in the study: mean age – 51.3 years old, SD 15.4; range – 22-83 years; male/female – 37/38. The patients filled out CSP Leuk-CML and generic quality of life (QoL) questionnaire SF-36 before second-line therapy, at 3 and 6 months after treatment start. Hematologists participating in the study were interviewed regarding the use of PROs in their decision-making. Analysis of symptom severity in different patient groups was performed using t-test.
Results: Practicability of CSP Leuk-CML was shown: patients needed about 15 min to answer it; the proportion of missing values was 0.56% for all questions; all items were easy for the patients to read and understand. Hematologists considered information about changes in symptom profile practical and useful for their decision-making. Of special importance for them was information about patient-reported treatment side effects. Usefulness of the tool to distinguish patients in terms of severity and number of disease- and treatment specific symptoms was demonstrated. The construct validity of CSP Leuk-CML was proven by factor analysis and "known-group" comparison. Factor analysis found five underlining constructs (domains) of the tool (explained 66% of the total variance) with Chronbach alphas varied from 0.72 to 0.94, which were clinically relevant and increased the practicality of the tool. Moderate correlations of five CSP Leuk-CML domains with the grades of QoL worsening were revealed (r=0.43-0.81; p<0.001): the higher symptom severity the more QoL worsening (r=0.43-0.81; p<0.001). A number of CML-specific symptoms were more severe in patients with side effects of treatment than in those without side effects (*fatigue, constant tiredness, decreased work energy, excessive sweat at rest, heat sensation, abdominal pain, diarrhea, edema, anxiety, restless dreams*; p<0,05). Sensitivity of the tool to clinical changes was demonstrated: symptom severity at 3 months after treatment start was reduced (*fatigue, excessive sweat at rest, muscle spasms, dry mouth, diarrhea, palpitation, shortness of breath, edema, numbness, restless dreams;* ES=0.36-0.46).
Summary and Conclusions: The CSP Leuk-CML is an appropriate, practical and sensitive tool to assess symptom profile and severity in CML patients. Further studies are needed before the wide-spread use of the CSP Leuk-CML in clinical practice and in clinical trials. Comprehensive symptom monitoring using patient-reported outcome measures may be recommended to clearly determine treatment outcomes in this patient cohort.

P627

PROGNOSTIC VALUE OF NEUROPSYCHOLOGICAL AND BIOLOGICAL FACTORS IN OLDER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES ADMITTED TO RECEIVE CHEMOTHERAPY

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Background: A Comprehensive Geriatric Assessment (CGA) is recommended to detect vulnerable cancer patients for whom chemotherapy may lead to severe impairment on functionality, quality of life, or survival. However, little is known about the reliability of G8 screening tool and the prognostic value of neuropsychological and biological factors in older patients with hematological malignancies admitted to receive chemotherapy.
Aims: To assess in "Go- and Slow-Go" patients with malignant hemopathies the reliability of G8 as a screening tool and to identify the predictive value of neuropsychological and biological factors assessed in the CGA in terms of one-year overall survival (OS).
Methods: G8 and CGA were proposed to 107 consecutive patients (65-89 yrs) with hematological malignancies admitted to receive chemotherapy. An initial full-dose or reduced-dose chemotherapy has been administrated to patients according to a multidisciplinary team decision.

Results: Ninety patients were evaluable for both scales, of which 72% and 80%, were defined as "vulnerable" when evaluated with G8 (≤14.5) or CGA (≥2 impairments), respectively. The area under ROC-curve of G8 compared to CGA was 0.749±0.051 (S=79.2% and Sp=55.6%). During the one-year follow-up, 27% (n=21) of older patients treated for hematological malignancies died. The leading cause of death (86%) was the disease progression. Retrospectively, neither the G8 nor the CGA total score had an impact with the initial treatment choice and were not predictive for one-year OS. Regarding the various CGA items, the multivariate Cox proportional hazards model adjusted for potential confounders showed that falls (OR=2.9) and ulcer disease (OR=4.4) were significantly and independently associated with mortality. Accordingly, we have found -using the log-rank test- a significant impact on one-year OS of acute or chronic ulcer disease (p<0.0001) and of ≥1 falls during the last year (p=0.014). Nevertheless, it seems that cognitive impairment (abnormal MoCA) (p=0.024) and renal impairment (p=0.083) may also have an impact on one-year OS.
Summary and Conclusions: Our small series of older patients admitted to receive chemotherapy for hematological malignancies suggests that G8 is a moderate screening tool for identifying patients who should benefit from a CGA and, in contrast with a population of solid tumor, is not predictive of OS. Some specific factors in the CGA (ulcer disease, falls during the last year, cognitive impairment and renal function) have more impact than other on one-year OS. Prospective trials are needed to further determine whether the combination of these specific items could be more adapted to "go- and slow-go" older patients with hematological malignancies.

P628

THE VALUE OF DASATINIB THERAPY IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC MYELOID LEUKEMIA FROM PHYSICIAN'S AND PATIENT'S PERSPECTIVE: "REAL WORLD" OUTCOMES

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Background: There is limited published data about the efficacy and safety of the second-line therapy with dasatinib in patients in chronic phase chronic myeloid leukemia (CML-CP) in a "real world" patients setting outside clinical trials. In addition, comprehensive evaluation of benefits and risks of the treatment is worthwhile to better define treatment outcomes in this patients' population.
Aims: We aimed to study clinical and patient-reported outcomes as well as safety of dasatinib treatment in a "real world" setting within the context of its approved indication through the analysis of prospectively collected data in patients with imatinib resistance or intolerance receiving dasatinib as the second-line therapy.
Methods: 75 CML-CP patients resistant or -intolerant to imatinib were enrolled in the prospective, multicenter, non-interventional study (mean age – 51.3 years old, SD 15.4; range – 22–83 years; male/female – 37/38). The median of disease duration was 5.8 years (0.75–17 years). 63 patients had resistance to imatinib; 12 patients were intolerant to imatinib; the median duration of imatinib treatment – 40 months (4–121 months). All the patients received dasatinib as the second-line therapy (100 mg daily). Median follow-up was 12 months. For quality of life (QoL) and symptom assessment patients filled out the SF-36 and Comprehensive Symptom Profile in Chronic Myeloid Leukemia Patients (CSP Leuk-CML), respectively, at base-line, in 1, 3, 6 months after treatment start and every 6 months thereafter. Statistical analysis was made using general linear model (repeated measures)with adjustment for age and gender. Mean symptom severity and percentage of patients with moderate-to-severe (ratings ≥5) symptoms was evaluated.
Results: After 12 months of treatment 79% patients achieved or maintained complete hematologic response and 63% – major cytogenetic response. Six cases of pleural effusion events were registered: they were easily managed in 5 cases; one patient died at 1 month after treatment start due to accompanied infection complication. Severe hematological adverse effects were observed in four cases (grade III-IV neutropenia and thrombocytopenia). Three patients died of disease progression at 6 months of follow-up; one more patient died of sepsis. At median follow-up of 12 months (range 1-24 months) event-free survival rate was 76% (95% CI; 54.7 - 88.4%). At 12 months of dasatinib treatment QoL parameters were stable for 5 out of 8 scales of the SF-36; vitality, social functioning and mental health significantly improved (p<0.001). QoL response in terms of stabilization or improvement was registered in 69% of patients; among them 39% improved, and 30% were stable. While treatment the number of patients with moderate-to-severe symptoms decreased (p<0.05). The majority of symptoms improved or were stable by 12 months of treatment. Positive changes in QoL and symptom severity preserved at 18 and 24 months of dasatinib treatment.
Summary and Conclusions: Thus, our study on "real world" patient data confirms that dasatinib as second-line therapy in CML-CP patients is effective both in terms of clinical outcomes and patient-reported outcomes, as well as exhibits good tolerability. Comprehensive evaluation of the outcomes of the second-line treatment of CML-CP allows to assess the benefits and risks of therapy both from physician's and patient's perspective.

P629

COVARIATION OF PSYCHOLOGICAL AND INFLAMMATORY VARIABLES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA RECEIVING IBRUTINIB

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Background: Ibrutinib has been shown to induce durable responses in patients with high risk relapsed/refractory (r/r) chronic lymphocytic leukemia (CLL) (Byrd *et al.*, 2013). Psychological factors, particularly stress, have been shown to covary with immunity and cytokines in solid tumor cancers.
Aims: This pilot study examines temporal changes and covariation in inflammatory/angiogenic markers and psychological variables among r/r CLL patients receiving Ibrutinib. We hypothesized that these variables would improve during treatment and would covary over time.
Methods: Patients enrolled in our single-institution phase-II trial of Ibrutinib with r/r CLL (N=148) provided blood and psychological data on day 1 (of 28) of cycles 1-3. For this pilot, ELISA assays were performed on a subset (N=24; mean age=65; 50% female) of patients representing a distribution of scores (0-33) on a cancer-specific stress measure. Depressive symptoms, mood disturbance, fatigue, and quality of life (QOL) were also assessed. Assays measured IL-6, TNF-alpha, VEGF, CRP, BAFF, CCL3, and IL-16 levels in the patients' plasma. Hierarchical linear modeling examined change over time for all variables and covariation between psychological and inflammatory/angiogenic markers.
Results: Improvements were observed in stress, depressive symptoms, fatigue, and QOL (p-values <0.05). Decreases were observed in IL-16, TNF-alpha, and CCL3 (p-values <0.05). Controlling for age, chromosome 17p deletion, and gender, covariation between the following variables was found: TNF-alpha with stress, depressive symptoms, mood disturbance, and QOL; IL-6 with mood disturbance, fatigue, and QOL; and VEGF with depressive symptoms (all p-values <0.05). That is, changes in inflammatory/angiogenic markers were correlated with changes in psychological variables.
Summary and Conclusions: These pilot data suggest that in addition to providing clinical benefit, Ibrutinib treatment contributes to improvements in psychological functioning and decreases in inflammation.

P630

A MULTINATIONAL OBSERVATIONAL STUDY IN MULTIPLE MYELOMA (PREAMBLE): ASSESSMENT OF DISEASE IMPACT ON WORK PRODUCTIVITY AND ACTIVITY

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Background: Cancer disease burden, treatment burden, and late effects of treatment have a potential impact on work productivity. Patients with multiple myeloma (MM) may experience anemia, renal impairment, and bone disease with associated symptoms of pain and fatigue. While data suggest that MM may impact work productivity, limited evidence currently exists.
Aims: PREAMBLE (Prospective Research Assessment in Multiple Myeloma: an Observational Evaluation) is a multinational, observational cohort study undertaken to prospectively evaluate the real-world effectiveness, healthcare resource utilization, and patient-reported outcomes (including work productivity) associated with the treatment of relapsed and/or refractory (R/R) MM. The aim of this report is to provide data on the initial (baseline) assessment of disease impact on work productivity for patients enrolled in PREAMBLE.
Methods: Patients completed questionnaires for quality of life and physical well-being, including the Work Productivity and Activity Impairment Questionnaire: General Health V2.0 (WPAI:GH). The WPAI:GH is a 6-item, patient-reported assessment of absenteeism, presenteeism (work impairment), and general activity impairment. As of the last data cut-off (November 22, 2013), 156 of 185 treated patients had completed the questionnaire at baseline. All patients had received prior therapy for MM.
Results: The mean time from initiation of current therapy for R/R MM to baseline WPAI:GH completion was 20 days. The majority of patients (94%) report-

ed that their activity level was impaired. Twenty-three of the 156 patients (15%) who completed the questionnaire were gainfully employed. Among the 16 of 23 employed patients who responded, a reported 22% of work time was missed. All 23 patients reported that health affected their work, with a mean impairment of 37%. Results are shown in the Table 1.

Table 1. Baseline WPAI:GH*.

Summary and Conclusions: Patients with R/R MM had already experienced a negative impact on general activity at baseline assessment. While 15% of the patients were employed, all reported impairment of work productivity to some extent. As all patients had prior treatment, it is possible that productivity may not have been impacted by MM alone. Additional follow-up will be needed to determine more fully the impact of both disease and therapy on work productivity.

P631

BONE MARROW ASPIRATION: A RANDOMIZED TRIAL ON THE QUALITY OF THE BONE MARROW SPECIMEN USING SLOW OR RAPID ASPIRATION TECHNIQUES AND ASSESSMENT OF THE PAIN INTENSITY

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Background: In bone marrow aspiration/biopsy (BMA), rapid aspiration technique is often recommended to reduce peripheral blood contamination in order to achieve best possible quality. There is limited evidence on whether speed of aspiration affects the quality of the bone marrow specimen. BMA is associated with pain and discomfort. However, it is not known if the intensity of pain is dependent of the aspiration technique.
Aims: To assess the quality of the bone marrow specimen and the overall pain intensity using slow (S) and rapid (R) aspiration techniques.
Methods: From Nov 2012 to Sep 2013 482 adult patients, among those scheduled for BMA at the Department of Hematology, Aalborg University Hospital, were randomized for either an S-technique (a slow, low differential pressure, uniform pull of a 10ml syringe, aspirating in the same pace as the appearance of the bone marrow for approx. 5 to 15 seconds), when having BMA, or an R-technique (a quick pull creating high differential pressure in a 10ml syringe for approx. 1 second and holding a high differential pressure during aspiration of marrow). The aspiration technique was blinded for the patients and pathologist. Bone marrow cellularity was graded as either no, few, moderate number or many nucleated cell. We defined no or few nucleated cells as inferior quality. The number of marrow particles (0 to >25) was assessed by the pathologist. We defined less than 11 marrow particles as inferior quality. One pathologist examined all samples. Patients assessed the overall pain intensity according to a Visual Analogue Scale (VAS) (0 to 10 where 0 is no pain and 10 is the worst pain imaginable). The BMA was, apart from the difference in aspiration technique, performed according to the standard procedure of the department. The biopsy site was the post. sup. iliac crest. Biopsies and aspirations were performed for all patients. The statistical analyses were carried out using Fisher's exact test for categorical and Wilcoxon's rank sum test for continuous variables, 5% was chosen as significance level, and effect estimates are provided with 95% one-sided confidence intervals. The analysis on cellularity and the number of marrow particles were performed as non-inferiority tests for the null-hypothesis that the number of inferior quality aspirates of the S-technique is no worse than the number of inferior quality aspirates using the R-technique.
Results: Before analysis, 38 patients were excluded for various reasons (hypoplasia/aplasia 5, fibrosis 13, samples lost/destroyed 10, other 10). No significant difference in the two groups. For bone marrow particles, the odds ratio (OR), for having a poor quality aspirate using the S- compared to the R-technique was 2.52 (1.62,∞). For cellularity, the OR was 3.046 (1.93,∞). We also found a statistical significant difference of 1 VAS point (P <0.001) of the median pain intensity in favor of the S-technique. The pathologist assessed whether the specimen as a whole (including information about blood, biopsy, and aspirated marrow) was sufficient to make a diagnosis. For this question there is no statistically significant difference (P=0.175) between the two groups.
Summary and Conclusions: The quality of BMA using the R-technique for aspirating bone marrow is higher than the quality using the S-technique. The pain intensity is statistically higher when using the R-compared to the S-technique. However, the difference in pain intensity is rather small compared to the general variability of the VAS pain score and thus the R-technique is preferred for BMA.

P632

IMPACT OF ANEMIA ON HEALTH-RELATED QUALITY-OF-LIFE AND CARDIAC REMODELING IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS). RESULTS OF GLOBQOL OBSERVATIONAL PROSPECTIVE STUDY.

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Background: Patients with lower-risk myelodysplastic syndromes (LR-MDS) have a diminished health-related quality of life (HRQoL), mainly due to fatigue and other anemia-related symptoms.
Aims: To assess the eventual changes in the HRQoL and cardiac remodeling of patients with LR-MDS during a one-year follow-up, and to analyse their clinico-biological correlations.
Methods: An observational, prospective, multicenter study was performed in 10 Spanish hospitals, including 39 adult patients (9 non-evaluable for not fulfilling selection criteria and/or lack of essential data) with LR-MDS (IPSS score <1.5 or Spanish Scoring System score <2), not receiving disease-modifying therapies. All patients gave informed consent. Socio-demographic, clinical and analytical data were collected at baseline and at 4 follow-up visits, including comorbidity, performance status, Short Physical Performance Battery, echocardiography, hemoglobin, and serum iron parameters. HRQoL was assessed using the SF-36 and EORTC QLQ-C30 questionnaires (at 0, 6 and 12m) and the FACT-An scale (at 3 and 9m), and cardiac remodeling through echocardiography. The perceived need for erythrocyte transfusions (assessed synchronically and independently by both patients and haematologists) was measured using the LASA scale. Seventeen patients (56.7%) were males, median age was 77.6, 18 (60%) were smokers/former smokers and 23 (76.7%) had one or more comorbid conditions (range 0-9, mean 2.4; cardiac disease 26.7%, diabetes mellitus 23.3%, arrhythmia 20.0%, moderate lung disease 20%). Most frequent subtype was Refractory Cytopenia with Multilineage Dysplasia (63.3%), twenty-one (70%) had a low IPSS score and median time since diagnosis was 5 months. Twenty-seven (90%) had an ECOG performance lower than 2, mean hemoglobin concentration was 10.1 g/dL (range 7-12, SD 1.5), 8 (26.7%) were receiving erythroid stimulating agents (ESA) and 4 (13,3%) were transfusion-dependent at study outset.
Results: During follow-up 17 patients (56.7%) initiated ESA therapy and 2 (6.7%) received iron chelating therapy, none of the patients evolved into other MDS subtype or acute myeloid leukemia, 7 (23.3%) developed cardiac failure or atrial fibrillation and 3 (10%) died. Mean hemoglobin concentration, serum ferritin levels, serum transferrin saturation as well as the overall HRQoL and functional parameters remained stable. Nevertheless, we observed statistically significant decrease in the SF-36 physical function and average Fact-An scores (p=0.029 in both cases), as well as a trend towards a lower score in the QLQ-C30 physical role domain (p=0.065). We also observed a statistically significant reduction in gait speed (p=0.038) and a trend towards a higher left ventricular end-diastolic diameter (p=0.081). In addition, patients communicated a non-significant slightly lower (Bland-Altman) perceived transfusion need than their physicians, according to the LASA scale.
Summary and Conclusions: Despite receiving active observation and supportive care as needed that kept stable their mean hemoglobin concentration and iron parameters, lower risk MDS patients suffered from a slight reduction

in their physical performance and showed a trend towards a higher left ventricular end-diastolic diameter during a one-year follow-up. Gate speed may be used to document their physical decline and to identify those patients that may need other disease- and HRQoL-modifying therapies during follow-up. Sequential echocardiography might also be of help for early detection of cardiac consequences of chronic anemia.

P633

THE BURDEN OF RELAPSE ON EUROPEAN HEMATOLOGISTS TREATING MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) accounts for 1% of all cancers and affects 0.003% of Europeans each year. Due to the incurable and chronic nature of the disease, almost all patients experience successive relapses. Each relapse typically requires a treatment alteration and increased symptom management. We hypothesize that the occurrence of a relapse may place a burden on the patient's physician, in terms of their emotional well-being, workload and stress levels.
Aims: To assess the impact that relapse events have on hematologists, by evaluating their experience of treating relapsed and/or refractory MM patients.
Methods: Thirty interviews were conducted with hematologists in the UK, France, Germany, Italy and Spain (6 per country). Hematologists were included if they had personally treated ≥10 MM patients in the previous month and ≥5 MM patients that had undergone a clinical relapse in the previous year. 'Myeloma UK' (a registered charity) was consulted for input into study design. During in-depth qualitative interviews, hematologists were asked open-ended questions relating to their observations, hopes for their patients, and how relapse affected consultations.
Results: Hematologists reported that consultations with relapsed MM patients often last 2–3 times longer than consultations with non-relapsed patients. This reduces the number of hours available for consultations with other patients, and thereby increases physicians' levels of stress. Information provided to MM patients depends on the physician's preference. Although some physicians felt obliged to provide full details to patients at all times, others reported that in instances where a patient was not yet symptomatic and did not require a treatment change – it was stressful determining whether to inform the patient of their relapse. Hematologists also reported that patients' reactions to the news that they have relapsed can be unpredictable. These factors make it difficult for physicians to prepare for consultations with MM patients who are no longer responding to treatment or who are experiencing a relapse event.
Hematologists reported that their hopes for their patients to remain relapse-free with long periods of remission are seldom realized. This can result in fear, anxiety and disappointment (feelings which are exacerbated by the gradual exhaustion of treatment options). As a result, some physicians described feeling reluctant to engage in emotional conversations with their patients, to minimize the emotional impact that relapse inflicts upon them. The most frequently expressed emotions by hematologists in relation to relapse were "uncertainty", "upset" and "stress". They also considered the wider implications of relapse on the healthcare system; specifically, resource use (*e.g.* the need to involve psychologists and pain specialists at relapse): "The burden of relapse on the healthcare service is a significant one – there is a need for more resources" (including physical, therapeutic and financial resources).
Summary and Conclusions: For hematologists treating MM patients, relapse is associated with failed hopes, difficult consultations and enhanced levels of stress which are collectively very challenging. As a result, relapse may cause hematologists to struggle with the emotional aspects of treatment (*i.e.* interaction with their patients). In order to provide holistic disease management, it is important to assess and evaluate the impact of relapsing patients on their physicians. The subsequent impact on patients (of physicians experiencing increased stress) should also be considered.

P634

A FEASIBILITY STUDY OF VIRTUAL REALITY EXERCISE USING WII FIT AMONG PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING CHEMOTHERAPY

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Background: Compared with patients with solid tumors, adherence rates of rehabilitation exercise are quite lower in patients with hematologic malignancies (60-85% and 22.5-45.8%, respectively) because of more intensive chemotherapeutic regimens. Virtual reality exercise is available even in the biological clean room and may improve adherence rates in those patients.
Aims: To clarify the feasibility of virtual reality exercise using Wii Fit among patients with hematologic malignancies receiving chemotherapy.
Methods: Sixteen hospitalized patients with hematologic malignancies aged 60 years or older participated in once a day, 5 times a week, 20-minutes exercise program using Nintendo Wii Fit (Nintendo, Kyoto, Japan) from the start of chemotherapy until their hospital discharge. Eight patients had acute myeloid leukemia, 2 had acute lymphoblastic leukemia, 1 had adult T-cell leukemia, 1 had T-cell prolymphocytic leukemia and 4 had non hodgkin lymphoma. Two activities, Hula Hoop and Basic Step, were chosen in patients' preference. Both of these exercises were light to moderate intensity; Hula Hoop was 4 Metabolic Equivalent of Task (METs) and Basic Step was 3 METs. Adherence rates, safety, physical and psychological performances were assesed. Adherence rates are calculated as the ratio of the number of days actually exercised to the number of days scheduled to exercise in advance.
Results: The median age was 66 years (range, 60-76) and 6 patients (38%) were female. Eight patients (50%) received their initial chemotherapy and the rest eight patients (50%) had received chemotherapy previously, ranging 1 to 4 regimens (median 1). The median duration of hospital stay was 26.5 days (range, 12-126). Nine (56.3%) participants completed the virtual reality exercise intervention with 88 sessions, and adherence rates were 61.1%. Four (25%) participants dropped out due to rapid progression of the disease and three (18.8%) dropped out due to patients' refusal after baseline asessment. We observed no intervention-related adverse effects more than grade 2 according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Table 1 shows changes in physical and psychosocial measures from baseline to post exercise. Compared with before and after exercise intervention, the median score of Barthel index assessing activity of daily life kept 100 (range 85-100). The median grip strength of the right hand slightly decreased from 36 kg (range 18-44) to 32 kg (range 18-42) and that of the left hand increased from 29 kg (range 16-46) to 32 kg (range 18-32). The median one leg standing time of the right leg kept 15.0 seconds during hospitalization and that of the left leg increased from 10 seconds (range 2.0-45.0) to 12.0 seconds (range 3.5-32.0). The median knee extension strength of the right leg increased from 0.79 kg (range 0.16-1.50) to 0.91kg (range 0.57-1.62) and that of the left leg increased from 0.79 kg (range 0.37-1.52) to 1.02 kg (range 0.61-1.41). The median Timed Up and Go Test (TUG) score increased from 8.20 (range 5.90-19.00) to 8.56 (range 8.56-13.00). The median Instrumental Activities of Daily Living score of men decreased from 4 (range 2-5) to 2 (range 2-4) and that of women decreased from 6 (range 5-7) to 2. The median anxiety score accoding to Hospital Anxiety and Depression Scale (HADS) decreased from 6.5 (range 1.0-14.0) to 5.0 (range 1.0-8.0) and the median depression score according to HADS decreased from 7.0 (range 4.0-17.0) to 6.0 (range 1.0-12.0). We observed maintenance of physical fitness and psychosocial performance during hospitalization receiving chemotherapy.

Table 1. Changes in physical and psychosocial measures from baseline to post exercise.

Summary and Conclusions: Virtual reality exercise using Wii Fit is feasible, safe and preliminary efficacious among patients with hematologic malignancies receiving chemotherapy.

P635

A EUROPEAN STUDY OF THE EMOTIONAL AND PHYSICAL IMPACT OF RELAPSE ON PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) affects 3/100,000 Europeans each year and remains incurable. Almost all patients (pts) experience successive clinical relapses (defined by calcium elevation, renal insufficiency, anemia and bone abnormalities), which may be accompanied by debilitating symptoms (*e.g.* infections, bone pain and fatigue). This, coupled with the chronic nature of the disease, is likely to impact on pts' emotional and physical well-being. However, there is currently no published evidence supporting this. It is important to understand the impact of relapse to inform clinical practice.
Aims: To provide a better understanding of the impact of relapse in MM patients, and how this changes over time.
Methods: Fifty qualitative interviews were conducted with relapsed and/or refractory MM pts in the UK, France, Germany, Italy and Spain (10 per country). 'Myeloma UK' (a registered charity) was consulted for input into study design. Only pts with ≥1 physician-diagnosed, clinical relapse were included. Pts with biochemical relapse (without symptoms) were excluded. The median number of treatment (Tx) lines for pts was 2 (1–6). To prepare for interviews, pts were asked to draw graphical diagrams illustrating changes in their emotional and physical well-being over time. During the interview discussions, pts were asked open-ended questions about the impact of MM on their lives (*e.g.* daily activities, relationships, quality of life), with a focus on individual relapse events.
Results: Pts reported a substantial decline in their emotional well-being at diagnosis (Dx) (Figure 1). This decline was most pronounced in younger pts who were struggling to accept their disease. Following initial Tx and symptom reduction, pts' emotional well-being improved, but not to pre-Dx levels. Well-being declined at the first relapse (the most emotionally challenging time since Dx); the most frequently expressed emotions were "scared", "depressed", "worried", "confused", "frustrated" and "powerless". Some pts reported that the impact of the second relapse was not as substantial as the first, as they knew what to expect. Others reported that multiple relapses were associated with loss of hope and increasing distress, as they felt that they were exhausting Tx options and "getting closer to the end". Pts also reported reduced physical ability (*e.g.* capacity to carry out daily activities) attributed to MM symptoms, Tx side effects and old age/frailty. As a result, pts reported an increased reliance on immediate family and a decline in social life. For some, attending family events (*e.g.* holidays, weddings and graduation ceremonies) improved emotional well-being. Tx side effects and frequent hospital visits (for monitoring and Tx) had social and practical impacts on pts' lives. The travel associated with hospital visits had financial implications.

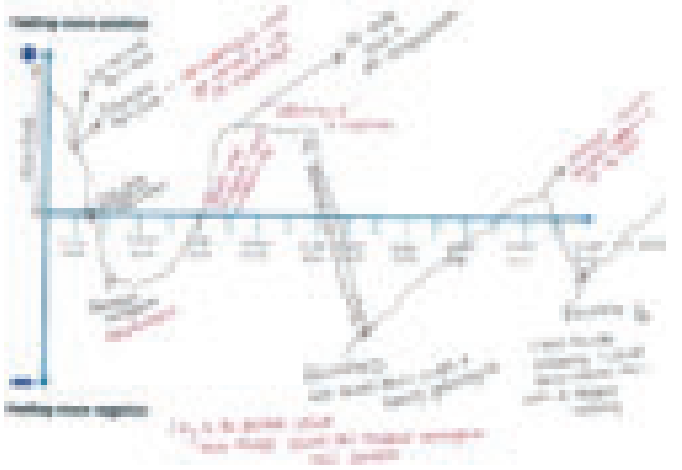

Figure 1.

Summary and Conclusions: Relapse impacts on the emotional and physical well-being of MM pts. The emotional response documented in this research varied according to pts' characters and disease histories, but included shock, anxiety, uncertainty and distress. Notably, pts' emotional well-being did not return to pre-Dx levels, indicating that the fear of relapse is a prominent emotion. Relapse was also associated with increased reliance on family members and reduced social interactions, particularly when there was a decrease in physical abilities. These findings highlight a potential unmet need for additional support, tailored toward individual pt needs. Further investigation is warranted to assess the impact of relapse on carers and family members.

P636

EDUCATIONAL INTERVENTION IN CANCER PATIENTS: THE IMPACT OF A CANCER SYMPOSIA ON PATIENT KNOWLEDGE

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Background: Patients diagnosed with cancer experience a significant information need. This knowledge deficit can negatively impact quality of life, contribute to anxiety, and decrease compliance with health care recommendations. Similarly, information provision may improve patient experience, symptom management, and interrelationships. In 2013 we evaluated a patient centered symposia of cancer patients for impact on symptom, quality of life, and knowledge level. 115 patients participated in the pre and post symposia questionnaires. The 2013 conference revealed no change in quality of life but did demonstrate significant knowledge deficits in the broad population of cancer patients that were improved by attendance. This inspired a deeper investigation into patient level of knowledge, information sources, and desired type of information provided in cancer patients.
Aims: The aim of this study is to build on the 2013 symposia data and evaluate the impact of a patient centered cancer symposium on knowledge level, reported symptom burden, and desired information from a broad population of cancer patients.
Methods: Surveys were distributed to the attendees of the third annual Mayo Clinic "Living with Cancer" patient symposium in January 2014. While 700 individuals registered for the event, only individuals with a past or present cancer diagnosis were asked to participate. Surveys included demographic data in addition to a questionnaire evaluating disease comprehension, symptom burden, patient desired information, and desired role in health care decision making process.
Results: 113 patients have completed the pre-intervention survey to date. There was slightly more female participants (60.2%). Disease types included 40% hematologic malignancies, 27% breast cancer, 20% prostate cancer and 13% other. The majority of patients were greater than 3 years from cancer diagnosis (62%). Most respondent reported understanding their disease quite a bit (51%) or very much (31%). Respondents reported the majority of their knowledge regarding their disease came from their oncologist (52%), previous symposiums (27%), or the internet (21%). Most respondents reported "quite a bit" or greater comprehension of screening tests (78%), monitoring disease response to treatment (74%), monitoring disease recurrence (68%), symptoms (74%) and treatment options (67%). There was no consensus among participants regarding understanding or limiting risk factors, symptoms associated with disease relapse, treatment side effects, fatigue and pain management, navigating the health care system, financial considerations, or confidence in their primary care physician's involvement in their cancer or post-cancer care. A large proportion of attendees reported "quite a bit" or greater desire for increased information/understanding regarding their disease (83%), risk factors (83%), nutrition (80%), screening tests (69%), and management of fatigue (69%) and stress (68%).
Summary and Conclusions: Cancer diagnosis, treatment and survivorship require significant health care and personal decisions. Individuals diagnosed with cancer experience a knowledge deficit which can negatively impact quality of life. Patients acquire information to decrease this knowledge deficit through multiple paths, including patient centered symposiums. Individuals, who choose to attend these conferences, seek to improve an already solid knowledge base. Further research regarding timing, focus, mode, and individualization of information provision are current and future needs in cancer care research. Further results of cancer knowledge and the impact of this intervention are being assessed and will be presented.

P637

PATIENTS' EXPERIENCES WITH CHRONIC LYMPHOCYTIC LEUKEMIA: EVIDENCE FROM QUALITATIVE RESEARCH WITH TREATED AND "WATCH AND WAIT" PATIENTS

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Background: We sought to better understand the patient experience with chronic lymphocytic leukemia (CLL) and its treatment using qualitative methods.
Aims: Specifically, we sought to document concerns about starting treatment expressed by "watch and wait" (WW) patients with CLL; and to examine differences in disease symptom reported among treated and WW patients.
Methods: Adults with CLL participating as members of the PatientsLikeMe online patient community were invited to complete a web-based survey about their experiences with the condition. Treatment status, CLL stage, performance status, and demographic variables were collected for descriptive analyses. Open-ended survey questions elicited descriptions of CLL symptoms and impacts; experiences with treatment (treated patients only); and perceptions of treatment (WW patients). Survey responses were coded for qualitative content analysis.
Results: Survey responses from 50 members of the online CLL patient community were coded for content analysis. The mean age of respondents was 60.5 years; 54% were female; 96% were White. When surveyed, 20% of respondents were receiving current treatment, 16% had received previous treatment, and 64% were under (WW) for their CLL. Most patients (78.0%) reported experiencing at least 1 symptom and 17% of patients reported their symptoms

required them to be in bed for up to 50% of their waking day. Among the 50 patients, 369 descriptions of CLL symptoms were coded in qualitative analyses. The five most frequently-expressed disease symptoms were the same across treated and WW patients, but more prevalent among treated patients compared to WW patients. These key symptoms included fatigue[pcyc1] (expressed by 25% of WW and 67% of treated patients), tiredness (34% *vs.* 55%), night sweats (34% *vs.* 44%), swollen lymph nodes (31% *vs.* 33%) and pain (19% *vs.* 33%). Conversely, the most frequently-expressed impact of CLL, worry and fear was more prevalent among WW (69%) than treated (61%) [pcyc2] patients. When asked about their concerns regarding use of CLL treatment, WW patients most-commonly expressed concern about potential treatment side-effects (noted by 63% of the 32 WW patients); becoming a burden to others (37.5% of WW patients), or having a decreased quality of life (31% of WW patients).Among the 18 respondents who had received treatment, ten (56%) reported experiencing difficulties from their treatment such as emotional and physical side-effects (*e.g.* "*It has left my immune system compromised*" and "*After treatment, I began to feel anxiety and depression"*) and disruption in their daily activities ("*Taking treatment makes it where I have to rest more*"). An equal number (56%) of treated patients described beneficial aspects of their treatment, including the elimination of specific symptoms (*e.g. "no more sweats"* and "*I'm not sick as often as I used to be*"); regaining energy (*e.g.* "*I started to feel like my old self*" or "*I have energy for a good portion of the day*") and entering remission (*e.g.* "*It eradicated my disease*").

Summary and Conclusions: Although many WW patients express concern about potential treatment side effects, patients who have received treatment describe positive and negative aspects of the treatment experience with similar frequency. Results identify little difference in the types of CLL symptoms experienced by WW and treated patients, but suggest a higher symptom burden on treated patients. Emotional effects (*e.g.*, worry, fear, depression) were the most frequently-expressed impacts of CLL, and appear to be slightly more relevant to WW patients.

P638

BURDEN OF ILLNESS OF PATIENS WITH CHRONIC LYMPHOCYTIC LEUKEMIA THAT ARE REFRACTORY TO FLUDARABINE AND ALEMTUZUMAB-CONTAINING REGIMENS IN SPAIN: AVERAGE COST ESTIMATION BY MONTECARLO SIMULATION

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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in western countries, accounting for more than 30% of total leukemias. Symptomatic CLL significantly impacts on patient's quality of life and is associated with high direct and indirect management costs. Though current CLL treatment has improved in recent years, no cure exists for this disease and a proportion of patients become refractory to both fludarabine- and alemtuzumab-containing regimens or "double refractory" (DRCLL). These patients were estimated from the Spanish Leukemia Registry data (2002 report) to constitute 2.96% of CLL patients in Spain. While there is a perception of high costs related to the management of these patients, there is no Spanish data on the burden of illness.
Aims: The aim of this study was to estimate the average management cost of DRCLL patients from the Spanish National Health System perspective.
Methods: MonteCarlo simulations are commonly used in rare diseases when the reduced number of patient makes it very difficult to obtain enough real-life data to perform statistical analysis. This technique assigns randomly generated values for patient parameters from mathematical distributions that describe real-life distributions. Since the prevalence of DRCLL patients in Spain is small, MonteCarlo simulation technique was deemed appropriate. In order to develop the mathematical distributions that describe life expectancy and resource consumption in these patients, the Spanish subgroup of patients (n=12) from a retrospective medical chart review study conducted in European centres in 2009 was used. Direct health care resource-use was obtained from the study database for patients diagnosed with DRCLL until death or loss of follow-up and classified into the following categories: hospital stay and physician visits, transfusions, laboratory analysis, imaging tests, other tests or medical interventions, drug treatment for cytopenia, chemotherapy and other drug costs. Unit costs were derived from national databases and official government journals and expressed in € 2014 applying the corresponding CPI variation when needed. Gamma distributions were then developed with the daily average cost of the 12 patients for each cost category and an exponential distribution was developed for overall survival with data from the 8 patients that were followed until death. One million simulations were performed.
Results: Mean and median life expectancy from DRCLL diagnosis were 230 and 159 days, respectively (P_{25-75}: 66-319 days). Mean cost per patient was €46,613.32 (P_{25-75}: €9,284.32 - €57,173.67). Hospital stays and physician visits accounted for 47.74% of mean daily cost, 3.42% of cost were attributed to transfusions, 7.79% to laboratory analysis, 5.18% to imaging tests, 11.83% to other tests or interventions, 19.77% to drug therapy for cytopenia, 3.95% to chemotherapy and 0.32% to other drug costs.

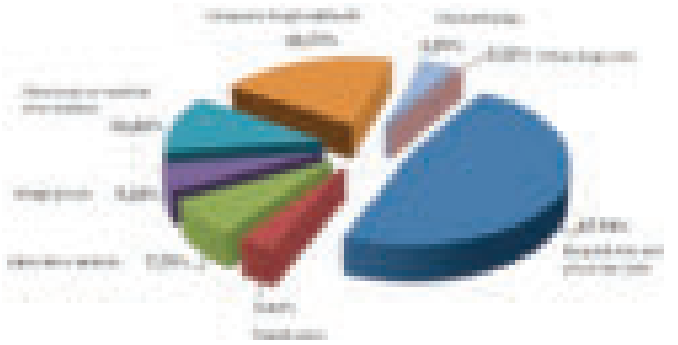

Figure 1.

Summary and Conclusions: DRCLL patients have poor prognosis with a life expectancy of less than a year and their management is associated with substantial direct medical costs. Hospital stays and physician visits were identified as the key drivers of total cost while chemotherapy cost accounted for less than 4% of total costs.

P639

SIGNIFICANT DISEASE BURDEN EVALUATED BY FACIT-FATIGUE AND EORTC QLQ-C30 IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA FROM A KOREAN PROSPECTIVE PNH REGISTRY

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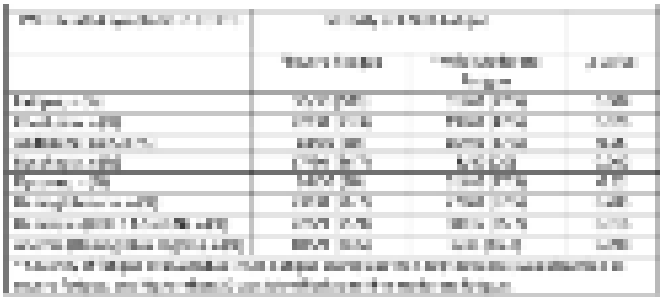
Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare and progressive disease driven by complement-mediated hemolysis leading to thromboembolism (TE), organ function impairment, and death. Beyond these risks, patients with PNH suffer from a poor quality of life, including disabling symptoms of fatigue, abdominal pain, chest pain, dyspnea, and hemoglobinuria.
Aims: To assess patient-reported severity of fatigue and incidence of PNH-associated clinical symptoms among 70 untreated patients enrolled in the Korean prospective PNH Registry.
Methods: Korean patients with a diagnosis of PNH are eligible for inclusion in the prospective registry study designed to identify key symptoms. Patient medical information and study questionnaire data were collected at study enrollment and the last 6 month follow-up, and included data based on the validated Functional Assessment of Chronic Illness Therapy Fatigue subscale version 4 (FACIT-Fatigue) and the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire C30, version 3 (EORTC QLQ-C30). Patients receiving eculizumab treatment or bone marrow transplantation (BMT) were excluded. Severe fatigue was identified as FACIT-Fatigue score of less than 30 (<30 score), and clinical symptoms, including abdominal pain, chest pain, dyspnea, swallowing difficulty, and hemoglobinuria, were based on patients' self-report in EORTC QLQ-C30.
Results: At the time of analysis, completed questionnaires were available for 70 patients. Among the 70 patients, the median FACIT-Fatigue score was 29 at enrollment and 31 at 6 month follow-up; there was no association between two different time points (p=0.102). Mean age was 45 years (range, 19-73; standard deviation, 13.2) and 41 patients (58.6%) were female. At 6 month follow-up time, median lactate dehydrogenase (LDH) was 4.75-fold above the upper limit of normal. Of the 70 patients, the majority of patients reported (EORTC-assessed) fatigue (61; 87.1%), dyspnea (55; 78.6%), hemoglobinuria (50; 71.4%), chest pain (41; 58.6%), abdominal pain (33; 47.1%), and dysphagia (25; 35.7%). Red blood cell transfusion was administered to 31 (44.3%) of the 70 patients during the last 6 month follow-up. Thirty patients (43%) were experiencing severe fatigue and 57% of patients had mild to moderate fatigue (assessed by FACIT-Fatigue). Incidence of selected PNH-related symptoms in those two fatigue groups is presented in Table 1. Regardless of the severity of fatigue, clinical symptoms, hemolysis (LDH≥1.5×ULN), and anemia were commonly experienced by PNH patients (Table 1). However, the prevalence of abdominal pain, chest pain, and dysphagia were significantly higher in the severe fatigue group.
Summary and Conclusions: Overall, results demonstrated that PNH patients commonly experience severe fatigue and this can have a negative impact on patients' quality of life and daily activities. The prevalence of severe fatigue remained constant from baseline through 6 months. These data confirm that

PNH patients with severe fatigue also frequently suffer disabling symptoms such as chest pain, dyspnea, abdominal pain, dysphagia, and hemoglobinuria. Interestingly, symptoms that could be associated with contributing to severe fatigue (ie, dyspnea, anemia, and hemolysis) did not predict the level of fatigue in PNH patients. While abdominal pain, chest pain, and dysphagia were higher with severe fatigue, the high prevalence of these symptoms with mild to moderate fatigue was still meaningful. PNH with moderate/severe fatigue should be evaluated as part of a full, comprehensive clinical workup to assess all PNH-related symptoms and disease burden. Patients' self-reporting questionnaires should be considered for routine use for the management of quality of life in patients with PNH.

Table 1.



P640

PERSPECTIVE OF CHILDREN WITH CANCER ABOUT CANCER RELATED FATIGUE: A SINGLE CENTER EXPERIENCE

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Background: Fatigue is the most common and incapacitating side effect in patients with Cancer. Although most cancer patients report that fatigue is a major obstacle to maintaining normal daily activities and quality of life, it is seldom assessed and treated in clinical practice.
Aims: to evaluate cancer- related fatigue and determine its possible causes according to perspectives of children with cancer.
Methods: This prospective study was conducted on a sample of 125 children with cancer (64 males and 61 females) at Oncology Unit at Zagazig University Children Hospital during a period from October 2011 to June 2013. Their age ranged between 6 -18 years. Five tools were used in this study, **The first** was A questionnaire interview sheet.**The second** was The Multidimensional Fatigue Symptom Inventory-Short Form to assess the principal manifestations of fatigue, **the third** was The cancer fatigue scale to assess physical, affective, and cognitive aspects of fatigue experienced by cancer patient., and the **fourth** was Fatigue assessment scale for assessment of severity of fatigue. **The fifth** was Multidimensional Assessment of fatigue scale which measures four dimensions of fatigue: severity, distress and degree of interference in activities of daily living, as well as timing degree of interference in activities of daily living.
Results: Our data revealed that 76.8% of children mentioned fatigue as a sense of physical tiredness, 75.2% of them mentioned it as not able to do things and 63.2% mentioned persistent tiredness and fatigue with rest. Also, 72% of children mentioned cancer treatment as a cause of fatigue and 91.2% of children mentioned that hospital had role in their fatigue. High score of physical fatigue type was reported by 93.6% of studied children, while 63.2% of them reported higher score of affective fatigue, in addition to 44% of them reported cognitive fatigue type. Moderate fatigue was reported by 71.2% of children; while 19.2% reported severe fatigue. About 93.6% of studied children mentioned high score of fatigue interference on daily activities.The The majority (96.1%) of studied children who did not practice exercise reported high score of total fatigue type with statistical significant relation.
Summary and Conclusions: The children with cancer oriented about definition, causes, and degree, as well as types of fatigue. Moreover, they oriented about multidimensional aspect of fatigue including fatigue effect on daily living activities. So, it could be recommended that an assessment and management of cancer –related Fatigue should be enrolled in daily routine care of cancer children.

SIMULTANEOUS SESSION I

Myeloma and other monoclonal gammopathies - Clinical 1

S641

RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 3 STUDY OF PANOBINOSTAT OR PLACEBO PLUS BORTEZOMIB AND DEXAMETHASONE IN RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA (PANORAMA 1)

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Background: Despite therapeutic advancements, patients (pts) with multiple myeloma (MM) relapse or become refractory to frontline therapies, and thus an unmet need remains. Panobinostat (PAN) is a potent pan-deacetylase inhibitor (pan-DACi) that demonstrated synergistic antimyeloma activity when combined with bortezomib (BTZ)+dexamethasone (Dex) in preclinical studies. Results from phase 1 and 2 studies demonstrated durable responses in pts with relapsed (Rel) or relapsed/refractory (Rel/Ref) MM treated with PAN/BTZ/Dex, including pts with BTZ-Ref MM.
Aims: The PANORAMA 1 study was conducted to determine if the addition of PAN to BTZ/Dex increased progression free survival (PFS) compared with placebo (Pbo)/BTZ/Dex in pts with Rel or Rel/Ref MM.
Methods: Eligible pts had Rel or Rel/Ref MM (excluding BTZ- and primary-Ref MM) following 1-3 prior regimens. Pts received oral PAN (20 mg) or Pbo 3 times per week+intravenous BTZ (1.3 mg/m^2; days 1, 4, 8, and 11) during weeks 1-2 with oral Dex (20 mg) on the days of and after BTZ in treatment phase (TP) 1, eight 3-week cycles. Pts demonstrating benefit could proceed to TP2, with PAN dosing maintained and BTZ/Dex less frequent. The primary endpoint was PFS with response assessed by modified European Society for Blood and Marrow Transplantation criteria as per investigator's assessment. Other endpoints included overall survival (OS), overall response rate (ORR), near complete/complete response (nCR/CR) rate, duration of response (DOR), quality of life, pharmacokinetics, and safety.
Results: A total of 768 pts (PAN+BTZ+Dex [n=387]; Pbo+BTZ+Dex [n=381]) were randomized. Median age was 63 years (range, 28-84 years), 55% had Eastern Cooperative Oncology Group performance status 1-2, and 47% had International Staging System stage II/III. Nearly half (48%) received ≥2 prior regimens. Prior therapies included BTZ (43%), thalidomide (51%), and lenalidomide (20%), with 25% receiving prior BTZ+immunomodulatory drugs. The primary endpoint was met: median PFS, 12.0 *vs* 8.1 months (*P* <.0001; hazard ratio [HR], 0.63; 95% CI, 0.52-0.76) for pts treated with PAN/BTZ/Dex *vs* Pbo/BTZ/Dex (Figure 1). ORR was 61% *vs* 55% in the PAN/BTZ/Dex and Pbo/BTZ/Dex arms, respectively, with DOR of 13.1 *vs* 10.9 months. Analysis of the rate of nCR+CR demonstrated a clear benefit for pts on the PAN/BTZ/Dex arm (27.6%; 95% CI, 23.2-32.4) *vs* the Pbo/BTZ/Dex arm (15.7%; 95% CI, 12.2-19.8). Among pts who achieved ≥nCR, median PFS was 19.4 months for the PAN/BTZ/Dex arm *vs* 15.2 months for the Pbo/BTZ/Dex arm (*P*=.00006; HR, 0.56; 95% CI, 0.37-0.86). PFS and ORR were confirmed by an independent review committee. OS data are not yet mature. Common grade 3/4 nonhematologic adverse events (AEs) regardless of study drug relationship in the PAN/BTZ/Dex and Pbo/BTZ/Dex arms, respectively, included diarrhea (26% *vs* 8.0%), asthenia/fatigue (24% *vs* 11.9%), and peripheral neuropathy (18% *vs* 15%). Common grade 3/4 hematologic laboratory abnormalities included thrombocytopenia (67% *vs* 31%) and neutropenia (35% *vs* 11%). In general, these AEs were manageable with dose reduction and supportive care. AEs led to discontinuation in 36% in the PAN/BTZ/Dex arm and 20% in the Pbo/BTZ/Dex arm. AEs leading to discontinuation included diarrhea (4.5%), peripheral neuropa-

thy (3.7%), asthenia and fatigue (2.9% each), and thrombocytopenia (1.6%) in the PAN/BTZ/Dex arm and fatigue (2.9%), pneumonia (2.1%), peripheral neuropathy (1.9%), and diarrhea (1.6%) in the Pbo/BTZ/Dex arm. On-treatment deaths occurred in 8% and 5%, respectively.

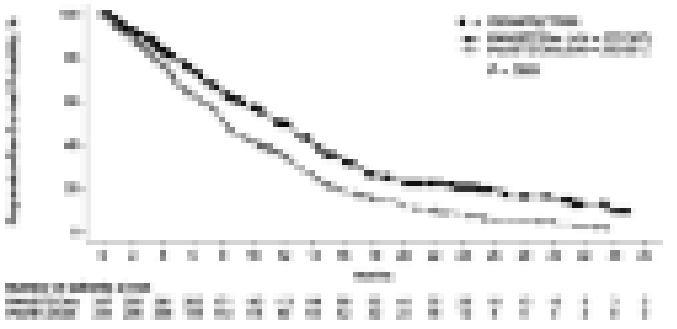

Figure 1.

Summary and Conclusions: The addition of PAN to BTZ/Dex led to a significant and clinically relevant improvement in PFS compared with Pbo/BTZ/Dex in pts with Rel or Rel/Ref MM, with improvement in DOR and nCR+CR rate.

S642

E1A06: A PHASE III TRIAL COMPARING MELPHALAN, PREDNISONE, AND THALIDOMIDE (MPT) *VERSUS* MELPHALAN, PREDNISONE, AND LENALIDOMIDE (MPR) IN NEWLY DIAGNOSED MULTIPLE MYELOMA MM)

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Background: Melphalan, prednisone and thalidomide (MPT) is an accepted regimen in newly diagnosed MM. Early studies suggested that lenalidomide (R) might be substituted for thalidomide (T) with equal efficacy and less toxicity. We present E1A06, a randomized, multicenter phase 3 trial comparing MPT *vs.* MPR in pts with untreated, symptomatic, transplant ineligible MM

Aims: The primary objective was PFS differences between pts receiving MPT: M 9 mg/m^2 and P 100 mg p.o. each on days 1-4 with T 100 mg daily *vs.* MPR: M 5 mg/m^{2}and P 100 mg p.o. each on days 1-4 with R 10 mg p.o. on days 1-21. MPT or MPR therapy was continued for twelve 28 day cycles followed by T 100mg or R 10mg daily until relapse. Aspirin prophylaxis was required.

Methods: Pts were stratified by ISS stage (I-II *vs.* III) and age (<65 *vs.* ≥65). Inferiority of MPR was defined as a PFS treatment hazard ratio (HR) of MPT/MPR ≤0.82. Secondary objectives included OS between the arms, toxicities, response rates, depth of response and quality of life (QoL) change.

Results: 306 pts were enrolled. Treatment arms were balanced for age, ISS stage and other major prognostic factors. Median age was 75.7y. The median follow-up was 40.7 months (m). Median time on therapy was 12m, and 23m for the 46% of pts on maintenance therapy, with no differences by arm. Per protocol partial response rate was 62% (MPT) *vs.* 61% (MPR) with no difference in VGPR/CR rates (18.8% *vs.* 23%). Grade ≥3 toxicity was 71.6% (MPT) *vs.* 56.7% (MPR); p=0.008. By ITT, the median PFS was 21m on MPT and 18.7m on MPR; HR 0.84 [95%CI: 0.64, 1.09]. The null hypothesis of inferiority of MPR was not rejected. Three year OS was identical by arm at 63% and median OS was not significantly different; p=0.476. Second primary malignancies were observed in 17 (MPT) *vs.* 14 (MPR) pts with incidence rates of 3.47 and 2.01 (/100 person years). DVT/PE occurred in 8.8% *vs.* 6.7% of pts. QoL analysis favored MPR by induction end; p=0.007.

Summary and Conclusions: This phase III trial compares the efficacy of MPT and MPR in elderly patients with newly diagnosed MM. Response rates, PFS and OS were similar between the two arms; however, there was significantly better QOL at 12m and lower toxicity with MPR.

S643

LENALIDOMIDE+LOW-DOSE DEXAMETHASONE (RD) *VS.* MELPHALAN-PREDNISONE-THALIDOMIDE (MPT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS (PTS): THE FIRST TRIAL

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Background: The MPT regimen is recognized as a standard therapy (Tx) for stem cell transplantation (SCT)-ineligible NDMM pts, based on a statistically significant advantage in overall survival (OS) and progression-free survival (PFS) *vs.* the combination of melphalan (MEL) and prednisone (PRED) (Facon, Lancet 2007; Fayers, Blood 2011; National Comprehensive Cancer Network, 2013). In the randomized ECOG E4A03 trial Rd increased OS and improved tolerability *vs.* lenalidomide (LEN) and high-dose dexamethasone (DEX) in NDMM pts (Rajkumar, Lancet Oncol 2010).

Aims: The FIRST trial (MM-020/IFM-0701) is a multicenter, open-label, pivotal phase 3 trial comparing the efficacy and safety of continuous Rd *vs.* MPT in SCT-ineligible NDMM pts.

Methods: SCT-ineligible NDMM pts or ≥65 years (yrs) were randomized to 1 of 3 arms: continuous Rd (LEN 25 mg/D, D1–21 and DEX 40 mg/week [wk] in 28-day cycles until disease progression); Rd18 (Rd in 28-day cycles for 72 wks [18 cycles]); or MPT (MEL 0.25 mg/kg/D, D1–4; PRED 2 mg/kg/D, D1–4; and thalidomide 200 mg/D in 42-day cycles for 72 wks [12 cycles]). Response was assessed using IMWG criteria after each cycle. Enrollment of pts with renal impairment was allowed with the exception of pts on dialysis. Thromboprophylaxis was mandatory. Stratification factors included age, International Staging System (ISS), and country. The primary endpoint was PFS (continuous Rd *vs.* MPT). Secondary endpoints included OS, overall response rate (ORR), time to response, duration of response (DOR), time to 2nd antimyeloma Tx, safety, and quality of life; exploratory analyses included PFS2.

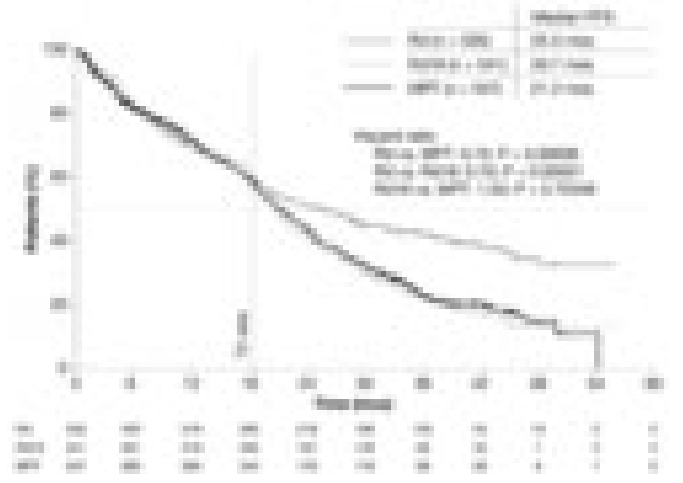

Figure 1. PFS in pts treated with continuous Rd, Rd for 72 wks (Rd18), or MPT for 72 wks.

Results: 1,623 pts were randomized to continuous Rd (535 pts), Rd18 (541 pts), and MPT (547 pts). Median age was 73 yrs, 35% of pts were ≥75 yrs, and 40% had ISS stage 3 disease. With a median follow-up of 37 months (mos), 23% of pts (121 pts) remained on continuous Rd. There was a 28% reduction in risk of PFS events with continuous Rd *vs.* MPT (HR=0.72; *P*=0.00006); there was also a reduction with Rd18 (HR=0.70; *P*=0.00001). Median PFS with continuous Rd, Rd18, and MPT were 25.5, 20.7, and 21.2 mos, respectively (Figure 1). The OS interim analysis showed a 22% reduction in risk of death in favor of continuous

Rd *vs.* MPT (HR=0.78; *P*=0.0168). Secondary endpoints consistently showed improvement with continuous Rd *vs.* MPT, respectively: ORR 75% *vs.* 62% (*P* <0.00001); median DOR 35.0 *vs.* 22.3 mos (HR=0.63; *P* <0.00001); and time to 2nd antimyeloma Tx 39.1 *vs.* 26.7 mos (HR=0.66; *P* <0.00001). Relevant grade 3–4 adverse events with continuous Rd *vs.* MPT, respectively, were neutropenia (28% *vs.* 45%), thrombocytopenia (8% *vs.* 11%), febrile neutropenia (1% *vs.* 3%), infection (29% *vs.* 17%), peripheral sensory neuropathy (1% *vs.* 9%), and deep-vein thrombosis and/or pulmonary embolism (8% *vs.* 5%). The incidence of hematologic second primary malignancies (SPMs) was 0.4% with continuous Rd *vs.* 2.2% with MPT; the incidence of solid tumor SPMs was similar in both Tx arms (2.8%).

Summary and Conclusions: In SCT-ineligible NDMM pts, Tx with the all-oral doublet continuous Rd significantly improved PFS, with an OS benefit at interim analysis, *vs.* the standard triplet MPT and *vs.* Rd18. The safety profile of continuous Rd was manageable with a lower incidence of hematologic SPMs *vs.* MPT. Thus, continuous Rd should be considered a new standard of care for SCT-ineligible NDMM pts.

S644

DO WE STILL NEED THE ALKYLATORS AS PART OF THE UPFRONT-TREATMENT OF ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS? UPDATED FOLLOW-UP OF GEM2005MAS65 SPANISH TRIAL COMPARING VMP *VS* VTP AS INDUCTION

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Background: Introduction: Melphalan (M) has been the most relevant drug for myeloma treatment for over 30 years and in combination with prednisone (MP) has been the backbone for new combinations development including thalidomide (MPT), lenalidomide (MPR) and bortezomib (MPV).

VMP has shown significantly increase the overall survival (OS) over MP in the VISTA trial. The main issue of VISTA is the toxicity profile and early discontinuations, which have been significantly reduced by the weekly and SQ administration of bortezomib. New alkylator-free schemes, such as lenalidomide plus low-dose dex, are emerging and challenging the role of alkylators in the treatment for elderly patients. The GEM2005 addressed this question in 2010 by comparing VMP with VTP as induction and the initial results, although with a short median follow-up, supported the value of alkylators upfront. In order to consolidate data on this hot topic, we have updated the outcome with very long follow-up (median of 72 months).

Aims: Pts and Methods: Between April 2005, and October 2008, 260 pts were randomized to receive 6 cycles, of VMP *vs* VTP as induction and those who completed the induction therapy were subsequently randomly assigned to maintenance therapy with VT or VP. The details were previously described (Lancet Oncology 2010), but during induction bz was given twice a week during the first cycle followed by five additional cycles in which bz was given weekly. Thalidomide was given at dose of 100 mg and melphalan and prednisone at conventional doses.

Results: Results: The median PFS was 32m and 23m for VMP and VTP arms, respectively (p=0.09). Deaths occurred in 73 pts treated with VMP (56%) and 91 pts (70%) in the VTP arm. VMP significantly prolonged the overall survival as compared with VTP (median of 63m *vs* 43m in VMP and VTP arms, respectively; HR:0,67; (p=0,01)). These results were not influenced by the maintenance received, VT or VP. Subsequent drugs used in first clinical relapse were balanced in both induction groups and included lenalidomide combinations in 40 (43.4%) pts, bz-based therapy in 19 (20.5%) pts, chemotherapy in 27 pts (29.1%) and six (6.5%) pts supportive care. OS from relapse was significantly longer for pts treated with VMP than VTP (17 *vs* 12 m, p=0,05). In multivariate analysis, to receive VMP as induction (HR:1,3), to have standard-risk cytogenetic abnormalities (HR: 1,85) and to achieve CR (HR=2,14) were independent factors associated with significantly longer OS. Pts who achieved CR had a significantly longer OS (median of 78 m) as compared with pts who didn't (median of 60 m) (HR: 2,26; p<0.0001) and this was more evident for pts who received VMP as induction (OS not reached: 53% at 8yrs) as compared with VTP (73m) (p=0,05). Moreover, median OS in the group of pts who achieved immunophenotypic CR has not been reached and 54% remain alive at 8 years while it was 58 months in patient who didn't achieve immunophenotypic CR. This benefit was also more evident for VMP and (median OS not reached: 66% alive at 8yrs) *vs* 73m for VTP (p=0,05).

Summary and Conclusions: Conclusions: Melphalan, in combination with bortezomib, should be maintained as one of the standards of care for treatment of elderly MM patients. VMP followed by maintenance with VT or VP significantly improved OS as compared with VTP. Patients who achieved CR and immunophenotypic response had significantly longer OS than patients who didn't and this benefit is even more evident in the VMP arm.

S645

CHARACTERISTICS AND OUTCOMES OF 714 PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS –ANALYSIS OF A PROSPECTIVE OBSERVATIONAL STUDY (ALCHEMY STUDY)

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Background: Systemic AL amyloidosis is a challanging condition to treat. Rarity of the disease as well as frailty of patients with advanced end organ damange makes clinical trials challanging and large prospective trials are lacking. We report the outcomes from ALChemy study (*AL Che*motherapy study) - one of the largest prospective observational trials in AL amyloidosis.

Aims: To assess the characteristics and treatent outcomes in a large prospectively followed cohort of patients with sytemic AL amyloidosis

Methods: We report the characteristics and outcomes of 714 patients with systemic AL amyloidosis seen at the UK National Amyloidosis centre from September 2009 to December 2013 who consented for recruitment in the ALChemy study. Treatment data on initial 500 patients is presented here - an update of all 714 will be presented at the meeting. Data on treatment/response/toxicity was collected monthly and with protocolized assessments every six months.

Results: Heart was involved in 68%, kidneys in 67% and liver in 13%. The Mayo disease stage was: stage I – 125 (18%); stage II - 287 (40%); stage III – 301 (42%). Median overall survival (OS) was not reached. The estimated survival at 12, 36 and 48 months was 67%, 56% and 51% respectively. Median OS was 8.8 months for Mayo stage III and 4.7 months for patients with NT-proBNP >8500 ng/L. In a landmark analysis of stage III patients at six months, the median survival was 49 months. Treatments were: thalidomide based 58%, lenalidomide based 3%, MelDex 6% and bortezomib based 25%. There was with no significant difference in survival with either regime but with estimated 75% *vs.* 61% survival at four months for patients with New Mayo stage 4 disease treated with Thalidomide-based/MelDex *vs.* bortezomib based regimes respectively. On an intention to treat basis, 51% achieved a ≥partial haematological response (PR) with a complete response (CR) in 14% and dFLC-VGPR in 24%. The median number of cycles was 4 and 59% experienced ≥grade 3. Fluid overload and infection were the commonest toxicities and cause of serious adverse events. Twenty-five percent patients reported ≥grade 3 toxicity in more than one cycle.

Summary and Conclusions: This large patient cohort confirms the improved survival in AL amyloidosis with median OS over 4 years. There is a significant problem of early deaths in patients with advanced stage disease. However, even patients with advanced systemic AL amyloidosis who surivive the first few months, have excellent longer term outcomes. There is substantial treatment related toxicity. Fluid overload, the commonest toxicity encountered, may be avoided in many cases by simple measures like daily weights and early diuretic adjustment helped by patient/physicial education. This trial confirms the utility of careful observational trials as a very important tool to study rare diseases.

Acute myeloid leukemia - Clinical 1

S646

ALL-TRANS RETINOIC ACID AS ADJUNCT TO INTENSIVE TREATMENT IN YOUNGER ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA – FINAL RESULTS OF THE AMLSG 07-04 RANDOMIZED TREATMENT TRIAL

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Background: Based on the WHO-2008 classification more than 70% of all acute myeloid leukemia (AML) patients can be categorized according to their underlying genetic aberrations with AML exhibiting nucleophosmin-1 gene (*NPM1*) mutations representing the largest genetic subgroup. In a biomarker study within a randomized trial of older AML patients, we could demonstrate that patients with mutated *NPM1* in the absence of a *FLT3*-ITD significantly benefit from *all*-trans retinoic acid (ATRA) as adjunct to chemotherapy (Schlenk *et al.* Haematologica 2009;94:54-69).
Aims: To evaluate the impact of ATRA in combination with chemotherapy on outcome, and to assess the *NPM1* mutational status in relation to ATRA as a marker for response in younger adult AML-patients entered in the prospective randomized treatment trial AMLSG 07-04 (ClinicalTrials.gov Identifier: NCT00151242).
Methods: Patients (18 to 60 years of age) were accrued between 2004 and 2009. They were randomized up-front for open-label treatment with ATRA and in the first 372 patients also for valproic acid (VPA). Induction therapy consisted of two cycles of ICE. For consolidation therapy, patients with high-risk AML, defined either by high-risk cytogenetics or induction failure, were assigned to receive allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a matched related (MRD) or unrelated donor. All other patients were assigned to 3 cycles of high-dose cytarabine or, if an MRD was available, an allo-HSCT in intermediate-risk AML. During induction cycles, ATRA was given in a dosage of 45mg/m² from day 6 to 8, and 15mg/m² from day 9 to 21. The primary and secondary end points of the study were event-free survival (EFS) and rate of complete remission (CR) as well as cumulative incidence of relapse (CIR) and overall survival (OS), respectively. The analyses were performed on an intention-to-treat (ITT) and a per-protocol (PP) basis.
Results: A total of 1100 patients were randomized, 556 in the standard arm, and 544 in the ATRA-arm; in both arms n=19 patients received or did not receive ATRA in contrary to their randomization. After a planned interim analysis on the endpoint CR-rate, the randomization for VPA was stopped in 2006 due to ineffectiveness. Median follow-up for survival was 5.1 years. *NPM1* mutational status was available in 1007 patients (92%) and a mutation was identified in 287 (29%) patients. Pretreatment patient characteristics were well balanced between the standard and the ATRA-arm, except for higher white blood counts (WBC) and peripheral blast percentages in the standard arm (p=0.003, each). In multivariable analyses on the endpoint CR, ATRA neither as single factor nor as interaction term with *NPM1* had a significant impact on an ITT basis. In contrast, the same analysis on a PP basis revealed a significant impact of the ATRA-*NPM1* interaction (OR, 2.07; p=0.03) indicating a higher probability for AML-patients with mutated *NPM1* treated with ATRA to achieve a CR. Almost similar results were found for EFS; in the ITT analysis the impact of the ATRA-*NPM1* interaction was not significant, whereas in the PP analysis this interaction had a significant impact (HR, 0.75; p=0.04) indicating a higher probability for AML-patients with mutated *NPM1* treated with ATRA to stay event-free. Explorative analysis in all patients on OS revealed a benefit for ATRA (ITT, p=0.09; PP, p=0.01). This beneficial effect of ATRA could be attributed to patients with ELN-favourable risk including core-binding factor AML, AML with *CEBPA*dm and AML with mutated *NPM1* in the absence of *FLT3*-ITD (p=0.04).
Summary and Conclusions: ATRA as adjunct to chemotherapy improved response to induction therapy and EFS in *NPM1* mutated-AML, as well as OS in patients with ELN favourable risk.

S647

RESULTS OF A RANDOMIZED MULTICENTER PHASE 2 STUDY OF A 5-DAY REGIMEN OF SGI-110, A NOVEL HYPOMETHYLATING AGENT, IN TREATMENT NAÏVE ELDERLY ACUTE MYELOID LEUKEMIA NOT ELIGIBLE FOR INTENSIVE THERAPY

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Background: SGI-110 is a second generation Hypomethylating Agent (HMA) given as a small volume subcutaneous (SC) administration. Relative to first generation HMAs, SGI-110 prolongs half-life and extends exposure to the cytidine analog decitabine. In the Phase 1 study, SGI-110 given daily for 5 days (QDx5) produced potent hypomethylation and clinical responses in MDS and AML patients previously treated with first generation HMAs (Kantarjian *et al.* 2012). The Biologically Effective Dose (BED) defined as the smallest dose producing maximum demethylation was 60 mg/m^2 QDx5 while 90 mg/m^2 QDx5 was the highest dose with no Dose Limiting Toxicities.
Aims: To assess efficacy and safety of two doses of SGI-110 given as a 5-day SC regimen every 28 days in elderly AML.
Methods: In a randomized Phase 2 study, treatment naïve elderly (≥65 years) AML patients who were not suitable for intensive induction chemotherapy due to secondary AML, unfavorable cytogenetics, poor Performance Status (PS), or major organ dysfunction were randomized to one of two SC SGI-110 doses – 60 mg/m^2 QDx5 or 90 mg/m^2 QDx5 every 28 days till progression. The primary endpoint was Overall Complete Remission (OCR=CR+CRi+CRp) rate at any time based on International Working Group Criteria (Cheson 2003). Adverse events (AEs) and Long Interspersed Nucleotide Element (LINE-1) DNA methylation pharmacodynamics were also reported.
Results: The study has completed enrolment with 51 patients treated: 24 and 27 patients were randomized to 60 and 90 mg/m^2 doses respectively. Patient characteristics were generally balanced between the 2 dose groups. However there were more patients with poor PS on the 60 mg/m^2 dose and more patients with poor risk cytogenetics in the 90 mg/m^2 dose: Median age (78y; 77y); ECOG PS 2 (46%, 26%), Males (58%, 59%), poor risk cytogenetics (33%, 44%) for the 60 and 90 mg/m^2 dose groups respectively. The primary endpoint of OCR was observed in 12 (8 CR+4CRi) and 12 (9CR+3CRi) patients or 50% and 44% for 60 and 90 mg/m^2 dose groups respectively. OCR for both doses combined was achieved in 24 of 51 patients (17 CR, 7 CRi, no CRp) or 47% (95% CI: 32.9%, 61.5%). LINE-1 DNA methylation data before and after treatment were available in 48 (94.1%) patients. The average maximum LINE-1 demethylation was similar for the 2 dose groups (-18.6% for 60 mg/m^2 and -21.4% for 90 mg/m^2). The 90 mg/m^2 dose showed similar safety profile to 60 mg/m^2 except for neutropenia for which the incidence was higher in the 90 mg/m^2 dose group. The most common adverse events (AEs) ≥Grade 3, regardless of relationship to SGI-110, for the 2 doses combined were febrile neutropenia (43%), thrombocytopenia (39%), neutropenia (33%), anemia (24%), and leukopenia (22%). SGI-110 treatment with the two dose groups combined was associated with relatively low all-cause mortality (compared to intensive chemotherapy) at 30/60 days of 5.9%, and 15.7% respectively.
Summary and Conclusions: Second generation HMA SGI-110 SC QDx5 treatment is well tolerated and clinically active in elderly AML patients with other poor prognostic criteria who are unlikely to benefit from intensive chemotherapy. CR, OCR, safety, and early mortality compare favorably with previous results reported for other HMA treatment and merit confirmation in a larger randomized trial. Efficacy and safety were largely similar for the 2 dose groups. These data support evaluation of the 60 mg/m^2 QDx5 every 28 days dose regimen in a Phase 3 investigation of SGI-110 in elderly AML not eligible for intensive chemotherapy.

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A PHASE I DOSE-ESCALATION STUDY OF PRI-724, A CBP/B-CATENIN MODULATOR IN PATIENTS WITH ADVANCED ACUTE MYELOID LEUKEMIA (AML)

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Background: PRI-724 is a first-in-class CBP/β-catenin modulator that inhibits the cAMP-response element-binding protein (CREB)-binding protein (CBP) and β-catenin interaction and decreases leukemic stem cell proliferation and survival. In pre-clinical models, PRI-724 and its active metabolite C-82 increase p300/β-catenin binding and promote differentiation of leukemia initiating cells, thereby increasing sensitivity to cytotoxic drugs.
Aims: The aims of this study are to evaluate PRI-724 in patients with AML for safety, toxicity, tolerability, efficacy and PK profile; and to delineate the pharmacodynamic marker of drug-on-target effect with gene expression in peripheral blood (PB) and bone marrow (BM) in patients.
Methods: Relapsed/refractory AML patients age over 18 years old were enrolled. PRI-724 was given as a continuous infusion X 7 days q 2 weeks starting at 640 mg/m^2/day with dose escalation in a 3+3 design up to 1280mg/m^2/day unless a dose limiting toxicity (DLT) occurred. Based on available PK and safety data from solid tumor phase I study, no dose escalation beyond 1280 mg/m^2 was planned. Survivin expression of both PB and BM were measured by RT-PCR and flow cytometory and CD44 by RT-PCR.
Results: Dose-escalation phase was completed with 3 cohorts (640, 920 and 1280 mg/m^2) including 14 pts. Median age was 62 years (range 42-83). 9 of 14 pts (64%) were evaluable for DLT (*i.e.*, completed 28-day observation); 5 other discontinued early for progressive disease without DLT. Evaluable patients completed a median of 3 cycles (range, 2 to 5). The maximum dose was determined to be 1280 mg/m^2/day. There was no DLT and no MTD at the doses evaluated. Of 104 reported AEs, only 4 grade-1 AEs (2 nausea, 1 vomiting and 1 diarrhea) were judged to be related to the study drug. All 16 reported SAEs were considered by the investigators not study drug related. Per protocol, BM blast assessment was done at pre-treatment and at the end of Cycle2. Within the evaluable subjects, 7 of 9 (78%) had samples available for PD evaluation; relative to baseline, 5 of 7 (71%) showed blast decrease at the end of Cycle2 with median decrease of 44% [range 14-78%]. 2 of 7 (29%) showed blast decrease to 10% in the BM; one from baseline of 46% and the other from 18%. Decrease of CD44 expressions in both PB and BM was closely correlated with the decrease of blast counts. BM survivin decrease was also seen. The median Cmax and $AUC_{0\text{-}t}$ for C-82 at 920 mg/m^2/day were 2512 ng/mL and 272912 h*ng/mL. The median elimination T½ was 1.96 h.
Summary and Conclusions: PRI-724 is well tolerated in this study with an acceptable toxicity profile. The down-regulation of CD44 expression in PB and BM and survivin expression in BM serves as a pharmacodynamic marker of drug-on-target effect. Studies combining PRI-724 with standard anti-leukemia chemotherapy are on-going for AML and CML patients.

S649

PHASE I/II STUDY OF VOLASERTIB, AN INTRAVENOUS POLO-LIKE KINASE INHIBITOR (PLK), IN PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA (AML): UPDATED PHASE I RESULTS FOR VOLASERTIB MONOTHERAPY

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Background: The prognosis of patients (pts) with relapsed or refractory (rel/ref) AML considered unlikely to benefit from, or tolerate, intensive salvage treatment is unfavorable and novel therapies are needed. Plks are critical in cellular division and mitotic progression. Volasertib (lab code: BI 6727) is a selective and potent cell cycle kinase inhibitor that induces mitotic arrest and apoptosis by targeting Plk. Here we present from the phase I/II study, the updated phase I results of volasertib monotherapy in pts with rel/ref AML considered ineligible for intensive salvage treatment.
Aims: This study aimed to determine the maximum tolerated dose (MTD), safety, and pharmacokinetics (PK) of volasertib monotherapy.
Methods: Pts with rel/ref AML received volasertib as a 1-hr intravenous infusion on Days 1 and 15 Q4W. Dose escalation for MTD determination followed a 3+3 design with de-escalation. Blood samples for PK analyses of volasertib were taken in Cycles 1 and 2, before and at several time points after infusion, up to 2 weeks. Bone marrow samples were collected in Cycle 1.
Results: Increasing volasertib doses (150-200 mg, 350-550 mg) were evaluated in 56 pts including an MTD extension cohort of 15 pts (median age: 70 yrs [range 26-84]; 84% aged ≥65 yrs). All pts had failed prior AML treatment (median of two previous lines of antileukemic therapy [range 1-5]).
Over all cycles, adverse events (AEs) were reported in all 56 pts. Drug-related AEs were reported in 38 pts (68%). Most frequent drug-related AEs (>10%) were anemia (27%), thrombocytopenia and neutropenia (25% each), leukopenia (13%), and alopecia (11%). Grade ≥3 drug-related AEs (occurring in ≥3 pts) included neutropenia and thrombocytopenia (25% each), anemia (21%), leukopenia (13%), mucosal inflammation (7%), and pneumonia (5%). Dose-limiting toxicities (DLTs) in Cycle 1 were: pneumonia (fungal; 150 mg, n=1); mucosal inflammation (400 mg, n=1); pyrexia (450 mg, n=1); thrombocytopenia and epistaxis (500 mg, n=1); gastrointestinal (GI) inflammation and oesophagitis (500 mg, n=1); mucosal inflammation (550 mg, n=1); pneumonia, mucosal inflammation, GI hemorrhage, hemoptysis, lung infiltration and dyspnea (550 mg, n=1). Based on reported DLTs the MTD was determined to be 450 mg.
At higher volasertib doses (≥350 mg), antileukemic activity was observed with 5/43 pts (12%) achieving a complete remission with incomplete blood count recovery (CRi), as best response. Pts achieving CRi stayed on treatment for a median of 3 cycles (range 2-9). Bone marrow samples showed a pattern of nuclear condensation and fragmentation characteristic of apoptosis following prolonged mitotic arrest by Plk inhibition (Polo-arrest). Volasertib is a moderate clearance drug with a volume of distribution >5000 L and a terminal half-life of ~113 hrs. No accumulation was observed between the first two analyzed treatment cycles.
Summary and Conclusions: This study determined the MTD of volasertib to be 450 mg on Days 1 and 15 Q4W. The safety profile of volasertib was clinically manageable in this heavily pretreated pt population at doses above the recommended phase II dose previously determined in solid tumors. Most of the higher grade drug-related AEs were expected from the antimitotic mode of action. These results demonstrate the clinical antileukemic effect of single agent volasertib in pts with rel/ref AML, further supporting Plk as a potential therapeutic target in AML. A phase III trial of low dose cytarabine, with or without volasertib, in older untreated pts with AML ineligible for intensive therapy, is ongoing.

S650

FIRST-IN-CLASS NAE INHIBITOR MLN4924 IN COMBINATION WITH AZACITIDINE FOR ACUTE MYELOID LEUKEMIA (AML) PATIENTS CONSIDERED UNFIT FOR CONVENTIONAL CHEMOTHERAPY: RESULTS FROM THE C15009 TRIAL

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Background: The management of AML in older patients considered unfit for standard chemotherapy is challenging. Hypomethylating agents are commonly used alternatives in this group of patients. In the case of azacitidine, complete (CR) and partial remission (PR) rates are generally limited and responses are typically seen after 4-6 cycles. MLN4924 is an investigational NEDD8-activating enzyme (NAE) inhibitor. Preclinical studies in AML cell lines and xenograft models are suggestive of synergistic activity with combined treatment (Smith *et al.*, ASH 2011, abstract #578).
Aims: The primary objectives of this phase 1 dose-escalation study were to define dose-limiting toxicities (DLTs), the maximum tolerated dose (MTD), and the safety and tolerability of MLN4924 in combination with azacitidine. Secondary objectives included assessments of pharmacokinetics (PK) and disease response.
Methods: Previously untreated AML patients aged ≥60 years who were considered unfit for or unlikely to benefit from standard induction chemotherapy received MLN4924 by IV infusion over 1 hour on days 1, 3, and 5. Azacitidine 75 mg/m^2 (IV, n=11, or SC, n=3 [SC only included after dose-escalation phase]) was given on days 1-5 and days 8-9. Cycles were repeated every 28 days. Adverse events (AEs) were evaluated per NCI-CTCAE v4.03. Response was assessed per revised International Working Group recommendations. Blood samples for PK analysis were collected on dosing days 1 and 5 of cycle 1 only.
Results: At data cut-off (Jan 6, 2014), 14 patients had received MLN4924 at 20 mg/m^2 (n=11) and 30 mg/m^2 (n=3). Median age was 76.5 years (63-85), 7/7 were male/female, 8 had *de novo* AML, 6 had MDS/AML, and 4 had adverse and 7 intermediate-risk cytogenetics (3 undetermined). Patients' baseline AML mutation profile will be presented. One patient each had grade 3 AST elevation and grade 2 increased blood bilirubin at MLN4924 30 mg/m^2 that was dose-limiting (Table); liver enzymes returned to normal following discontinuation of MLN4924. None of the 11 patients treated at the MTD (MLN4924 20 mg/m^2 plus IV/SC azacitidine 75 mg/m^2) experienced further DLTs. Ten patients remain on treatment. The most common AEs (Table) included anemia, constipation, and thrombocytopenia (each n=4); 8 patients had drug-related AEs, and 5 had drug-related grade ≥3 AEs, with only anemia and thrombocytopenia (each n=2) seen in >1 patient. The safety profile of azacitidine is well established and no new toxicities were observed with the addition of MLN4924. MLN4924 PK has been previously described in single-agent studies and remained unchanged in com-

bination with IV azacitidine (based on preliminary data: individual PK profiles from 8 patients on days 1 and 5). In the 10 patients evaluable for response, the CR/PR rate was 60%, including 4 CRs and 2 PRs as best response; for five of the six patients reporting a response, the first evidence of response was documented within the first two cycles of therapy (Table 1).

Table 1.

Summary and Conclusions: These data suggest that azacitidine plus MLN4924 20 mg/m^2 is generally well tolerated in older patients with AML who are unfit for standard chemotherapy. A randomized phase 3 trial is currently being planned.

Indolent Non-Hodgkin lymphoma - Clinical

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POST-INDUCTION THERAPY FDG-PET IS PROGNOSTIC FOR PROGRESSION-FREE SURVIVAL IN RELAPSED FOLLICULAR LYMPHOMA: A PRELIMINARY ANALYSIS OF THE GAUSS STUDY

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Background: Use of fluorodeoxyglucose positron emission tomography (FDG-PET) as a prognostic tool after post-induction therapy (post-IT) in follicular lymphoma (FL) has not been fully investigated, particularly following immunotherapy alone, in patients (pts) with relapsed indolent NHL and in regimens that include maintenance. The three evaluation criteria; International Harmonization Project (IHP), Deauville 5-point scale (D5-PS) and European Organisation for Research and Treatment of Cancer criteria (EORTC-c) have not been validated for indolent NHL after induction treatment.

Aims: To determine whether post-IT FDG-PET based response assessment using IHP, D5-PS and EORTC criteria is a suitable prognostic endpoint for PFS in pts with relapsed FL treated with obinutuzumab (GA101) or rituximab monotherapy.

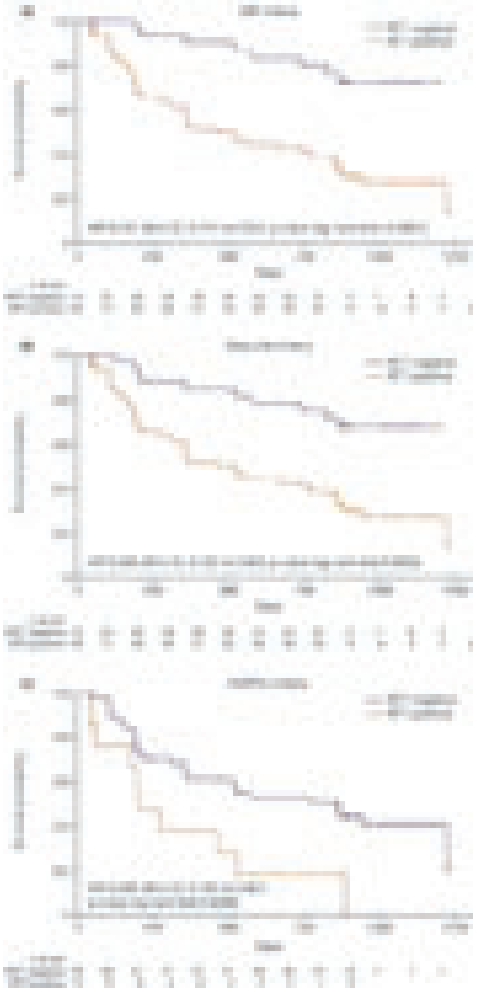

Figure 1.

Methods: In the phase II GAUSS trial (BO21003; NCT00576758), pts with FL were randomized to treatment with obinutuzumab or rituximab monotherapy. FDG-PET was performed at baseline and 4-6 weeks post-IT and was interpreted using IHP as the primary reading criteria, with D5-PS and EORTC-c as secondary reading criteria. The reduction in standardized uptake values (ΔSUV_{max}) between baseline and post-IT was assessed in only those tumours with the highest FDG uptake. Receiver-operating characteristics (ROC) analysis was used to determine an optimal cut-off for ΔSUV_{max} in predicting disease progression or death. Multivariate Cox analyses were performed to assess various factors (post-IT PET result, conventional response and three baseline factors; β2-microglobulin, Follicular Lymphoma International Prognostic Index and bulky disease) as prognostic indicators for PFS.
Results: The clinical results of this study have been presented elsewhere (ASH 2011: Sehn *et al.* Blood 2011;118: Abstract #269). Of 147 evaluable pts, 132 (median age=62 years) were analysed at baseline and 118 at post-IT, using IHP as the primary reading criteria. After a median follow up of 32.1 months, 44.6% of pts in the obinutuzumab arm and 56.1% in the rituximab arm had died or progressed. The post-IT PET results significantly correlated with response by all criteria (IHP, p=0.0002; D5-PS, p=0.0003; EORTC, p=0.0012). Using IHP criteria, the median PFS was 517 days for PET+ pts *versus* 'not reached' for PET- pts (95% confidence interval [CI], 309-772). The risk of disease progression was reduced in PET- compared with PET+ pts using IHP (hazard ratio [HR], 0.25; 95% CI, 0.117-0.5220.117-0.522; p<0.0001), D5-PS (HR 0.31; 95% CI, 0.155-0.6030.155-0.603; p=0.0003) and EORTC criteria (HR 0.39; 95% CI,0.191-0.8070.191-0.807; p=0.0083; Figures 1A-C). Post-IT PET results (p=0.0041) and conventional response (overall p=0.0442) were significant prognostic factors for PFS. The ROC analysis yielded an optimal ΔSUVmax cut-off of 49% in predicting disease progression or death (HR 0.31; 95% CI, 0.161-0.6080.161-0.608; p=0.0003).
Summary and Conclusions: Overall, post-IT PET status was a strong predictor of PFS regardless of the assessment criteria used in this pre-treated FL cohort. The optimal ΔSUVmax cut-off in predicting PFS was around 50%. Further evaluation using quantitative parameters is underway. These data provide further proof that post-IT PET may be used in risk-adapted clinical trials as a surrogate for PFS.

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SUBCUTANEOUS RITUXIMAB AND CHEMOTHERAPY ACHIEVES SIMILAR OVERALL RESPONSE RATES TO INTRAVENOUS RITUXIMAB IN FIRST-LINE FOLLICULAR LYMPHOMA: EFFICACY AND SAFETY RESULTS OF THE PHASE III SABRINA STUDY

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Background: Rituximab (R) plus chemotherapy induction followed by R maintenance is the backbone therapy for follicular lymphoma (FL). Intravenous R (R^{IV}) administration time and patient convenience could be improved by the development of a subcutaneous formulation (R^{SC}).
Aims: Pharmacokinetic non-inferiority was demonstrated in Stage 1 of the study with a $C_{trough,SC}$:$C_{trough,IV}$ ratio of 1.62 (90% confidence interval [CI]: 1.36-1.94) and provisional efficacy was comparable between R^{SC} and R^{IV} based on 127 randomised patients (pts; Davies *et al.* Lancet Oncol 2014). An additional 283 pts were randomised in Stage 2 to assess efficacy and safety of R^{SC} in a larger patient population.
Methods: SABRINA (BO22334; NCT01200758) is a two-stage phase III study of R^{SC} 1400mg or R^{IV} 375mg/m^2 plus chemotherapy (≤8 cycles CHOP [cyclophosphamide, doxorubicin, vincristine, prednisone] or 8 cycles CVP [cyclophosphamide, vincristine, prednisone]) every 3 weeks during induction (first cycle R^{IV} on both arms) followed by R^{SC} or R^{IV} maintenance every 8 weeks in pts with FL. The primary objective of stage 2 was to provide additional efficacy data and estimate the overall response rate (confirmed [CR] and unconfirmed [CRu] complete response, and partial response) at the end of induction treatment. Here we report pooled efficacy and safety analyses from Stage 1 (127 pts) and Stage 2 (283 pts).
Results: Pts with previously untreated confirmed CD20-positive grade 1, 2, or 3a FL (n=410) were randomised to R^{SC} (n=205) or R^{IV} (n=205). Pts were stratified by Follicular Lymphoma International Prognostic Index score, chemotherapy, and region. In each arm, approximately 64% pts received CHOP and 36% received CVP chemotherapy. Investigator-assessed overall response rates (ORR) at the end of induction were 83.4% (95% CI: 77.6%, 88.2%) in R^{SC} pts and 84.4% (95% CI: 78.7%, 89.1%) in R^{IV} pts. CR/CRu rates were 32.7% (67/205 pts) for R^{SC}, and 31.7% (65/205 pts) for R^{IV}. After a median follow-up of approximately 14.4 months, similar rates of adverse events (AEs; 184/197 [93%] SC; 194/210 [92%] IV) were reported in each arm. The majority of AEs were grade ≤2 in severity (1469/1651 AEs [90%] in the R^{SC} arm; 1225/1386 AEs [88%] in the R^{IV} arm). The incidence of pts with at least one grade ≥3 AE was similar between R^{SC} (96/197, 49%) and R^{IV} (99/210, 47%). Serious AEs (SAEs) were reported in 57 pts (29%) R^{SC} and 55 pts (26%) R^{IV}, respectively. Infections were the most frequently reported SAEs (20/197 [10%] SC; 16/210 [8%] IV) followed by febrile neutropenia (11/197 [6%] SC; 9/210 [4%] IV). Other common haematological AEs were neutropenia and anaemia (both ≤5% of pts in each group). Administration-related reactions occurred in 93 (47%) R^{SC} pts and 70 (33%) R^{IV}, pts and were predominantly grade 1 or 2. The difference was mainly due to grade 1 injection site erythema (10% *vs* 0%) which was anticipated following the change in route of administration.
Summary and Conclusions: R^{SC} demonstrated comparable ORR and CR rates with R^{IV}. The safety profile of R^{SC} and R^{IV} was similar and there were no new safety concerns with the SC formulation. Availability of R^{SC} administration over approximately 5 minutes is expected to have a positive impact on pt convenience and health care resource savings without compromising the anti-lymphoma activity of rituximab.

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RISK STRATIFICATION IN SMZL PATIENTS: VALIDATION OF THE SIMPLIFIED PROGNOSTIC SCORE FOR SPLENIC MARGINAL ZONE LYMPHOMA OF THE SPLENIC MARGINAL ZONE LYMPHOMA WORKING GROUP

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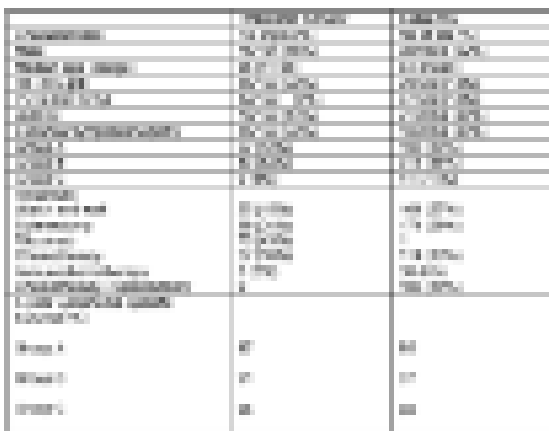
Background: splenic marginal zone lymphoma (SMZL) is generally characterized by an indolent clinical behavior. However a substantial proportion of patients presents with a more aggressive clinical course and short survival. Several prognostic factors have been proposed. Recently, a new simplified prognostic score has been proposed by the SMZLWG.
Aims: In this collaborative study we evaluate the reproducibility of the simplified risk stratification score in a larger series of SMZL patients.
Methods: The prognostic index was based on the combination of 4 factors: haemoglobin level<9.5 g/dL, platelet counts<80×10^9/L, elevated LDH and the presence of extrahilar lymphadenopathy. All variables were assigned the same value of one point each. Three risk groups were identified: low (A) 0 points, intermediate (B) 1-2 points and a high risk group (C) 3-4 points. Kaplan-Meier method was used to estimate survival and the log-rank test to compare survival curves.
Results: Among 164 SMZL patients, 159 had complete data for all variables and were included in this analysis (Table 1). The median age was 65 years (range, 41-95) with a slight female predominance (89/164 or 54%). Anemia and thrombocytopenia were present in 35/159 (22%) and 12/159 (8%), respectively. Serum lactate dehydrogenase was elevated in 44% (70/159) while 35/159 (22%) of SMZL patients had extrahilar lymphadenopathy at diagnosis. The stratification of our patients in the three risk groups were as follows: 67/159 (42%) were stratified into group A, 83/159 (52%) into group B and 9/159 (6%) into group C. Treatment differed among the three risk groups. The 5- and 10-year LSS was 97% & 93% for group A, 91% & 66% for group B and 65% at 5 years (but not applicable at 10 years due to shorter follow-up) for group C (p=0.0001). The 5- and 10-year OS was 93% & 89% for group A, 81% & 57% for group B and 49% at 5 years (but not applicable at 10 years) for group C (p=0.0001).

Table 1. Comparison of the clinical characteristics between the present study and the SMZLWG pts.



Summary and Conclusions: Our results confirm the applicability of the recently proposed simplified prognostic system of SMZLWG in an independent series of 159 SMZL patients.

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A PHASE II STUDY OF RITUXIMAB PLUS LENALIDOMIDE IN PATIENTS WITH EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF THE MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

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Background: Extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma) is a distinct entity thought to arise from marginal zone B-cells related to plasma cells. MALT lymphoma shares some clinical features with multiple myeloma (*e.g.* immunoglobulin production by lymphoma cells and plasmacytic differentiation) and a recent study with lenalidomide (LEN) monotherapy reported activity in MALT lymphoma.
Aims: To further improve the activity of LEN in MALT lymphoma, we initiated a phase II study of rituximab (RIT) plus LEN in pts with MALT lymphoma. Patients (pts) with histologically verified gastric or extragastric MALT lymphoma received RIT 375 mg/m² i.v. day 1 and oral LEN at a dose of 20 mg daily given on days 1 - 21. In gastric MALT lymphoma with evidence of *Helicobacter pylori* (HP) infection, pts had to be refractory to, or relapsing after successful HP-eradication; pts without evidence of HP were directly eligible. Treatment was repeated every 28 days with restaging after 3 and 6 cycles. Patients with stable disease or better after 3 cycles were given another 3 cycles. If remaining lymphoma was detected after 6 cycles another 2 cycles were given for a maximum of 8 cycles. Patients with evidence of progressive disease were taken off study. All pts received prophylactic ASA (100 mg daily) for the duration of treatment, and allopurinol (300 mg daily) for the first 4 weeks of therapy. The primary endpoint of the study was objective response rate and the secondary endpoint was safety and tolerability.
Results:: To date, 43 patients have been enrolled (28 female, 15 male); 30 are evaluable for efficacy, having undergone at least 1 restaging investigation (3 after 8, 20 after 6, and 5 after 3 courses of therapy, and 1 pt after withdrawal following the first course of therapy). The most common primary localizations were the ocular adnexa (13 pts), stomach (10 pts), and lung (5 pts); the remaining pts had MALT lymphoma of the parotid, colon, small intestine, liver, and subcutaneous tissue, respectively. Nine pts were pretreated; 2 pts having relapsed after CR from LEN monotherapy[AM1]. At the time of enrollment 13 pts had localized disease, while the remaining 30 had disseminated disease. The overall response rate was 26/30 (87%), with 14 complete (47%, including the 2 patients pretreated with LEN) and 12 partial remissions (40%). Three pts had stable disease, and 1 pt progressed during therapy and was given salvage therapy. All 43 pts were evaluable for safety. Toxicities were mostly non-haematologic and of grade I/II with exanthema gr I/II in 15 patients being the most common event. Two pts had diarrhea, nausea and joint-pain III, 7 pts presented with exanthema/pruritus III, 1 pt had vertigo III, and 2 pts had infections necessitating hospitalization. Haematologic side effects included leucopenia (10 pts; 1 pt grade III), thrombocytopenia (5 pts; I/II), and anemia (6 pts; 2 grade III). Dose reductions were performed in 10 pts (8 to 15 mg and 2 to 10 mg), while 4 pts discontinued treatment early due to toxicity.
Summary and Conclusions: This is the first study on efficacy of LEN plus RIT in pts with MALT lymphoma. The promising interim analysis CR rate (currently 47%) response rate of 87% is likely to increase in terms of with continued therapy in pts who had not undergone the full scheduled courses. As expected, the response rate is higher than the activity reported with LEN monotherapy in a pilot study in MALT lymphoma (RR 61%), and toxicities appear manageable and mostly non-haematological.

S655

BENDAMUSTINE AND RITUXIMAB COMBINATION IS SAFE AND EFFECTIVE AS SALVAGE REGIMEN IN WALDENSTROM'S MACROGLOBULINEMIA

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Background: Rituximab (R) plus chemotherapy is a standard of care in pts with indolent lymphoprolipherative disorders. In the Stil trail the combination Bendamustine (B) plus R demonstrated a PFS benefit and improved tolerability comapred with CHOP-R when administered in first line treatment in Waldenstrom's Macroglobulinemia (WM). Limited data are evaluable on the setting of pretreated patients.
Aims: To define the efficacy and tolerability of BR combination as salvage regimen in symptomatic WM patients.
Methods: We retrospectively analyzed the outcome of symptomatic WM patients treated with BR in 11 Italian centers. All patients receiving at least one day of treatment were included in the study. Treatment consisted of: R 375 mg/sqm iv day 1 and B either at the dosage of 90 or 70 mg/sqm iv days 1,2. Therapy was administered every 4 weeks up to 6 courses.
Results: Fifty-four patients are included in the study. At treatment, median age was 70 years (49-86), sex ratio M/F 33/21. Median number of prior regimens was 2 (range 1-6), being median time from WM diagnosis and from last treatment to BR administration of 85 and 15 months respectively. Eighteen patients (33%) presented with refractory disease The 89% of patients had previously received rituximab based immunochemotherapy, in 32% previous treatment included Fludarabine. In the majority of patients (63%) reason for starting treatment was anemia, 17% of patients presented with hyperviscosity syndrome. Median IgM level at treatment was 3995 mg/dL (range 1028-8040). Lymphadenopathy and splenomegaly were present in 31% and 18% patients respectively. Fifteen patients (28%) received B at the lower dosage of 70 mg/sqm. Overall 282 courses of BR treatment were administered, median number 6 (range 2-6). Sixteen patients (29,6%) received fewer than 6 cycles, cytopenia was the major cause of discontinuation 5/16 (31%). No difference in terms of treatment discontinuation was observed according to B dosage administered and patients age. Overall response rate (ORR) was 83.4%, including 5.6% complete remissions CR), 18.5% very good partial remissions (VGPR) and 59.3% partial remissions (PR), 13% of patients remained in stable disease while disease progression rate was 1.9%. One death (1.9%) due to infection was recorded. An amelioration of response was observed in 4 cases: 1 PR converted in VGPR and 3 SD reached a PR, leading to a final ORR of 88.8%. None of the clinical and biological characteristics considered (age, sex, disease status, previous lines of treatment, previous fludarabine, bulky dissease, Hb and IgM level, β 2 microglobulin, B dosage, had an impact in quality of response achievement (CR plus VGPR *versus* PR). A trend, even if not significative (*P=0.05*), of higher ORR achievement was observed among patients treated with the higher dosage of B. Considering that most of the patients received prophylactic growth factors, grade 3-4 neutropenia developed in only 13% of courses, 38% of patients. Dose modification or delayed treatment administration was necessary in 5 and 10% of courses respectively. Twelve episodes of FUO and 3 major infections, leading death in one case, were recorded during treatment. Seven (14.5%) of the 48 responding patients showed a disease progression after a median of 12 m. After a median follow up of 15 monts none of the patients developed secondary myelodisplastic syndrome, acute leukemia or diffuse large B-cell lymphoma. In 2 cases a solid cancer was observed.
Summary and Conclusions: BR combination showed to be as effective as more intensive salvage regimens in pretreated WM patients effective. Treatment showed to be well tolerated even in elderly patients with limited episodes of myelosuppression and infections when compared to purine analogues including regimens.

Chronic lymphocytic leukemia and related disorders - Biology

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ACQUIRED INITIATING MUTATIONS IN EARLY HEMATOPOIETIC CELLS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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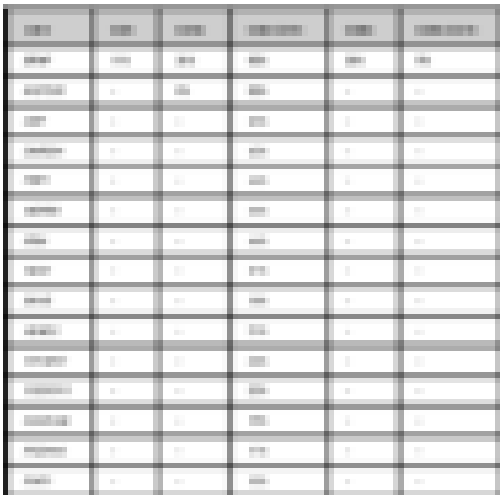
Background: Chronic lymphocytic leukemia (CLL) is a clonal accumulation of mature B-lymphocytes and is the most frequent adult leukemia in western countries. The cell of origin of this incurable disease is still elusive.
Aims: In the present study we aimed to investigate the repartition of acquired mutations in the hematopoietic differentiation tree of CLL patients using a combination of whole-exome and targeted deep resequencing.
Methods: Diagnostic pretreatment blood samples of 24 CLL patients (17 *IGHV*-unmutated and 7 *IGHV*-mutated) were flow-sorted for $CD3^+$ T-lymphocytes, $CD5^+CD19^+$ tumor cells, $CD14^+$ monocytes, and $CD34^+CD19^-$, progenitors. Acquired mutations were identified by comparison of exome sequences of tumor cells and T-lymphocytes. Subsequently we used targeted deep resequencing to simultaneously validate and quantify the mutation burden of candidate mutations in DNA from all sorted cell fractions. In order to establish multipotent progenitor involvement, we genotyped colonies from single $CD34^+CD19^-$, cells from 18 of the investigated patients, grown *in vitro* in myeloid conditions.
Results: Whole-exome sequencing identified a total of 415 somatic mutations predicted to result in protein-coding changes of 361 different genes with a median of 17 mutations/ patient. Among the 24 patients analyzed, only 3 did not exhibit detectable mutations in the $CD34^+$ progenitor or the $CD14^+$ monocyte fractions, all of which exhibiting mutated *IGHV* rearrangements in their CLL cells. In all other 21 patients, at least one mutation was detectable in the $CD14^+$ or in the $CD34^+$ fractions (see Table 1). Genotyping of $CD34^+CD19^-$–derived myeloid colonies confirmed the presence of CLL mutations in myeloid cells in 13 patients. These mutations included classical CLL mutations, such as *SF3B1* or *NOTCH1*, but also *EGR2* and *NFKBIE*, an inhibitor of NFKB.
Recurrence analyses in a cohort of 168 advanced stage patients treated within a French multicentric trial (www.clinicaltrials.gov: NCT00931645), confirmed the recurrent nature of mutations in *EGR2* and in NFKBIE observed in 8.3% and 10.7% of patients, respectively. Missense mutations in *EGR2* associated with shorter time to treatment (median: 15.4 *vs* 1.2; p=0.0006) and a shorter 5-year overall survival (56.2 *vs* 80.4 months; p=0.04). The impact of *EGR2* mutations was confirmed by over expression studies in hematopoietic cell lines. RNA-seq data from CLL patients showed that *EGR2* mutated patients showed a signature of *EGR2* activity that overlap with BCR activation signatures.

Table 1. Representative example of the mutation repartition in a CLL patient.



Summary and Conclusions: Our findings establish the presence of acquired mutations in multipotent haematopoietic progenitors of CLL patients. Early mutations were observed in genes that belong to the most frequent mutated ones in CLL (*NOTCH1, SF3B1, TP53,* and *XPO1)* and in genes that have been reported to be recurrent in other cancers (*e.g. FUBP1, MED12* or *PEG3)*. Together with the previous report of differentiation bias of CLL progenitor cells in xenograft experiments (Kikushige *et al.* Cancer Cell 2011), our results suggest that abnormalities in hematopoietic progenitors and early B-cell differentiation is an early step during CLL pathogenesis. They also support the hypothesis that early CLL mutations, despite their diversity, commonly affect B-cell differentiation, through deregulation of pre-BCR signaling.

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SF3B1 MUTATIONS INDUCE COMMON AND LINEAGE SPECIFIC ABERRANT MESSENGER RNA SPLICING IN MALIGNANCIES INCLUDING CHRONIC LYMPHOCYTIC LEUKEMIA AND CONFER SENSITIVITY TO SPLICEOSOME INHIBITION

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Background: Recurrent heterozygous mutations of the spliceosome protein SF3B1 have been identified in chronic lymphocytic leukemia (CLL), myelodysplastic syndromes, and solid tumors. In CLL, SF3B1 mutations are associated with poor prognosis and are enriched in fludarabine-refractory CLL. In MDS, SF3B1 mutations are enriched in subtypes with ringed sideroblasts. SF3B1 is part of the U2 snRNP complex which binds to the pre-mRNA branch point site and is involved in recognition and stabilization of the spliceosome at the 3' splice site.
Aims: Here, we sought to determine the impact of SF3B1 mutations and explore opportunities for therapeutic intervention in SF3B1 mutated cancers.
Results: A comparison of RNAseq data from tumor samples with SF3B1 hotspot mutations ($SF3B1^{MUT}$) or wild-type SF3B1 ($SF3B1^{WT}$) in CLL, melanoma and breast cancer revealed significant increases in the usage of alternative 3' splice sites generating known and new alternative splice junctions in $SF3B1^{MUT}$, samples. These events induce expression of alternative mRNAs that are translated into novel proteins or aberrant mRNAs that are decayed by cells. We identified a common set of aberrantly spliced mRNAs (*e.g.* ZDHHC16 and COASY) across different hotspot mutations and lineages; however, unique alternative splicing profiles were also observed suggesting lineage specific effects. In particular, in CLL we identified multiple aberrantly spliced genes in the NF-KB pathway, FOS/JUN transcriptional network and BCR signaling pathway. RNAseq analysis of several cell lines with endogenous SF3B1 hotspot mutations confirmed the presence of the same spliced isoforms as observed in tumor samples. To prove that $SF3B1^{MUT}$ were inducing alternative splicing, transient transfection of several SF3B1 hotspot mutations in 293FT cells induced the expression of the common alternatively spliced genes suggesting functional similarity. Selective shRNA depletion of mutant SF3B1 allele in $SF3B1^{MUT}$ cells resulted in downregulation of the same splice isoforms. Furthermore, isogenic B-cell lines (NALM-6) expressing the most frequent SF3B1 mutation (K700E) were generated and profiled by RNAseq. As expected, similar alternatively spliced genes were observed only in NALM-6 $SF3B1^{K700E}$, cells. To investigate the role of nonsense-mediated mRNA decay (NMD) in eliminating certain aberrant mRNAs induced by $SF3B1^{MUT}$, we treated NALM-6 $SF3B1^{K700E}$ cells with the NMD inhibitor cylcoheximide. In the treated samples we uncovered expression of several aberrant mRNAs, some of which were shown to be downregulated in patient samples. $SF3B1^{MUT}$, CLL samples displayed evidence of impaired NMD pathway activity since many aberrantly spliced genes that are subject to NMD pathway degradation could be detected in the absence of cycloheximide treatment. Taken together, these results confirm the association between different SF3B1 hotspot mutations, the presence of novel splice isoforms and lineage specific pathway defects. To evaluate therapeutic approaches to the treatment of malignancies with SF3B1 mutations we evaluated a potent and selective inhibitor of SF3B1, E7107. We demonstrated that E7107 binds and inhibits both wild-type and mutant SF3B1 protein. Furthermore, E7107 represses the expression of several common aberrant splice mRNA products in $SF3B1^{MUT}$, cells *in vitro* and *in vivo*. When tested in a NALM-6 mouse model, E7107 induced tumor regressions and increased the overall survival of animals implanted with NALM-6 $SF3B1^{K700E}$, cells.
Summary and Conclusions: These data suggest splicing inhibitors as a promising therapeutic approach for malignancies with SF3B1 mutations, including CLL and MDS.

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IL23 RECEPTOR (IL23R) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): MODULATION, MICRORNA (MIRNA) REGULATION AND PREDICTION POWER. A PROSPECTIVE MULTICENTER O-CLL STUDY

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Background: The complex biology underlying how the CLL cell is influenced by the surrounding immunological and stromal environment is an area of active investigation. IL23R is a heterodimeric construct consisting of the common IL12Rβ1 chain and a unique IL23Rα subunit.
Aims: We investigated i) the expression pattern of the IL23R subunit and its correlation with progression free survival (PFS); ii) the role of the microenvironment in controlling the IL23R complex subunits; iii) the epigenetic regulation of IL23R expression and turn-over; iii) the down-stream signalling induced by IL23R engagement in CLL clones.
Methods: CLL patients were prospectively enrolled from diagnosis (O-CLL1 protocol, clinicaltrial.gov identifier NCT00917540). MiRNA analysis was performed by Agilent's Human V2 platform and by quantitative PCR. The heterodimeric construct of IL23R, consisting of IL23Rα and of IL12Rβ1 sidechains was determined by flow-cytometry using the anti-human IL23R-PE mAb and anti-Human IL-12Rβ1 FITC monoclonal antibodies (R&D Systems). MirVANA microRNA mimics and inhibitors used in this study were purchased from Ambion, Inc. For 3'UTR luciferase reporter experiments, miRNA target reporter vectors were purchased from Origene.
Results: A totale of 233 CLL cases were defined according to a median IL23Rα cut-off value. The 3-year PFS probability of IL23Rα-low patients was 91% as compared to 75% of IL23Rα-high cases [χ2 9.1, P=.003; HR=3.2, 95%CI (1.4-7.1)]. The IL23Rα-high group was enriched in *IGVH* unmutated cases and showed preferential usage of VH1-69 and VH4-34 sequences. In a multivariate model, IL23Rα expression still remained significant in predicting PFS together with CD38 expression and *IGHV* mutational status, while ZAP-70 and FISH lost their significance. IL12Rβ1, was not expressed in circulating B-cells, however *in situ* analyses revealed its expression in association with IL23Rα, within CLL tissue infiltrates, suggesting that regulation of IL12Rβ1 expression in CLL cells occurred according to microenvironment-derived signals, whose prototype is the CD40/CD40L axis. Accordingly, a significant increase of both IL23Rα and IL12Rβ1 expression was documented after co-culturing CLL B-cells in the presence of CD40L-expressing NIH-3T3 cell line. Similar results were obtained by stimulating CLL cells with autologous T cells activated by CD3/CD28 MACSiBead™ (Miltenyi) particles and IL2. CLL cells concomitantly expressed higher levels of CD38, lower levels of CXCR4 and CD5dim or bright, thus indicating that the IL23R complex is expressed by the activated and dividing B-cells. The expression of both chains of the IL23R complex was observed in the spleen of a xenografted mouse (NSG) in the context of proliferating Ki67 positive CLL cells. Global miRNA expression analysis identified miRNAs that inversely correlated with IL12Rβ1 or IL23Rα expression. These miRNAs were tested in a dual luciferase reporter system, in which miR-324-5p and miR-146b-5p were found to target the 3'UTR region of IL23Rα and IL12Rβ1, respectively. Down-regulation of the IL23Rα side chain protein could be achieved in CLL B-cells by the transfection of miR-324-3p mimic, while up-regulation of the IL12Rβ1 subunit was observed through inhibition of miR-146b-5p expression. Finally, the engagement of IL23R complex induced phosphorylation of Stat-3.
Summary and Conclusions: These findings underscore a novel paradigm in the epigenetic regulation of pro-inflammatory cytokine sensing by CLL clones with biological and prognostic relevance.

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ISOTYPE MATTERS: FUNCTIONAL DIFFERENCES AFTER SIGM *VERSUS* SIGD TRIGGERING OF THE BCR IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: B-cell receptor (BCR) signaling plays an important role in CLL pathogenesis. CLL cells carrying *IGHV*-unmutated BCRs (U-CLL) are more responsive to surface IgM (sIgM) stimulation in terms of intracellular signaling activation, while cells with *IGHV*-mutated receptors (M-CLL) are usually more anergic. In contrast, previous studies demonstrated that a vast majority of CLL clones are responsive to sIgD stimulation, irrespective of the mutational status.
Aims: The aim of the present study is to characterize functional differences of BCR signaling after surface IgM and IgD triggering in CLL cells.
Methods: Purified CLL cells were stimulated with anti-IgM and anti-IgD or the combination of both anti-IgM and anti-IgD; phosphorylation levels of the BCR-related molecule HS1 (tyrosine^{Y397}) was analyzed in serial samples after 2, 5, 15, 30, and 60 minutes of stimulation by Western Blot and flow cytometry; BCR surface receptor expression and 48-hour cell viability were analyzed by flow cytometry; CCL3 and CCL4 secretion by CLL cells was measured by ELISA after 24-hour stimulation.
Results: HS1 is an early BCR signaling and cytoskeletal molecule, whose phosphorylation correlates with the clinical outcome of CLL patients. We observed that HS1 activation peaked after 2-5 minutes following stimulation, and then returned to baseline at later time points with different patterns. We noted robust and protracted HS1 phosphorylation after anti-IgM stimulation in most samples (19/22; 86%). In contrast, protracted HS1 phosphorylation after anti-IgD stimulation was seen only in 8/22 samples (36%), while 14/22 samples showed only transient HS1 activation that rapidly returned to baseline. The transient nature of IgD signaling may, at least in part, be explained by the rapid receptor internalization following stimulation, as compared to sIgM molecules, as analyzed in 10 CLL samples. The analysis of HS1 activation kinetics in U-CLL and M-CLL samples showed greater responsiveness of U-CLL cells (n=8) to both IgM and IgD stimulation as compared to M-CLL cases (n=6). In line with this finding, the surface expression of IgM (P=0.0004) and IgD (P=0.0042) molecules was detected to be higher in U-CLL (n=22) as compared to M-CLL (n=16) samples. IgM stimulation protected CLL cells from *in vitro* apoptosis to a greater extent than IgD stimulation, as demonstrated in 20 patients following 48 hours of *in vitro* culture. Interestingly, IgD stimulation did not induce secretion of CCL3 or CCL4 chemokines by the CLL cells, whereas IgM induced robust release of these chemokines into CLL cell supernatants (see Figure 1), based on analyses of 10 different patients' samples after 24-hour stimulation with anti-IgM and anti-IgD. sIgM-induced protection from *in vitro* apoptosis and chemokine production were greater in U-CLL as compared to M-CLL cases. Treatment with the combination of both anti-IgM and anti-IgD increased signaling activation, but did not have a significant impact on cell viability and chemokine production, with respect to anti-IgM stimulation alone.

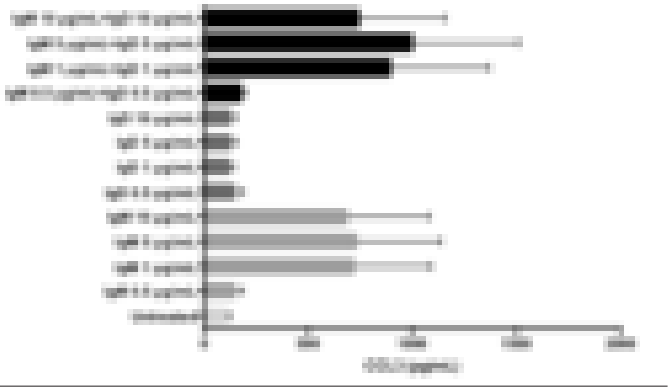

Figure 1.

Summary and Conclusions: sIgM stimulation induces protracted signaling activation, protection from *in vitro* apoptosis and CCL3 and CCL4 production to a greater extent than sIgD stimulation, particularly in U-CLL cells. Taken together, these observations suggest differential functional relevance of sIgM and sIgD triggering in CLL, which is further modulated by BCR signaling differences in patient subsets. Deeper insight into the fine-tuning of BCR signaling in CLL will help understanding and guide us towards pathophysiology-based use of BCR signaling inhibitors.

S660

CLL INDUCES SEVERE SKEWING IN THE MYELOID COMPARTMENT IN PATIENTS AND IN THE TCL1 MOUSE MODEL

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Background: Previous *in vitro* studies have demonstrated that myeloid cells play an important role in providing Chronic Lymphocytic Leukaemia (CLL) cells with survival stimuli. When cultured with CLL cells, blood monocytes can differentiate into adherent Nurse-Like Cells (NLCs) which protect leukaemic cells from undergoing spontaneous apoptosis.
Aims: In the current study, we investigated the changes induced by CLL cells in myeloid cells and their role in disease progression *in vivo*.
Methods: For *in vitro* experiments, human blood monocytes were cultured for 2 days with either CLL cells or normal B cells. Cells were then harvested and

analyzed by flow cytometry. For *in vivo* experiments, we used the well-established transgenic Eμ-TCL1 mouse model of CLL. In addition, we injected young wild-type (WT) C57Bl/6 recipients with CLL cells from spleens from leukaemic mice, and sacrificed them when they exhibited symptoms of progressive CLL. Using an 8-color flow cytometry panel, we performed in depth phenotypic analyses of myeloid cells in spleen, blood and peritoneal cavity. More than 140 serum cytokines from leukemic mice and clinically well-annotated CLL patients were screened with antibody arrays.

Results: Compared to healthy WT mice, mice with CLL exhibited fundamental changes in the composition of myeloid cells with skewing towards subsets with documented immunosuppressive functions. This corresponded with an increased production of pro-inflammatory and immunosuppressive cytokines. In addition, multiple chemo-attractants for myeloid cells, such as MCP-5 were upregulated. This resulted in the recruitment of myeloid cells to tissue sites where they were enriched in the proximity to tumor vasculature. Accordingly, several matrix-remodelling proteins and angiogenic factors including MMP-9 and osteopontin were highly enriched in serum, indicating that CLL might be associated with an induction of tumour angiogenesis. Of interest, conventional dendritic cells were drastically reduced in leukemic mice. This was accompanied by deregulation of key cytokines such as GM-CSF and FLT3-L. Furthermore, similar to many other tumours, peritoneal cavity macrophages harboured a M2-like phenotype. A direct induction of M2 macrophage differentiation by CLL cells was confirmed in co-cultures of human cells with monocytes from healthy donors. Functionally, the described alterations in the myeloid compartment were accompanied by failure to stimulate anti-tumor immune responses. Ongoing work focuses on the molecular characterization of dysregulated cell subsets, and the manipulation of the myeloid compartment *in vivo*.

Summary and Conclusions: Our *in vivo* data suggests that the myeloid compartment contributes as a key player in the pathogenesis of CLL. Skewing of these cells to immunosuppressive subtypes along with their potential role in tumor angiogenesis makes them attractive targets for immunotherapy complementing current standard chemo-immunotherapy approaches.

Red cell clinical

S661

DEFERASIROX-DEFEROXAMINE COMBINATION THERAPY REDUCES CARDIAC IRON WITH RAPID LIVER IRON REMOVAL AFTER 24 MONTHS IN PATIENTS WITH SEVERE TRANSFUSIONAL IRON OVERLOAD (HYPERION)

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Background: The prospective, Phase II, multinational HYPERION study evaluated the efficacy and safety of deferasirox-deferoxamine (DFX–DFO) in combination followed by DFX monotherapy in patients with severe transfusional cardiac siderosis. During the first 12 months (mo), improvement in cardiac T2* and reduction in liver iron concentration (LIC) were observed in patients with severe liver and cardiac iron overload (IO) while the safety profile was consistent with established monotherapy (Aydinok, *Blood* 2013;122(21) Abst 2257).

Aims: The aim of this HYPERION analysis is to evaluate the efficacy and safety of combination treatment followed by DFX monotherapy for up to 24 mo.

Methods: Study design/inclusion-exclusion criteria have been described (Aydinok, *Blood* 2013;122(21) Abst 2257). Patients aged ≥10 years with CMR-measured cardiac T2* 5–<10 ms, left ventricular ejection fraction (LVEF) ≥56%, and R2-MRI LIC ≥7 mg Fe/g dw were enrolled. Patients achieving cardiac T2* ≥10 ms accompanied by a relative T2* increase of ≥10% from baseline (BL) after 6 mo were switched to DFX monotherapy, but switched back to combination therapy if cardiac T2* fell to <10 ms with a relative decrease of ≥10%. At 24 mo, geometric mean (GM) cardiac T2* ratio (GM_{24}/GM_{BL}), is presented with a 95% CI. Proportion (%) of patients and time to achieving T2* ≥10 ms with ≥10% increase from BL was evaluated. Efficacy was analyzed for all evaluable patients in the full analysis set (FAS) who received ≥1 dose of study drug and had a BL and a post-BL assessment within each visit window.

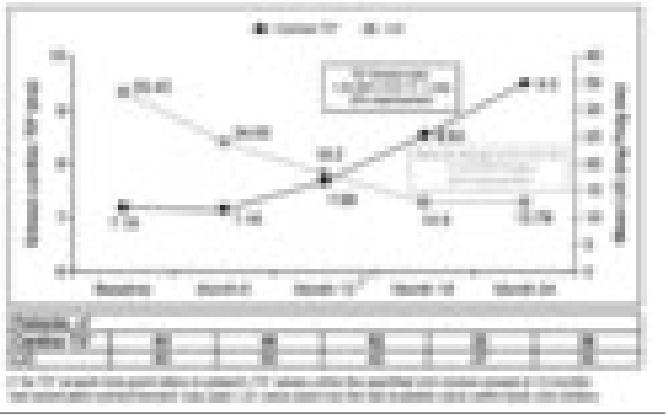

Figure 1. Geometric mean cardiac T2* and mean LIC in patients treated with DFX-DFO in combination followed by DFX monotherapy over 24 months (Full analysis set).

Results: Results are presented for the cumulative 24 mo study. Of the 60 patients enrolled, 34 (56.7%) completed 24 mo. Discontinuations were due to consent withdrawal (6), lost to follow up (6), adverse event (AE) (5), abnormal test procedure ((5), 2 consecutive T2* values were <5 ms), administrative (2), protocol deviation (1), and death (1); majority occurred during the first 12 mo. Mean dose (mg/kg/d) was DFX, 30.5±7.13, 7 d/wk, and DFO, 36.3±6.66, 5 d/wk. Median (range) duration of exposure was DFX, 719.0 (21.0–737.0) and DFO, 407.5 (29.0–737.0) days. Serum ferritin levels decreased by 44% (Median: BL, 5551; 24 mo, 2491 ng/mL). Cardiac T2* increased by 30% (GM: BL, 7.19; 24 mo, 9.5 ms), and LIC decreased by 52% (Mean (SD): BL, 33.43 (14.55); 24 mo, 12.79 (11.72) mg Fe/g dw) (Figure 1). Patients with BL LIC <30 and ≥30 mg Fe/g dw, cardiac T2* improvement was 35% (8.04 to 10.59 ms, n=15) and 26% (6.83 to 8.78 ms, n=21), respectively. Overall, 12.5% (n=6), 19.2% (n=10), 33.3% (n=11) and 47.2% (n=17) of patients achieved T2* ≥10 ms and ≥10% relative increase from BL at Months 6, 12, 18 and 24, respec-

tively. Median time to T2* ≥10 ms and ≥10% relative increase from BL was 722.0 days. 11 patients transitioned to DFX monotherapy. LVEF remained stable during the study. AEs with suspected relationship to either drugs or combination (≥5%) included: abdominal pain, increased urine protein/creatinine ratio (each 8.3%); increased serum creatinine (SCr), diarrhea, and nausea (each 6.7%); 2 patients had increased SCr >33% from BL and >upper limit of normal at 2 consecutive visits; 1 death reported in a patient with pyrexia and altered state of consciousness (suspected DFO). Majority of AEs, including the death, were reported during the first 12 mo.
Summary and Conclusions: In this large clinical trial of DFX–DFO combination therapy, continued improvement in T2* with a clinically significant decrease in LIC was demonstrated in patients with severe liver and cardiac IO. Liver iron removal was rapid and cardiac iron removal was steady with nearly 50% of patients who remained on study achieving T2* ≥10 ms and ≥10% relative increase from BL. LVEF remained stable and there were no new episodes of arrhythmias or cardiac failure, despite severe cardiac IO at BL in all cases (cardiac T2* 5–<10 ms). Safety of the combination therapy was consistent with established monotherapy profiles with no unexpected findings.

S662

INTERIM RESULTS FROM A PHASE 2A, OPEN-LABEL, DOSE-FINDING STUDY TO DETERMINE THE SAFETY, EFFICACY, AND TOLERABILITY OF SOTATERCEPT (ACE-011) IN ADULTS WITH BETA-THALASSEMIA

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Background: β-thalassemias are characterized by ineffective erythropoiesis leading to anemia, erythroid hyperplasia, iron overload, and organ failure. Sotatercept (ACE-011), a novel and first-in-class activin type IIA receptor fusion protein, acts on late-stage erythropoiesis to increase release of mature erythrocytes into circulation (Carrancio S, *et al.* Br J Haematol 2014; in press). In healthy volunteers, sotatercept therapy was associated with increased red blood cell (RBC) parameters, including hemoglobin (Hb) level (Sherman ML, *et al.* J Clin Pharmacol 2013;53:1121-30). RAP-011, a murine version of sotatercept, was effective in a β-thalassemia mouse model, supporting clinical development of sotatercept (Dussiot M, *et al.* Nat Med 2014; in press).
Aims: To determine a safe, tolerable, and effective dose of sotatercept in β-thalassemia major (TM) patients (pts) with transfusion-dependent thalassemia (TDT), or β-thalassemia intermedia (TI) pts with TDT or non-transfusion-dependent thalassemia (NTDT).
Methods: In this ongoing phase 2a, multicenter, open-label dose-finding study, pts received sotatercept subcutaneously (SC) once every 3 weeks; dose escalation to 0.75 mg/kg is ongoing. Efficacy is assessed by Hb increase from baseline (BL) for NTDT pts and RBC transfusion burden reduction for TDT pts. Safety is assessed by NCI-CTCAE v4.0. All pts provided informed consent.
Results: Of the 25 pts in the sotatercept 0.1, 0.3, and 0.5 mg/kg dose groups, 18 (72%) had NTDT (17 TI, 1 Hb-E/β-thalassemia) and 7 (28%) had TDT (5 TM, 2 TI). Six NTDT pts were treated in each dose group. Median BL Hb for NTDT pts was 8.7 g/dL (range 6.1–10.7), 8.3 g/dL (range 6.0–9.5), and 8.2 g/dL (range 6.4–9.3) in the 0.1, 0.3, and 0.5 mg/kg dose groups, respectively. In the first 3 cycles, one or more Hb increases of ≥1.0 g/dL were seen in 0 (0%), 5 (83%), and 5 (83%) NTDT pts in the 0.1, 0.3, and 0.5 mg/kg groups, respectively. Hb increases of ≥2.0 g/dL were seen in 0 (0%), 1 (17%), and 2 (33%) NTDT pts, respectively. Area under the curve and peak concentration of sotatercept increased proportional to dose. Increased exposure was associated with higher Hb increases in the first 3 cycles for NTDT pts (n=17; r=0.77, P<0.001). No appreciable reduction in transfusion burden was seen for TDT pts in the 0.1 and 0.3 mg/kg groups. Treatment of the 0.5 mg/kg group is ongoing. Sotatercept was well tolerated; 19 (76%) pts remain on treatment. Three grade ≥2 treatment-related adverse events leading to discontinuation were seen: worsening grade 3 bone pain in 1 TDT pt with history of severe osteoporosis and grade 2 phlebitis in 1 NTDT pt with underlying high D-dimer at BL, both in the 0.1 mg/kg group, and grade 3 ventricular extrasystoles in a pt with history of ventricular extrasystoles at BL in the 0.5 mg/kg group. Three pts discontinued due to lack of efficacy: 2 and 1 in the 0.1 and 0.3 mg/kg groups, respectively.
Summary and Conclusions: These preliminary data suggest sotatercept given SC once every 3 weeks increases serum Hb, improving anemia. Sotatercept may provide clinical benefit to β-thalassemia pts with a favorable safety profile, addressing a significant unmet need. These data suggest a dose-dependent effect for sotatercept, supporting further study of the exposure–effect relationship in β-thalassemia pts. Updated safety and efficacy data will be presented.
JP, MDC, and OH contributed equally to this abstract.

S663

A PHASE 1 DOSE-ESCALATION TRIAL OF TOPICAL SODIUM NITRITE IN PATIENTS WITH SICKLE CELL ANEMIA AND LEG ULCERS: EVIDENCE OF IN HUMAN EFFECT ON BLOOD FLOW

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Background: Chronic leg ulcers are a debilitating complication of sickle cell disease (SCD). There is a need for well-tolerated, effective therapies. Sodium nitrite functions as a reservoir of nitric oxide, whose vasodilating, angiogenic, antiplatelet and anti-microbicidal activities make it an attractive investigational therapy for wound healing.
Aims: We assessed safety and tolerability of escalating doses of a novel preparation of topical sodium nitrite and examined changes in microcirculation to investigate mechanism of action.
Methods: A phase 1, dose-escalation study of topical sodium nitrite (ClinicalTrials.gov NCT01316796) was conducted in adults with SCD and chronic leg ulcers. Sodium nitrite was applied twice a week for 4 weeks to one ulcer per patient in five cohorts of escalating concentrations. Safety, tolerability and pharmacokinetic of nitrite and nitrate were obtained. Laser speckle blood flow images and infrared (IR) thermal images of the ulcer peri-wound area were obtained before and after application. Ulcer size and pain were measured at each visit. A brief pain inventory (BPI) was administered before and after the trial.
Results: 18 subjects enrolled and completed the trial. Tolerability was excellent, with no serious adverse events. In five subjects diastolic blood pressure decreased transiently below 50mmHg. Methemoglobin did not exceed safety thresholds. There appeared minimal systemic absorption after cream application; increases in whole blood nitrite concentration were attributable to the oral administration of hydroxyurea. We observed a dose-dependent decrease in leg ulcer size (p<0.0001) and pain (p<0.0001). Wound size decreased by: 30% for cohort 1, 4% for cohort 2, 38% for cohort 3, 68.5% for cohort 3a (one healed) and 88% for cohort 4, (two healed). BPI improved significantly in pain severity (p<0.005) and interference (p<0.001) and overall opioid use decreased. The Visual Analogue Scale (VAS) mean score was 3.7±3.4 cm at enrollment, decreasing to 1.2+1.4 cm (p<0.0003). In the 9 patients that had more than one ulcer, VAS scores for non-treated ulcers did not change significantly, mean of 2.7± 2.6 cm *vs.* 2 ±2.4 cm (p=0.8) at the end. There was a statistically significant decrease in total WBC, from 9.9±4.5K/ul to 8.7±3.1K/ul, p=0.001. Significant increases in peri-wound temperature (p<0.01) and blood flow (p=0.00016) were observed after nitrite application.
Summary and Conclusions: On the basis of these safety, pharmacokinetic and tolerability data, and promising efficacy results, topical sodium nitrite warrants further clinical evaluation in patients with sickle cell disease and/or other types of leg ulcers

S664

ACE-536 INCREASES HEMOGLOBIN LEVELS IN ADULTS WITH BETA-THALASSEMIA: PRELIMINARY RESULTS FROM A PHASE 2 STUDY

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Background: ACE-536 is a recombinant fusion protein containing modified activin receptor type IIB, being developed for the treatment of anemias due to ineffective erythropoiesis (IE), such as β-thalassemia (β-thal). In β-thal, IE is due to intracellular α-globin aggregates causing premature death of late-stage erythroid precursors. Patients with β-thal often have elevated levels of EPO and are unresponsive to erythropoiesis-stimulating agents (ESAs). ACE-536 binds to ligands in the TGF-β superfamily and promotes late-stage erythroid differentiation via a mechanism distinct from ESAs. In a healthy volunteer study, ACE-536 was well-tolerated and increased hemoglobin (Hgb) levels (Attie K *et al.*, Am J Hematol 2014). Murine ACE-536 (RAP-536) increased Hgb levels and decreased RBC inclusion bodies and hemolysis in a mouse model of β-thal (Suragani R *et al.*, Blood 2012;120:248).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study to evaluate the effects of ACE-536 in adults with transfusion-dependent (TD) or non-transfusion dependent (NTD) β-thalassemia. Study outcomes include erythroid response (either Hgb increase in NTD patients or reduced RBC trans-

fusion burden in TD patients), safety, tolerability, PK, and pharmacodynamic biomarkers.

Methods: Inclusion criteria included age ≥18 yr and anemia defined as either Hgb <10.0 g/dL (NTD, defined as <4 units RBCs/8 wks prior to baseline) or requiring ≥4 units RBCs/8 weeks prior to baseline (TD). ACE-536 was administered in sequential cohorts (n=6) at dose levels of 0.2, 0.4, 0.6, 0.8 mg/kg by subcutaneous (SC) injection once every 3 weeks for up to 5 doses with a 2-month follow-up. Further dose escalation cohorts and an expansion cohort (n=30) are planned, contingent on periodic safety data review.

Results: Preliminary baseline data were available for the 24 patients (12M/12F, 20 NTD/4TD) enrolled in the first 4 cohorts as of 07Feb2014. Median age was 34.5 yr (range: 20-57 yr); 79% had prior splenectomy. Genetic analysis and disease history were consistent with β-thal intermedia for all patients enrolled to date, including 5 with α-globin triplication/quadruplication. Median (range) baseline Hgb (over 28 days prior to treatment) for the NTD patients was 8.4 (6.5-9.6) g/dL. Preliminary efficacy data were evaluated for the NTD patients in the first 3 cohorts and demonstrated a mean (SD) Hgb increase after 3 cycles (~9 weeks) of 0.0 (0.8), 0.9 (0.4), and 1.4 (0.4) g/dL in the 0.2 (n=6), 0.4 (n=5), and 0.6 (n=5) mg/kg groups, respectively (see Figure 1). Preliminary efficacy data were available for the 4 TD patients enrolled to date (1 pt at 0.6 and 3 pts at 0.8 mg/kg) and demonstrated reductions in transfusion requirement. ACE-536 was generally well tolerated. No related serious adverse events (AEs) have been reported to date. One patient discontinued treatment early due to a related AE (ankle pain in the 0.8 mg/kg cohort). No notable changes in mean platelets or WBC were observed.

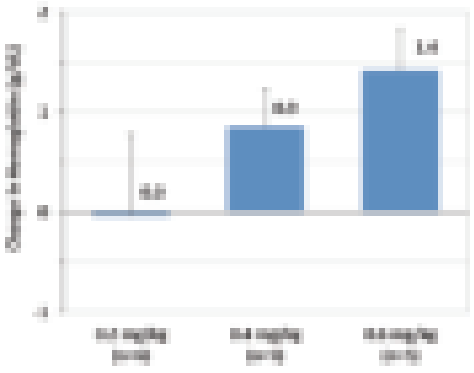

Figure 1. Mean (± SD) change in hemoglobin after 3 cycles non-transfusion dependent patients.

Summary and Conclusions: Based on preliminary data, ACE-536 administered SC every 3 weeks increased Hgb levels in NTD patients and decreased transfusion requirement in TD patients with β-thalassemia via a novel mechanism of action with a favorable safety profile. These data strongly support further evaluation of ACE-536 in patients with β-thalassemia.

S665

CLINICAL AND MOLECULAR HETEROGENEITY OF 47 PATIENTS AFFECTED BY CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II (CDAII)

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Background: CDAII, the most frequent type of congenital dyserythropoietic anaemia, is an autosomal recessive disease characterized by ineffective erythropoiesis, peripheral hemolysis, erythroblast morphological abnormalities and hypoglycosylation of some RBC membrane proteins. SEC23B gene, encoding the COPII coat component involved in protein trafficking from the endoplasmic reticulum to the Golgi apparatus, has been identified as the causative gene for CDAII; approximately 60 different mutations have been reported.

Aims: To describe the phenotype and genotype features of 47 CDAII patients (22 females, 25 males) from 42 unrelated families, diagnosed over a period of 30 years.

Methods: The diagnosis was made by presence of congenital anaemia/jaundice, ineffective erythropoiesis, typical morphologic appearance of bone marrow erythroblasts, hypoglycosylation of band 3 in SDS-PAGE and presence of mutations in *SEC23B* gene.

Results: The age at diagnosis (median 5 yrs, range birth-49 yrs) drastically decreased in patients born in the last decade, likely due to the availability of SDS-PAGE analysis and molecular testing. About 2/3 of patients had more than one misdiagnosis, in particular HS (15 cases). Clinical presentation was mild in 11 patients, moderate in 28, and severe in 8. Median Hb and reticulocytes levels were 10.3 g/dL (range 3.1-12.4) and 79 $x10^9$/L (range 3-369) respectively. Two cases presented with hydrops foetalis: one died at 11 yrs, and the other underwent BM transplantation. Eight were transfusion-dependent and 10 needed occasional support only. Fourteen had been splenectomised (mean age at splenectomy 20 yrs, range 7-39) and 13 patients underwent iron chelation (maximum ferritin levels: median 504, range 32-4000). The patients were characterized at molecular level by direct sequencing: 29 different mutations (15 disruptive and 14 missense) were identified among the 94 mutated alleles, 8 were new (Glu10X, Arg18Cys, delLeu245, delAsp377Phe fs, Arg550X, Asp600Asn, His757Pro, Ser617Pro). Missense mutations affect highly conserved residues in multiple domains of SEC23B. Taken together the two most frequent mutations, c.325G>A (Glu109Lys) and c.40C>T (Arg14Trp) account in our series for the 56% of mutant alleles. Out of the 47 patients, 15 were homozygous (14 carrying c.325G>A), 10 compound heterozygous for two different missense mutations and 18 for one missense and one drastic mutation; 2 unrelated subjects showed only one mutation at heterozygous level. Interestingly, two unrelated patients with mild/moderate clinical presentation were compound heterozygous for one stop and the splicing mutation c.221+31a>g, in spite of the commonly reported opinion that combination of two drastic mutation could be lethal. The 14 cases with homozygous Glu109Lys mutation (12 unrelated) displayed median Hb levels of 9.2 g/dL (range 7-11.2) and maximum ferritin levels: 701 mg/ml, (range 110-1740); 5 patients need occasional blood transfusion, and 4 had been splenectomised for a previous diagnosis of HS.

Summary and Conclusions: The analysis of this large series of patients confirms the clinical and molecular heterogeneity of this disease and underlines the usefulness of molecular testing to avoid misdiagnosis.

Bleeding disorders

S666

THE OCCURRENCE AND IMPACT OF JOINT BLEEDS IN VON WILLEBRAND DISEASE

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Background: Von Willebrand disease (VWD) is a heterogeneous inherited bleeding disorder that affects up to 1% of the population. Joint bleeds are not predominant, but have been reported to occur in 8-45% of patients with VWD, especially in those with a more severe phenotype. Joint bleeds can lead to structural joint damage. The most severe type 3 VWD patients develop similar rates of joint range of motion limitation over time as moderate hemophilia A patients. However, the severity, onset and impact of joint bleeds and its complications in VWD patients are largely unknown.
Aims: The aim of this study is to assess the incidence, onset and treatment of joint bleeds and its impact on quality of life and joint integrity in moderate and severe VWD.
Methods: In the Willebrand in the Netherlands (WIN) study 804 moderate and severe VWD patients (VWF activity ≤30U/dL) completed a comprehensive questionnaire after giving informed consent. We quantified joint bleed occurrence as reported in the questionnaires and examined the medical files for documentation on joint bleeds and joint problems from all patients who had reported treatment for joint bleeds with desmopressin or clotting factor concentrate (CFC) and from as many patients without joint bleeds for comparison, matched for gender, age, FVIII and VWF activity.
Results: Twenty three percent of the patients (184/804) self-reported joint bleeds in the questionnaire, mostly in the knee, followed by the ankle and elbow. These patients had more severe VWD (type 3 VWD 12% *vs.* 4%, p<0.01, OR 3.4, 95%CI 1.8-6.2; FVIII level ≤10 IU/dL in 9% *vs.* 3%, p<0.01, OR 3.8, 95%CI 1.9-7.8), reported more joint damage (54% *vs* 18%, p<0.01) and large joint surgery more often (25% *vs* 11%, p<0.01) compared to the patients not reporting joint bleeds. Also, patients with joint bleeds reported lower quality of life (SF36 mental component summary ß -2.78, 95%CI -4.74 to -0.83, p<0.01 and physical component summary ß -4.03, 95%CI -6.92 to -1.14, p<0.01), still significant after correction for age, comorbidity, epistaxis and menorrhagia in women. In the medical files, we found documentation of joint bleed treatment with CFC or desmopressin in 55 of the 79 patients (70%) who had self-reported treatment for joint bleeds. Of these 55 patients 38% had type 3, 40% type 2 and 22% type 1 VWD. The first joint bleed occurred in childhood in most cases (65% before age 16, median age 12 years, range 3-69). More than 10 joint bleeds were documented in the medical files of 9/55 (18%) patients. Compared to 55 matched control VWD patients without joint bleeds, patients with joint bleeds used CFC prophylaxis more often (29% *vs.* 2%, p<0.01). The frequency of joint bleeds decreased >50% in 14/16 VWD patients with joint bleeds who started CFC prophylaxis. We found documented X-ray joint damage in 44% of the patients with joint bleeds compared to 11% of the controls (p<0.01). Orthopedic joint surgery, performed in 14 patients and 6 controls (26% *vs.* 11%, p=0.03), was performed because of arthropathy due to joint bleeds in 10 of the 20 joint bleed patients undergoing joint surgery. Impaired mobility occurred in 7 cases and 1 control patient (p=0.08). We found notes on joint pain in 24 cases and 10 controls (p<0.01), but pain medication prescriptions in only 6 cases and 3 controls (p=0.49).
Summary and Conclusions: Joint bleeds in VWD mostly occur at young age, in all three VWD types, are associated with more severe VWD, lower quality of life and significantly more radiologic and self-reported joint damage. Radiologic joint damage also occurs in 11% of moderate and severe VWD patients without clear clinical joint bleeds. The relatively low proportion of VWD patients with joint bleeds using CFC prophylaxis and pain medication suggests that treatment of VWD patients with joint bleeds can be improved.

S667

BIOCHEMICAL MARKERS OF JOINT DAMAGE ARE INCREASED AFTER A JOINT BLEED; AN EXPLORATIVE HUMAN AND CANINE *IN VIVO* STUDY

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Background: Haemophilic arthropathy is one of the major causes of morbidity amongst haemophilia patients. At the moment, the only way to evaluate joint damage is by radiologic examination. Detection of early and small changes due to a joint bleed, for instance by the use of biomarkers, would be favourable to monitor joint disease progression, adapt treatment, and evaluate its efficacy in haemophilia patients. It is previously shown that a panel of four biomarkers for cartilage degradation (uCTX-II, sCOMP, sC1,C2, sCS846) is associated with overall radiographic joint damage (Jansen *et al.*, Arthritis Rheum 2009).
Aims: The purpose of this study is to investigate whether those biochemical markers are sensitive to changes in joint tissue turnover induced by a joint bleed.
Methods: Blood and urine samples were collected from 10 haemophilia patients after they reported a joint bleed: within two days, after 3-5 days, 12-14 days and 90 days (last time point considered baseline). Commercially available assays for serum and urine biomarkers were performed: urinary C-terminal telopeptide of type II collagen (uCTX-II), serum cartilage oligomeric matrix protein (sCOMP), serum cartilage cleavage product C1,2C, and serum chondroitin sulfate (sCS846). The same panel of biomarkers was explored in dogs (n=7) after induction of a joint bleed by intra-articular blood injections. Biomaterials were collected at baseline, day two, one and two weeks later.
Results: In haemophilia patients, levels of uCTX-II and sCS846 increased five days after the bleed when compared to baseline (uCTX-II +52%; p=0.021; sCS846 +14%; p=0.011; see Figure 1).
In dogs, uCTX-II increased statistically significant from day two to day seven (from 75% to 154% of baseline; p=0.018), and sCOMP from baseline to day two (+46%; p=0.028).

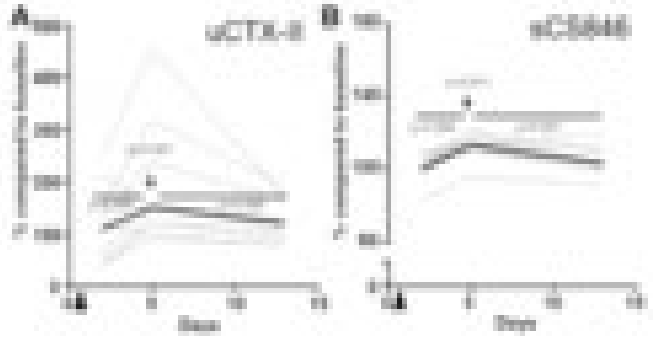

Figure 1. Biomarkers of joint demage increase after an acute joint bleed in haemophilia patients.

Summary and Conclusions: This study demonstrates that biochemical markers of joint tissue damage increase already after a single joint bleed, both in a clinical and an experimental setting. This might be useful in monitoring the impact of a joint bleed and in evaluation of treatment in haemophilia patients.

S668

THROMBOPOIETIN RECEPTOR AGONISTS SHIFTS THE BALANCE OF FCGAMMA RECEPTORS TOWARDS THE INHIBITORY FCGAMMA RECEPTOR IIB ON MONOCYTES IN IMMUNE THROMBOCYTOPENIA

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Background: The human FcγR receptor (FcγR) system comprises the activating FcγRs (FcγRI, FcγRIIa and FcγRIII) and the inhibitory FcγR (FcγRIIb), the balance of which determines the magnitude of inflammatory response. Impaired homeostasis in FcγR expression has been implicated in the pathogenesis of primary immune thrombocytopenia (ITP). Clinical studies using thrombopoietin (TPO) receptor agonists to stimulate platelet production have yielded favorable outcomes in ITP patients.
Aims: The study is aimed to investigate the effects of thrombopoietic agents on modulation of FcγRs on monocytes in ITP patients and murine models.
Results: In the present study, expression of FcγRs on monocytes was determined in 17 corticosteroid-resistant/relapsed chronioc ITP patients before and after eltrombopag treatment. Eltrombopag was given orally with an initial dose of 25 mg/d, and adjusted between 25 and 75 mg/d to maintain platelet counts ≥50 000/μL. Results showed that 6 weeks after the initiation of eltrombopag administration, mRNA and protein expression levels of FcγRIIb were significantly elevated. Concurrently, levels of FcγRI and IIa were decreased remarkably, whereas FcγRIII expression remained unchanged. *In vitro* phagocytosis assay of IgG-opsonized platelets indicated that the shift of FcγR balance towards the inhibitory FcγRIIb on monocytes of ITP patients was accompanied by a considerable decrease in monocyte phagocytic capacity after eltrombopag treatment. Modulation of monocyte FcγR balance by TPO receptor agonists was

also found in a murine model of ITP in which splenocytes from CD61 knockout mice immmunized against CD61+ platelets were transferred into C57BL/6 severe combined immunodeficient (SCID) (CD61+) mouse recipients, and their platelet counts and phenotypes were observed. When thrombocytopenia was induced in the SCID mouse recipients, romiplostim was injected subcutaneously at a dose of 100 μg/kg every 3 days. After 2 weeks of romiplostim administration, platelet counts in SCID mice elevated remarkably, and a significant increase in the inhibitory FcγRII expression together with a decrease in expression of activating FcγRI were observed.

Summary and Conclusions: These findings suggested that the recovery of platelet counts after TPO receptor agonist treatment in ITP is accompanied by a change in the FcγR balance toward the inhibitory FcγRIIb on monocytes, and the thrombopoietic agents have profound effects on immune modulation in ITP.

S669

THE IMPACT OF BLEEDING COMPLICATIONS IN PATIENTS RECEIVING NOVEL ORAL ANTICOAGULANTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Vitamin K antagonists (VKAs) have been the standard of care for treatment of thromboembolic diseases including venous thromboembolism and stroke prevention from atrial fibrillation. Novel oral anticoagulants (NOACs) have been developed and found to be at least non-inferior to VKAs with regards to efficacy. However the risk of bleeding with NOACs remains controversial. We therefore performed a systematic review and meta-analysis of phase III randomized controlled trials (RCTs) evaluating on NOACs compare with VKAs for VTE and stroke prevention from atrial fibrillation.
Aims: We aimed to assess the bleeding side effects of NOACs compared with warfarin (target INR 2.0 to 3.0) in the patients with VTE and atrial fibrillation.
Methods: We searched MEDLINE, EMBASE, CENTRAL, conferences abstracts and www.clinicaltrials.gov up to the last week of January 2014 with no language restriction. Two reviewers independently performed study selection, data extraction and study quality assessment. Selected outcomes were major bleeding, clinically relevant non-major bleeding, gastrointestinal hemorrhage and intracranial hemorrhage. We analyzed the pooled relative risk (RR) and corresponding 95% confidence internal [CI] for major bleeding and other secondary outcomes using the Mantel-Hanszel random effect model.
Results: A total of 13 RCTs (4 evaluating dabigatran, 4 rivaroxaban, 3 apixaban and 2 edoxaban) involving 102,638 patients were retreived. NOACs significantly reduced the risk of overall major bleeding (4.01% *versus* 4.62%, RR 0.73; 95% confidence interval [CI] 0.63-0.85, number needed to harm (NNH)=164), clinically relevant non-major bleeding (10.20% *versus* 11.04%, RR 0.78; 95% CI 0.68-0.89, NNH=122) and intracranial bleeding (0.52% *versus* 1.12%, RR 0.43; 95% CI 0.37-0.49, NNH=166). There was no significant difference in gastrointestinal hemorrage between NOACs and VKAs (2.27% *versus* 1.89%, RR 1.09; 95% CI 0.88-1.34) (Table 1).

Table 1.

Summary and Conclusions: When compared to VKAs administered to a target INR of 2.0 to 3.0, NOACs cause less major bleeding, clinically relevant non-major bleeding and intracranial hemorrhage. Additionally, NOACs do not increase the risk of gastrointestinal hemorrhage.

S670

HEMORRHAGIC RISK IN HEMODIALYSIS PATIENTS WITH ATRIAL FIBRILLATION TAKING ORAL ANTICOAGULATION THERAPY: RESULTS FROM A MULTICENTER ITALIAN STUDY

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Background: The prevalence of atrial fibrillation (AF) in patients with end-stage renal disease (ESRD) on hemodialysis (HD) is high, estimated to be between thirteen and 27%. Oral anticoagulation therapy (OAT) is the recommended treatment for thromboembolism prevention in patients with AF. Traditional OAT with dicumarolic drugs such as warfarin is the most widespread choice, however its use is controversial in HD population in which the risk of bleeding is dramatically increased: HD patients taking OAT indeed, show major bleeding episode rates twice as high as HD patients without OAT treatment ongoing.
Aims: Safety of dicumarolic OAT was studied in a population of patients with AF managed in 10 Italian HD centers.
Methods: A Cox model was used to relate OAT to bleeding events, adjusted for possible confounders such as antiplatelet therapy, age, dialytic duration, comorbidities, percentage time in therapeutic INR range (TTR) according to the linear prediction method of Rosendaal(1) and INR variability (variance growth rate or VGR) according to Fihn method (2). VGR method takes both INR variability and the TTR into consideration, as it shows the degree of variation from the target INR over a period of time.
Results: All alive patients under observation in the centers at 31/10/2010, with paroxysmal, persistent or permanent documented AF, were recruited and followed up for two years. At recruitment, 134 patients over 290 were taking OAT. The overall median TTR was 54% (IQR: 42 to 67%). The median of INR variability (VGR) was equal to 0.34 (IQR: 0.16 to 0.87). Table 1 shows HAS-BLED score values according to hemorrhagic episodes. During follow-up 42 patients experienced hemorrhagic events for a total of 77 events, 3 of which were hemorrhagic strokes. After adjustment for possible confounders, OAT at recruitment resulted to be associated to an increased risk of bleeding episodes (HR 5.79, p=0.010). Having experienced hemorrhagic events before recruitment was related to a higher risk of a new bleeding episode (HR 2.39, p=0.015). In patients taking OAT the higher was the TTR, the lower was the risk of bleeding (HR 0.10, p=0.046), while higher VGR values were associated to a higher rate of bleeding during follow-up (HR=1.41, p=0.027).

Tablel 1.

Summary and Conclusions: In HD patients with AF, dicumarolic therapy greatly increases the incidence of bleeding, however hemorrhagic risk is reduced in subjects in whom INR is strictly kept within the therapeutic range and managed with low INR variability. Moreover patients with previous hemorrhagic episodes have a risk of bleeding more than doubled. Our data show that both TTR and VGR values are useful indicators of OAT management also in this setting of HD population. Large randomized controlled studied are needed in order to establish if HD patients, like people without severe renal impairment, could really benefit from OAT introduction.

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Myelodysplastic syndromes - Biology

S671

COMPREHENSIVE ANALYSIS OF MUTATION STATUS, GENE EXPRESSION PROFILES, BLOOD AND BONE MARROW COUNTS AND OUTCOME IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: The combined analysis of the genome and transcriptome in cancer may illuminate the impact of recurrent molecular abnormalities on gene expression, thus affording a better understanding of the causes of the observed variability in the transcriptome, disease pathogenesis and outcome.
Aims: We aimed to determine the impact of the common mutations on the transcriptome in myelodysplastic syndromes (MDS).
Methods: We have recently performed a large mutation screen of 111 cancer genes (including *SF3B1, SRSF2, TET2, ASXL1* and many others) in 738 MDS patients. In this current study, we have linked this genomic data with gene expression microarray data on bone marrow CD34+ cells of 124 MDS patients and 17 controls. We used linear models to deconvolute the expression of genes into contributions stemming from genetic and cytogenetic abnormalities, providing deep insights into how driver mutations affect the transcriptome.
Results: To obtain an overview of the main patterns of gene expression changes, we performed a principal components analysis. The expression changes associated with the first component PC1 are dominated by genes related to hematopoietic differentiation; for example the stem cell factors *KIT*, *CD34* and also *FLT3* have positive values in PC1 while members of the α and β globin gene clusters have negative values. The second component PC2 has low levels of multiple chemokines and high levels of eosinophil- and neutrophil-related genes as well as the hematopoietic transcription factor *KLF1*. When overlaying the status of 12 recurrent genetic and 4 cytogenetic alterations, we found that PC1 can be largely attributed to the antagonistic occurrence of *SF3B1* and *SRSF2* mutations (having low and high values in PC1 respectively). These data suggest that the mutational status shapes the gene expression landscape. To investigate the association of gene expression and mutation data, we fitted a linear model to each gene using the 16 genetic and cytogenetic variables, gender and age as covariates. The MDS transcriptome was globally perturbed by genetic and cytogenetic driver alterations, with approximately 20% of genes showing differential expression due to any mutation. *SF3B1* mutations had the largest number of differentially expressed genes (n=605). Patterns of mutational co-occurrence were reflected by the overlap of associated expression changes: correlated mutations show greater overlap of their expression targets and mutually exclusive abnormalities tend to have different target sets. Distinct differentially expressed genes were associated with the most common splicing gene mutations (*SF3B1, SRSF2, U2AF1* and *ZRSR2*) in MDS, suggesting that different phenotypes associated with these mutations may be driven by different effects on gene expression and that the target genes are different. Most genes deregulated by *EZH2* and *ASXL1* mutations had higher expression compared to non-target genes. Data from the NIH roadmap epigenome consortium show an enrichment of repressive H3K27me3 signal for these genes in normal CD34+ cells. This provides additional evidence that *EZH2* and *ASXL1* mutations lead to derepression of certain Polycomb group target loci. We modelled the influence of mutations, cytogenetics, gene expression and blood/bone marrow counts on MDS patient survival. The transcriptome was the most powerful predictor of outcome, suggesting that the incorporation of gene expression data into existing MDS prognostic scoring systems should be considered.
Summary and Conclusions: This study provides important insights into the impact of the common gene mutations and cytogenetic alterations on the transcriptome in MDS.

S672

MULTIPLE CONCOMITANT MUTATIONS TARGETING RAS-PATHWAY IN JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) SUGGEST A DOSE-RELATED EFFECT OF RAS ONCOGENESIS

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Background: JMML is a rare myeloproliferative/myelodysplastic syndrome of early childhood. It is initiated by mutations, classically described as mutually exclusive, in RAS (NRAS, KRAS) or regulators of the RAS pathway (PTPN11, NF1 or CBL) leading to the hyperactivation of the RAS signaling pathway. In most cases, JMML is a severe disease and the only curative treatment is bone marrow transplantation. Nevertheless, clinical presentation and evolution of JMML are highly heterogeneous since transformation to AML occur in one third of patients while some patients exhibit an indolent disease or even experiment spontaneous remission. This heterogeneity is only partially related to the initiating mutation and could be explained by the presence of additional mutations.
Aims: Additional genetic events were analyzed in the whole french cohort of JMML in order to evaluate their frequency, their repartition in the different JMML genetic subtypes and their potential impact on JMML evolution.
Methods: Paired tumoral and germline DNA from the 120 patients of the JMML french cohort was analyzed by SNP array (N=70) and/or whole exome sequencing (N=10) and/or high-throughput targeted sequencing (N=87). The order of appearance of these mutations was investigated by sequencing isolated colonies obtained by culturing myeloid progenitors on methylcellulose.
Results: The screening for additional mutations in 120 JMML, of which the initiating mutation had been previously identified, could uncover additional molecular abnormalities (mutations, deletions or acquired uniparental disomy) in 26 JMML cases (21%). When including karyotype abnormalities, 48/120 (40%) JMML presented at least 1 additional clonal abnormality. Unexpectedly, in 13 JMML, at least one of these additional events consisted in a second RAS-pathway activating event. Various combinations, involving up to 3 such mutations, were observed. Clonal analysis demonstrated the coexistence of these mutations in myeloid progenitors. The most frequent association was a secondary somatic inactivation of NF1 in 6/33 (18%) of JMML with PTPN11 mutation. Interestingly, 11/19 (58%) JMML with NRAS initiating mutation had at least 1 clonal additional abnormality. The additionalmutation directly impacted the RAS pathway in 3 cases, including a mutation of RRAS in two. These 3 later patients developed a particularly aggressive disease with blast excess at diagnosis and rapid transformation to AML with myelodysplasia. On the other hand, none of the NRAS patients who survived without bone marrow transplantation ("long survivors") had additional genetic events. Moreover, NRAS mutated patients who displayed no additional mutation had none of the following markers of severity: death, post-transplantation relapse, medullar blast excess (>10%) at diagnosis or blast crisis. On the contrary, all patients with one or more additional abnormality had at least one of these severity criteria.
Summary and Conclusions: The finding in sporadic JMML of multiple concomitant genetic events targeting the RAS-pathway challenges the dogma that these mutations are mutually exclusive and seems to mark very aggressive and rapidly progressing JMML. These observations suggest a dose effect of RAS-pathway activation, especially in a NRAS-mutated context. They also provide a clinical confirmation for RAS-mutated conditional expression studies in mice which first demonstrated the dose-related effect of RAS oncogenesis. The use of RAS inhibitors could be of particular therapeutic interest in this sub-group of very aggressive JMML.

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SF3B1 PLAYS AN IMPORTANT ROLE IN THE REGULATION OF HEMATOPOIETIC STEMS CELLS, BUT HAPLOINSUFFICIENCY OF SF3B1 MAY NOT BE SOLELY RESPONSIBLE FOR MYELODYSPLASIA

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Background: SF3B1 is a core component of the mRNA splicing machinery and is frequently mutated in MDS, particularly characterized by the presence of increased ring sideroblasts (RS). Most of *SF3B1* mutations reported in MDS are clustered in 5 hot-spot amino-acid positions. However, the molecular mechanism by which the *SF3B1* mutation leads to myelodysplasia and promotes the formation of RS is unknown. Previously we reported hematological phenotype of *Sf3b1*$^{+/-}$ mice aged 3 months. In this study, we additionally performed more detail analysis with *Sf3b1*$^{+/-}$ mice older than 6 months of age.
Aims: To clarify the role of *SF3B1* in hematopoiesis and to obtain insights into how the deregulation of *SF3B1* leads to the development of MDS phenotypes,

we investigated the hematological phenotype of *Sf3b1*$^{+/-}$ mice.
Methods: Analysis of peripheral blood (PB) count and RS were performed 3–13-month-old mice. Examinations of hematopoietic stem cells (HSCs)/progenitor cells, and splenic B cells were performed in 3- and 8-month-old mice. *In vitro* colony assays and competitive transplantation assays were conducted using 3-months-old mice.
Results: There was no significant change in complete PB counts between *Sf3b1*$^{+/+}$ and *Sf3b1*$^{+/-}$ mice at any time points up to 13 months. Total bone marrow (BM) cellularity, lineage composition and spleen weights of *Sf3b1*$^{+/-}$ mice were examined at 3 months and 8 months, and no obvious differences were detected between *Sf3b1*$^{+/+}$ and *Sf3b1*$^{+/-}$ mice. Morphologic abnormalities in PB and BM cells were not recognized. In contrast to a previous report describing increased formation of RS in the same *Sf3b1*$^{+/-}$ mouse strain, we observed very few sideroblasts and no RS formation until 13 months of age. To further assess the hematopoietic system in the BM, we evaluated HSCs and progenitor cells by flow cytometric analyses. The frequency and the absolute number of HSCs, defined as CD34^{-}KSL cells, were significantly decreased in *Sf3b1*$^{+/-}$ mice aged 3 months. Whereas, there were no significant differences in the number of progenitor cells including HPC fraction (CD34^{+}KSL cells), MEPs, CMPs, and GMPs between *Sf3b1*$^{+/+}$ and *Sf3b1*$^{+/-}$ mice. *SF3B1* mutations were also seen in a subset of CLL cases, but no obvious changes in a CLPs population as well as in splenic B cell populations were observed in *Sf3b1*$^{+/-}$ mice. Similar results were obtained from 8-month-old mice. Consistent with the reduction in the HSC fraction, *in vitro* colony assays indicated the significantly lower number of hematopoietic colonies in *Sf3b1*$^{+/-}$ mice BM cells than that in *Sf3b1*$^{+/+}$ mice. Subsequently, we assessed the reconstitution capacity of whole BM cells, using competitive repopulation assays. The chimerism of *Sf3b1*$^{+/-}$-derived cells was significantly lower than that of *Sf3b1*$^{+/+}$-derived cells. To confirm this finding further, we performed competitive repopulation assays using purified HSCs (CD34^{-}KSL cells). Similarly, the chimerism of donor-derived cells was also reduced in mice transplanted with *Sf3b1*$^{+/-}$ mice-derived HSCs. These findings were confirmed by competitive repopulation assays using enriched long-term HSCs (CD150^{+}CD34^{-}KSL cells). Furthermore, we performed serial transplantation experiments of whole BM and HSCs to assess the long-term reconstitution capacity of *Sf3b1*$^{+/-}$ HSCs more precisely. *Sf3b1*$^{+/-}$ mice showed reduced chimerism of donor-derived cells in the primary transplantations, and the reduced chimerism was even more pronounced after secondary transplantations.
Summary and Conclusions: HSCs from *Sf3b1*$^{+/-}$ mice showed a reduction in their number and compromised repopulation capacity of hematopoiesis. *Sf3b1*$^{+/-}$ mice aged older than 6 months exhibited similar hematological phenotype to 3-month-old *Sf3b1*$^{+/-}$ mice. There was no increase in RS or evidence of myeloid dysplasia in *Sf3b1*$^{+/-}$ mice, at least until 13 months of age. These results that indicate haploinsufficiency of *Sf3b1* leads to compromised stem cell function but not to myelodysplasia, were compatible with the fact that most of *SF3B1* mutations in MDS are suggested to be a gain-of-function.

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MOLECULAR PROFILING OF 944 PATIENTS WITH MYELODYSPLASTIC SYNDROMES USING DEEP SEQUENCING

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of chronic myeloid neoplasms characterized by varying degrees of cytopenias and a high propensity to acute myeloid leukemia. Diagnosis and prognostication of MDS may be improved by High-throughput mutation/copy number profiling, which also provide a new insight into their pathogenesis.
Aims: The purpose of this study is to investigate the role of gene mutations in the pathogenesis/clonal evolution of MDS and their impact on prognostication in MDS.
Methods: A total of 944 patients with various MDS subtypes were screened for gene mutations and deletions in 104 known/putative genes relevant to MDS using targeted deep-sequencing and/or array-based genomic hybridization. Somatic mutations were identified through in-house pipeline, correlations of different gene mutations and their clonal architecture were investigated. Impact of genetic lesions on overall survival (OS) was investigated by univariate analysis and a conventional Cox regression to build a new prognostic model based on gene mutations.
Results: After excluding sequencing/mapping errors and known or possible polymorphisms, a total of 2,764 single nucleotide variants and insertions/deletions were detected in 96 genes as high-probability somatic changes. A total of 47 genes were considered as statistically significantly mutated (q<0.01), of which only 6 genes (*TET2*, *SF3B1*, *ASXL1*, *SRSF2*, *DNMT3A*, and *RUNX1)* were mutated in >10% of the cases. Significant correlations were observed for different gene mutations, particular combinations of gene mutations occurred more frequently than expected, suggesting the presence of functional interactions between these mutations. Intratumor heterogeneity was evident in as many as 456 cases (48.3%), even though the small number of gene mutations available for evaluation was thought substantially to underestimate the real frequency. The number of observed intratumoral subpopulations tended to correlate with the number of detected mutations and therefore, advanced WHO subtypes and risk groups with poorer prognosis. Mean variant allele frequencies (VAFs) showed significant variations among major gene targets and VAF values were significantly biased for some combinations of genes, suggesting the presence of chronogenic hierarchy among these common mutations during clonal evolution in MDS. The impact of these genetic lesions on clinical outcomes was initially investigated in 875 patients. In univariate analysis, 25 out of 48 genes tested significantly affected overall survival (P <0.05), and only *SF3B1* mutations were associated with a better clinical outcome. Importantly, the impact on survival was observed even for subclonal mutations. Finally, to evaluate the combined effect of these multiple gene mutations/deletions, together with common clinical/cytogenetic variables used for IPSS-R, OS was modeled by a Cox regression. We found that when combined with traditional prognostic valuables, such as age, white cell counts, hemoglobin, platelet counts, cytogenetic score in IPSS-R, the mutation/deletion status of a set of genes could be used to build a prognostic system more accurately predict the survival of patients.
Summary and Conclusions: Large-scale genetic and molecular profiling using deep sequencing not only provided novel insights into the pathogenesis and clonal evolution of MDS, but also enabled to build a more robust prognostic model based on both gene mutations and other clinical variables that could potentially outperform the conventional prognostic systems.

S675

LIN28B DEFINES AN AGGRESSIVE SUBTYPE OF JUVENILE MYELOMONOCYTIC LEUKAEMIA

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Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive clonal myeloid neoplasm of early childhood associated with mutations in Ras pathway genes (*PTPN11*, *KRAS*, *NRAS*, *CBL* and *NF1*). Elevated fetal hemoglobin (HbF) levels and monosomy 7 are frequently observed. Stem cell transplantation is the only available curative treatment option but only provides an event-free survival of about 50%.
Aims: Gain insight in the molecular networks involved in JMML pathogenesis based on mRNA, microRNA and long non-coding RNA transcriptome analysis of JMML samples.
Methods: Expression of 27958 mRNA probes and 23042 lncRNA probes was assessed in diagnostic bone marrow or peripheral blood mononuclear cells of 63 JMML patients and 5 healthy donors, using a custom designed Agilent array. In addition, cDNA of 768 microRNAs was pre-amplified and quantified using miRNA specific Taqman probes.
Results: Unsupervised clustering of an initial cohort of 14 patients generated two subgroups with *let-7e* and RNA-binding protein *LIN28B* amongst the most significantly differentially expressed genes. In the final cohort, relative higher *LIN28B* expression was observed in 35 of 63 cases (55.6%) and was defined as the average of the healthy donors plus three standard deviations. Univariable Cox regression showed that logarithmic *LIN28B* expression as a dichotomous variable can predict overall survival (p=0.035, exp(B)=4.227, CI(95%)=1.108-16.125). Patients with higher *LIN28B* mRNA levels experience a significant worse overall survival (Kaplan-Meier plot, p=0.022). HbF and platelet count were also significant prognostic factors, as described previously (p=0.023 and 0.027 respectively). There was no association between *LIN28B* expression and Ras pathway mutation status. We observed the strongest miRNA anti-correlation between *LIN28B* and five *let-7* family members (d, b, g, e and a), and the second highest positive mRNA correlation between *LIN28B* and

HMGA2. Recently, it was shown that the *LIN28B – let-7 – HMGA2* axis determines higher self-renewal of fetal hematopoietic stem cells (Copley, 2013). This indicates that *LIN28B* confers augmented self-renewal to leukemic hematopoietic stem cells in JMML and – since this is an early childhood disease – this is potentially already initiated during embryogenesis.
JMML patients frequently show elevated HbF levels at diagnosis. A positive correlation was found between *LIN28B* expression and HbF levels (r_s=0.64, p<0.001, N=41). Interestingly, our gene expression profiling data showed that both probes corresponding to *HBG1* (encoding the human γ globin chain) and *HBBP1* (encoding a lncRNA-affiliated hemoglobin β pseudogene) were strongly correlated with *LIN28B* expression in our patient series. This emphasizes the central role for *LIN28B* in the fetal (leukemic) hematopoietic stem cell system. Strikingly, patients with monosomy 7 (n=7/56) never displayed increased *LIN28B* expression (Chi-square p=0.0017), suggesting the presence of a *LIN28B* activating transcription factor on chromosome 7. We identified *MNX1* (*HLXB9*) as a possible activator of *LIN28B* based on a very strong correlation and siRNA knockdown (Figure 1).

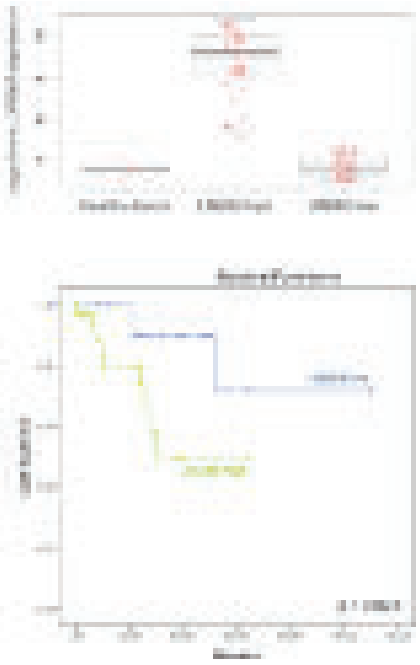

Figure 1.

Summary and Conclusions: *LIN28B* is overexpressed in more than half of JMML patients and significantly predicts poor outcome. Strong correlation with HbF levels, *HBG1* and *HMGA2* gene expression suggests that *LIN28B* regulates a leukemic hematopoietic stem cell network. Moreover, patients with monosomy 7 seem to be protected against *LIN28B* overexpression due to the absence of the putative activator *MNX1*. All together, *LIN28B* has a link with most characteristics of the disease and plays a central role in regulatory networks in JMML.

Chronic myeloid leukemia - Clinical 1

S676

EFFICACY AND SAFETY OF IMATINIB IN CML OVER A PERIOD OF 10 YEARS: DATA FROM THE RANDOMIZED CML-STUDY IV

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Background: Tyrosine kinase inhibitors (TKI) have changed the natural course of CML. With the advent of 2nd generation TKI and the now available choice of drugs, safety issues have gained interest.
Aims: We have used 1551 patients of the randomized CML-Study IV for a long-term safety and efficacy evaluation of imatinib (IM) and to comparatively analyze adverse drug reactions (ADR) for IM 400 mg, IM 800 mg and IM 400 mg+interferon (IFN).
Methods: ADR were reported at each follow-up visit, coded and graded according to the WHO CTC AE list. Molecular analyses for residual BCR-ABL transcripts were adjusted by the international scale. Efficacy was evaluated by intention to treat. ADR were analyzed as treated. For the general safety analyses, patients were only counted as long as they solely received imatinib. For the treatment comparisons, patients were kept within their randomized groups but only counted as long as they received the randomized treatment. Because of practicality, it was not possible to consider different imatinib doses.
Results: 1501 patients have received imatinib alone or in combination and were evaluable. Median age at diagnosis was 53 years, 88% were EUTOS low risk. At the last evaluation (11.4.2013), 1003 patients still received imatinib, 164 had died, 275 had been switched to a 2nd generation TKI, 106 had been transplanted (numbers in part overlapping). The longest observation time was 11.5 years, the median observation time 6.5 years. Efficacy: 10-year progression-free and overall survival probabilities were 81% and 84%, respectively. 10-year cumulative response rates reached 89% for MMR, 81% for MR^4, 74% for MR$^{4.5}$, and 63% for MR^5. Safety: 1375 patients received imatinib alone, at least for a certain time. In 1018 (74%), non-hematologic ADR were reported during imatinib treatment, in 199 grade 3/4 ADR (14%). 8-year probabilities, all grades, with 95% confidence intervals (CI) were: fluid overload or edema 41 (36-46)%, gastrointestinal 38 (34-43)%, myalgia or arthralgia 25 (21-29)%, rash 20 (17-24)%, muscoloskeletal 17 (13-22)%, fatigue 17 (13-20)%, neurological 11 (8-13)%, and flu-like 10 (8-13)%. By treatment, 8-year probabilities of any non-hematologic ADR, all grades, with 95% CI were 72 (65-78)% for IM 400mg, ≥83 (77-87)% for IM 800mg (last value at around 7 years) and 87 (76-94)% for IM 400mg+IFN and for grade 3/4 ADR, 16 (12-21)% for IM 400mg, ≥34 (24-44)% for IM 800mg and 35 (24-47)% for IM 400mg+IFN. These numbers represent minimum values, since some results were not evaluable and ADR might have been present. Probabilities over time were calculated for each ADR. In 5 patients, peripheral arterial occlusive disease of grade 2 or 3 was reported, but none could be clearly assigned to imatinib. A definite association between any ADR and death was not found. Most patients had their first ADR during their first three years of treatment with decreasing frequencies later on. ADR could be well managed by dose reduction, interruption, symptomatic medication or permanent discontinuation.
Summary and Conclusions: Given that no imatinib-related death was recorded and that grade 3/4 ADR could typically be symptomatically managed we consider imatinib a safe, comparably well tolerated TKI even after prolonged treatment, also at 800 mg and in combination with IFN. After 10 years, imatinib continues to be an excellent choice for most patients with CML.

S677

ENESTND 5-YEAR FOLLOW-UP: CONTINUED BENEFIT OF FRONTLINE NILOTINIB (NIL) COMPARED WITH IMATINIB (IM) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP)

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Background: The ongoing Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Pts (ENESTnd) study has continually demonstrated superior efficacy of frontline NIL over IM, with higher rates of major molecular response (MMR; BCR-ABL^{IS} ≤0.1%) and MR$^{4.5}$, (BCR-ABL^{IS} ≤0.0032%), more likely achievement of early molecular milestones (BCR-ABL^{IS} ≤10% at 3 months), lower rates of progression to accelerated phase (AP)/blast crisis (BC) and fewer new BCR-ABL mutations on treatment, in pts with newly diagnosed CML-CP.
Aims: This analysis aims to demonstrate long-term efficacy and safety of NIL compared with IM in ENESTnd, with a minimum follow-up of 5 calendar y (≈ 4.5 months longer than any follow-up previously presented).
Methods: In this phase 3, open-label, multicenter study, 846 adult pts with newly diagnosed CML-CP were stratified according to Sokal risk score and randomized to receive NIL 300 mg twice daily (BID; n=282), NIL 400 mg BID (n=281), or IM 400 mg once daily (n=283). Efficacy analyses were based on all randomized pts. Molecular response assessments were collected during study treatment. Data on progression to AP/BC and overall survival (OS) were prospectively collected during study follow-up and after treatment discontinuation.
Results: After a minimum follow-up of 5 calendar y, over 80% of pts remained on study in all arms (Table 1). Rates of MMR and MR$^{4.5}$, continued to be significantly higher in the NIL arms *vs* the IM arm, with more than half of NIL-treated pts achieving MR$^{4.5}$, by 5 y. For pts achieving MR$^{4.5}$, Kaplan-Meier estimates of remaining in MR$^{4.5}$, for 24 mo were similar across treatment arms (72.0%, 77.8%, 75.5% for NIL 300 mg BID, NIL 400 mg BID, and IM, respectively). No pts had treatment-emergent BCR-ABL mutations since the 4-y analysis or progressed to AP/BC on core treatment since the 2-y analysis. When including progression events occurring after core treatment discontinuation, estimated rates of freedom from progression to AP/BC on study remained higher in NIL-treated pts (Table 1). Overall, fewer deaths occurred on study in the NIL arms *vs* the IM arm. When considering only deaths in advanced CML (ie, the principle cause of death was either "study indication" or "unknown" or not reported but occurred subsequent to a documented progression to AP/BC), estimated rates of survival were higher in the NIL arms *vs* the IM arm. Reasons for death in pts without advanced CML were similar between arms. Safety data after 5 y of follow-up was similar to the known safety profiles of the drugs. The frequency of adverse events (AEs) leading to discontinuation was lowest in the NIL 300 mg BID arm (12.2%), followed by the IM arm (13.9%) and the NIL 400 mg BID arm (19.9%). Newly occurring or worsening total cholesterol abnormalities (all grades) were seen in 27.6%, 26.7% and 3.9% of pts in the NIL 300 mg BID, NIL 400 mg BID, and IM arms, respectively. Cardiovascular events are shown in the Table; rates of these AEs were higher in the NIL 400 mg BID arm than in the NIL 300 mg BID arm.

Table 1.

Summary and Conclusions: The 5-y data confirm the sustained efficacy of frontline NIL over IM in pts with newly diagnosed CML-CP, including achievement of earlier and deeper molecular responses and increased freedom from progression to AP/BC. When considering both safety and efficacy, NIL 300 mg BID continues to be a standard-of-care frontline therapy option in pts with newly diagnosed CML-CP.

S678

DASATINIB DAILY DOSE OPTIMIZATION BASED ON RESIDUAL DRUG LEVELS RESULTED IN REDUCED RISK OF PLEURAL EFFUSIONS AND HIGH MOLECULAR RESPONSE RATES: FINAL RESULTS OF THE RANDOMIZED OPTIM DASATINIB TRIAL.

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Background: Dasatinib first line (Sprycel®, Bristol-Myers Squibb) induces higher levels of cytogenetic and molecular responses as compared to imatinib in chronic phase chronic myelogenous leukemia (CP-CML) patients (pts). However, dasatinib is associated with the occurrence of pleural effusions (PE). Cumulative incidence of all grades PE (CI-PE) in the Dasision trial (median age 47y) was reported to be 19% by 36 months with an overall discontinuation rate of 29%. (Jabbour *et al.,* Blood 2014).
Aims: The aim of the Optim dasatinib trial was to validate a dose optimization strategy driven by dasatinib residual plasmatic level (Cmin) in terms of CI-PE and molecular response. (EudraCT 2008-006854-17).
Methods: 289 newly diagnosed CP-CML pts from french and canadian CML centers were assigned to dasatinib 100 mg/d. Dasatinib Cmin levels were assessed 24+/-2h after intake by tandem mass spectrometry at day 15. Pts with high Cmin values (Cmin ≥3 nM) (n=82) were randomized between a dose adaptation arm (A1, 20 mg decrement every 15 days in order to obtain a Cmin value <3nM, minimal dose level 40 mg/d) or no dose modification (A2). Patients with Cmin values <3nM were followed in arm B (n=207). All patients were treated according to the ELN 2009 recommendations. The CI-PE observed in each arm was compared. Intent to treat analysis was used to determine molecular outcomes in each treatment arm.
Results: Patients were recruited between May 2009 and December 2012. Database was locked in January 2014. Median follow-up was 32 months (14-57). Sokal scores were high for 21%, intermediate for 34% and low for 45% of pts. The median age was 53 years (18-86). Median dasatinib Cmin value was 2.1nM (0.1-18.7) with higher Cmin values in pts aged over 50y as compared to younger pts (2.5 *versus* 1.6, p<0.001). Accordingly, median age was higher in arms A1 and A2 as compared to arm B (54 *vs* 42, p=0.0032). After dose optimization in arm A1, median Cmin value was reduced from 5.1nM at randomization to 2.1nM at 12 months. Corresponding median Cmin values were 4.6nM and 4nM in arm A2 and 1.6nM and 1.9nM in arm B respectively. Dose intensity in arm A1 was 57 mg/d and mean daily dose after optimization was 51 mg. Higher dose intensity was observed in arm A2 (96 mg/d) resulting in high discontinuation rate (27%) as compared to arm A1 (13%). The overall CI-PE by 36 months was 16.2% (95%CI: 7.3-28.3). CI-PE was 10.7% (95%CI: 2-27.9) in pts with low Cmin values (arm B), as compared to 29.6% (95%CI: 13.8-47.3) in pts with high Cmin values (arms A1 and A2) (p=0.0002). A dramatic reduction in CI-PE was observed in pts randomized to the optimization strategy (arm A1, 11.3%; 95%CI 0.4-44.2) as compared to pts allocated to a standard follow-up (arm A2, 48.9%; 95%CI 26.4-68.1) (p=0.008). Age and Cmin were independent determinants of PE but the effect of age was overcome by dose optimization. Remarkably high cumulative molecular response rates by 24 months were observed in arms A1, A2 and B for MMR (88%, 82% and 83%), MR4 (69%, 67% and 67%) and CMR4.5 (39%, 32% and 34%), despite a significant dose reduction in arm A1. Of note, emerging adverse events were recorded in this trial such as pulmonary hypertension (2 cases, 0.6%), Raynaud syndrome (2 cases, 0.6%) and follicular hyperplasia (7 cases, 2.4%).
Summary and Conclusions: Dasatinib dose optimization is an efficient strategy to overcome the risk of pleural effusion in high risk patients (defined by a Cmin value ≥3nM) and to reduce the discontinuation rate. The proportion of

patients eligible to dasatinib dose optimization is increasing with age (up to 43% over 60y) reflecting the CP-CML population. Our results suggest that personalized dasatinib dose schedule in this high risk population may be close to 50 mg/d and remains associated with high levels of deep molecular responses.

S679

EPIC: A PHASE 3 TRIAL OF PONATINIB *VS* IMATINIB IN PATIENTS (PTS) WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CP-CML)

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Background: Ponatinib is a potent oral tyrosine kinase inhibitor active against native and mutated forms of BCR-ABL, including T315I. The phase 2 PACE study demonstrated that ponatinib is highly active in heavily pretreated Philadelphia chromosome–positive (Ph+) leukemia pts.
Aims: The efficacy and safety of ponatinib were evaluated in newly diagnosed CP-CML pts in the EPIC (Evaluation of Ponatinib *vs* Imatinib in CML) trial.
Methods: EPIC was an international, phase 3 trial of ponatinib (45 mg QD) *vs* imatinib (400 mg QD) in newly diagnosed CP-CML pts, with randomization stratified by Sokal risk score. The primary endpoint was major molecular response (MMR) rate at 1 yr. On 18 Oct 2013, EPIC was terminated due to accumulating vascular events in long-term follow-up of the PACE trial. Data as of 7 Oct 2013 are presented; median (range) follow-up was 3 (0.03-12) months. NCT01650805.
Results: At the time of analysis, 306 pts were randomized and gave informed consent. Baseline characteristics were balanced for ponatinib *vs* imatinib: 55 *vs* 52 yrs median age, 17% *vs* 16% high Sokal risk score, 62% *vs* 66% received prior hydroxyurea. Data were available on 267 treated pts (133 ponatinib, 134 imatinib). At analysis, 77% ponatinib and 84% imatinib pts were ongoing; 14 ponatinib and 6 imatinib pts discontinued (discontinuation due to adverse events [AEs]: 9 ponatinib pts [most common: thrombocytopenia and rash]; 1 imatinib pt). Response rates are shown in the Table 1 (evaluable pts defined as pts assessed at 3, 6, 9 months). Most common (≥25%) all-grade treatment-emergent AEs in the ponatinib arm were rash (36%), abdominal pain (32%), headache (31%), lipase increased (26%), and myalgia (26%); in the imatinib arm, nausea (32%) and muscle spasms (31%). 11% ponatinib and 2% imatinib pts had grade 3/4 thrombocytopenia; 3% ponatinib and 8% imatinib pts had grade 3/4 neutropenia. Serious treatment-emergent AEs (SAEs) occurring in ≥3 ponatinib pts were pancreatitis (5 pts), atrial fibrillation (3), and thrombocytopenia (3); no individual SAEs occurred in ≥3 imatinib pts. 9 (7%) ponatinib and 5 (4%) imatinib pts experienced vascular occlusive events (SAEs: 6 ponatinib, 1 imatinib). Updated data will be presented.
Summary and Conclusions: While ponatinib demonstrated early activity in frontline CP-CML, EPIC was terminated because its objectives could not be met, since ponatinib dose reductions were implemented mid-trial due to safety observations in PACE. Further investigation of ponatinib safety is warranted. Ponatinib remains an important treatment option for pretreated CML and Ph+ acute lymphoblastic leukemia pts for whom the need and benefit outweigh the potential risks.

Table 1.

	At 3 months		At 6 months		At 9 months	
	Ponatinib	Imatinib	Ponatinib	Imatinib	Ponatinib	Imatinib
MMR	29% (28/95)	0% (0/98)	66% (27/41)	21% (9/42)	83% (10/12)	44% (7/16)
MR4.5	4% (4/95)	0% (0/98)	10% (4/41)	0% (0/42)	25% (3/12)	0% (0/16)
≤10% BCR-ABL transcripts	94% (89/95)	68% (67/98)	100% (41/41)	83% (35/42)		

S680

THE EUTOS POPULATION-BASED REGISTRY: EVALUATION OF BASELINE CHARACTERISTICS AND FIRST TREATMENT CHOICES OF 2983 NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA (CML) PATIENTS FROM 20 EUROPEAN COUNTRIES

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Background: Most of the knowledge about treatments and outcome of CML patients originates from clinical studies. To get new and unbiased insights in the epidemiology, treatment and outcome of CML, the EUTOS population-based registry of newly diagnosed CML patients was established, - as part of the European Treatment and Outcome Study (EUTOS) for CML.
Aims: The aim was to collect the data of all adults with newly diagnosed CML, irrespective of treatment and of enrolment in studies.
Methods: The EUTOS population-based registry collected data of newly diagnosed CML patients, 18 years or older, over a specified period of time from 2008 till 2012 living in defined regions. The data were collected by 22 study groups in 20 European countries. Data were gathered via a web-based CRF-system.
Results: Till 31.12.2013 2983 patients were registered. Treatment data are currently available for 1948 patients. 94.2% of the patients were diagnosed in chronic phase (CP), 3.6% in accelerated phase (AP), and 2.2% in blastic phase (BP). 54% of the patients were male. The median age at diagnosis was 57 (range: 18-99) years, varying from 50 yrs. in Russia to 62 in Sweden. Stratified by decades, 0.6% patients were 18 or 19 years old, 6.8% 20-29 years, 11.6% 30-39 years, 16.8% 40-49 years, 22.1% 50-59 years, 19.2% 60-69 years, 16.0% 70-79 years, 6.4% 80-89 years, and 0.5% more than 90 years old. According to the WHO PS score, in CP 57.4% of patients were asymptomatic, 36.6% were symptomatic but completely ambulatory, 4.2% were symptomatic but less than 50% in bed during the day, 1.2% were symptomatic with more than 50% in bed during the day, and 0.6% were bedbound. For patients in AP or BP at onset, the corresponding Figures were 31.9%, 41.3%, 18.1%, 7.2%, and 1.4%. For 55.4% of the patients comorbidities were recorded. 18.4% of patients were current smokers, while 16.6% were former smokers, and 65.0% were non-smokers. For the calculation of the prognostic scores 432 patients had to be excluded as they had already been treated before measuring the hematologic values. By EUTOS Score (Hasford *et al.*, 2011) 88.7% of CP patients were low risk, and 11.3% were high risk. By Euro Score 37.8% were low, 51.4% intermediate and 10.9% high. By Sokal Score 34.5% were low, 40.8% intermediate, and 24.8% high. CP patient distribution by spleen volume, blood cell count, and differential are shown in Table 1. 83.3% of the patients received imatinib firstline, 10.9% nilotinib and 3.8% dasatinib. During the first year after diagnosis, 9.4% of patients received nilotinib and 6.0% dasatinib second-line.

Table 1.

Variable	Hb [g/dl]	WBC [109/l]	Platelets [109/l]	Blasts [%]	Basophils [%]	Eosinophils [%]	Spleen*
N	2332	2338	2333	2304	2310	2303	2290
N miss	6	0	5	34	28	35	48
Min	5.0	10.2	11	0	0	0	0 [54%]
Max	18.3	932	4812	29	20	30	35
Median	12.1	84	395	1	3	2	0

**palpated, cm under costal margin.*

Summary and Conclusions: This is the first report of the demographic and baseline characteristics of an unselected, population-based, series of patients with newly diagnosed Ph+, BCR-ABL+ CML. The age distribution and some baseline characteristics are different from many prospective studies. This should be taken in due consideration before extrapolating the results of treatment studies to the overall population. With further follow-up, this registry will provide a population-based insight on treatment, survival, and causes of death.

Acute lymphoblastic leukemia - Biology 1

S681

THE INCIDENCE OF THE HIGH-RISK PH-LIKE ALL SUBTYPE MAY ACCOUNT FOR THE MAJORITY OF NON BCR-ABL1+ B ALL CASES IN ADULTS

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Background: Adult patients with Acute Lymphoblastic Leukaemia (ALL) have a universally poor outcome, particularly those with *BCR-ABL1+* ALL, though the addition of tyrosine kinase inhibitor (TKI) therapy to the upfront regimen has improved outcomes. In contrast, contemporary therapy for childhood ALL results in 80% event free, and 90% overall 5 year survival. Despite this, relapsed ALL remains the major cause of non-traumatic death in children. Recent genomic profiling of childhood ALL has identified a novel subtype of B-ALL with a gene expression profile similar to *BCR-ABL1+* ALL, but harbouring novel kinase activating rearrangements (Ph-like ALL). Importantly, *in vitro* studies and anecdotal cases suggest that Ph-like ALL, a high-risk subtype, may respond to current TKIs. The frequency of Ph-like ALL rises with increasing age[1], but the frequency and spectrum of lesions in adults is unknown.
Aims: To identify the frequency of Ph-like disease in AYA and adults, and determine *in vitro* sensitivity to kinase inhibitors.
Methods: Ph-like ALL cases were identified by Taqman Low Density Arrays (TLDA) using 20 genes selected from prior paediatric reports. Bioinformatic modelling was used to derive a 9-gene signature. Phospho-flow cytometry (p-Flow) was used to detect pathway activation, resulting in phosphorylation of known kinase targets (ie: CrkI and STAT5) and to assess responsiveness to kinase inhibitors. CRLF2 surface expression was detected by flow cytometry. Candidate RT-PCR for previously identified fusions, sequencing and FISH were used for identification.
Results: Sixty-one AYA/adult *BCR-ABL1* neg ALL diagnosis samples were analysed using TLDA; 27 were AYA (16-39y) and 34 adults (40+y). 15/61 (25%) patients; 3 AYA (11%) and 12 adult (35%) demonstrated a Ph-like signature by TLDA. In 2/3 AYA cases this was accompanied by expression of pSTAT5 and CRLF2. *In vitro* treatment with JAK inhibitors resulted in attenuation of p-STAT5, and candidate PCR identified CRLF2 fusion partners {IGH and P2RY8}. Sequencing revealed a JAK mutation in the IGH-CRLF2 case. In the remaining Ph-like AYA case expression of p-CrkI was attenuated by TKIs and candidate PCR revealed an EBF1-PDGFRB fusion, confirmed by sequencing. In the adult cohort 15/34 patients demonstrated indicative Ph-like positivity by TLDA alone (n=5); by p-Flow (and CRLF2 expression), but not TLDA (n=3); or by both (n=7). Of this latter cohort 5/7 have confirmed fusions, and identification of fusions in the remaining patients are in progress. In all cases CRLF2 cell surface expression by flow correlated with increased mRNA expression and the presence of a *CRLF2* rearrangement. In 4/6 patients with CRLF2 rearrangement non-synonymous (n=2) and synonymous JAK2 mutations (n=2: T875N and R683S) were detected. The presence of JAK alterations has been associated with sensitivity to JAK inhibition detected by reduction in p-STAT5. Where the genetic alteration has been identified the concordance between TLDA and p-Flow was 100% in the AYA cohort and 80% in the adult (Table 1).

Table 1. Summary of the screening of 61 AYA and adult ALL patients for Ph-like disease.

Summary and Conclusions: These preliminary data demonstrate a significantly increased frequency of Ph-like cases in patients aged over 40. This rise is concurrent with the rise in *BCR-ABL1+* ALL, and may suggest in adults, these

two groups constitute the majority of the B cell ALL cohort. Both TLDA and p-Flow analysis enable rapid detection of these cases, and candidate drug sensitivity can be determined using p-Flow. Importantly, this may guide intervention with targeted therapies matched to the identified causative genetic lesion in this high-risk cohort.

Reference

1. ASH.2013.122.21:825.

S682

BCR-ABL1-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA: A NOVEL SUBTYPE IN ADULTS CHARACTERIZED BY HIGH RELAPSE RATE AND NON-RESPONSE TO TREATMENT

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Background: In pediatric patients with precursor B-cell acute lymphoblastic leukemia (BCP-ALL), the *BCR-ABL1*-like expression signature and *IKZF1* deletion were identified as unfavorable prognostic markers (Den Boer *et al.*, Lancet Oncol 2009; van der Veer *et al.*, Blood 2013). *BCR-ABL1*-like BCP-ALL is identified based on a gene expression signature, which is similar to that of *BCR-ABL1* positive ALL, although these cases do not harbor the *BCR-ABL1* translocation. Approximately 40% of pediatric *BCR-ABL1*-like ALL cases have deletions in the B-cell development gene *IKZF1*.
Aims: We investigated whether a *BCR-ABL1*-like group would be present in adolescent and adult BCP-ALL, and its association with *IKZF1* deletion and prognosis.
Methods: Adolescent and adult patients with newly diagnosed BCP-ALL (127 cases, median age 36 years, range 16-71) were included in four consecutive clinical trials of the Dutch-Belgium HOVON study group between 1993 and 2008 (102 cases), or treated according to similar protocols between 1987 and 2009 (25 cases). *BCR-ABL1*-like BCP-ALL cases were identified using hierarchical clustering with 110 probe sets (Den Boer *et al.*, Lancet Oncol 2009) in Affymetrix U133Plus2 expression data. *IKZF1* deletions were detected using the SALSA P202 *IKZF1* Multiplex Ligation-dependent Probe Amplification assay. Frequencies were compared between groups of samples using Fisher's Exact test. We compared the complete response rate, cumulative incidence of relapse, and event-free survival between *BCR-ABL1*-like, *BCR-ABL1*-positive, *MLL*-rearranged, and remaining BCP-ALL cases.
Results: We identified 21 *BCR-ABL1*-like adult BCP-ALL cases (17%, median age 25 years, range 16-59). The complete response rates were statistically different between the four groups, with lower response rates in the *BCR-ABL1*-positive and *BCR-ABL1*-like BCP-ALL subgroups of about 70%, *versus* more than 90% in the other BCP-ALL groups (p<0.001). Event-free survival showed a trend towards a lower probability compared with the remaining cases for *BCR-ABL1*-like BCP-ALL (HR 1.7, p=0.06), similar to the cytogenetically high-risk *BCR-ABL1*-positive BCP-ALL (HR 1.7, p=0.08). The cumulative incidence of relapse was highest in the *BCR-ABL1*-like BCP-ALL (5-year CIR 67%), compared with *BCR-ABL1*-positive (5-year CIR 32%), *MLL*-rearranged (5-year CIR 29%), and remaining BCP-ALL (5-year CIR 47%). *IKZF1* deletions were detected in 56/122 evaluated BCP-ALL cases (46%). Deletion of *IKZF1* was found in a high percentage of *BCR-ABL1*-positive BCP-ALL patients (74%), but was not increased in *BCR-ABL1*-like BCP-ALL patients (35%) compared with the remaining BCP-ALL cases (37%), and showed a trend towards lower event-free survival in *IKZF1*-deleted cases (p=0.1).
Summary and Conclusions: Our findings suggest that *BCR-ABL1*-like BCP-ALL is a new subgroup comprising 17% of adult BCP-ALL, similar to the frequency in pediatric BCP-ALL. This group was characterized by poor treatment response and high relapse rate, compared with *BCR-ABL1*-positive BCP-ALL and remaining BCP-ALL. *IKZF1* deletions in adults were equally common in *BCR-ABL1*-like BCP-ALL cases compared to the remaining BCP-ALL cases. Further research is needed to study outcome in a larger number of patients and to identify possible genomic lesions underlying the *BCR-ABL1*-like BCP-ALL subtype in adults that may provide a rationale for targeted or intensified treatment.

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IDENTIFYING THE DEVELOPMENTAL LEVEL OF LEUKEMIA INITIATION IN ETV6-RUNX1+ ACUTE LYMPHOBLASTIC LEUKEMIA BY STUDYING MONOZYGOTIC TWINS

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Background: Investigating monozygotic twins with concordant leukemia provides a unique opportunity to analyze the early genetic steps of leukemogenesis. We previously revealed that identical *ETV6-RUNX1* gene fusion is present in both siblings confirming its early (*in utero*) occurrence and implicating its role in the pathogenesis.
Aims: Identification of the fetal cell type which is transformed by *ETV6-RUNX1*.
Methods: Diagnostic bone marrow samples from five monozygotic twin pairs concordant for pediatric *ETV6-RUNX1*$^+$ precursor B-cell ALL were screened for all varieties of immunoglobulin (*IG*) and T-cell receptor (*TCR*) gene rearrangements which are hallmarks of various stages of lymphoid cell development. Identical rearrangements shared by the siblings and indicative of the *IG/TCR* status of the cells giving rise to pre-leukemic clones were identified. The study was conducted in accordance with the Declaration of Helsinki.
Results: Clonal rearrangements were detected in *IGH*, *IGK*, *IGL*, *TCRD*, *TCRG* and *TCRB* genes in 10, 8, 4, 4, 6 and 2 patients, respectively. At least two affected genes were found in each child. In total, 65 rearrangements were identified in the five twin pairs. Five identical junctions (including N bases) shared by the siblings were observed with the following distribution: twins 1A/B *TCRD* VD2-DD3; 2A/B *IGH* V(D)J; 3A/B *IGH* DJ and *IGK* VKde; 5A/B *IGH* DJ. All these rearrangements must have occurred in the single pre-leukemic clone formed *in utero*. The vast majority of clonal markers proved to be patient-specific and are likely to have arisen post-natally in twin-specific sub-clones. Oligoclonality was observed by analyzing the *IGH*, *IGK* and *TCRD* genes and the combined in-depth analysis of these rearrangements with those shared by the twins revealed the long-term (if not permanent) accessibility of both B-lineage specific and cross-lineage loci during the whole period of pre-diagnostic oncogenesis. Siblings in one twin pair (2A/B) reported a significant difference in age at diagnosis (42 *vs* 58 months) and more mature immunogenotype was detected in the older child, supporting the hypothesis that the longevity of the pre-leukemic phase influences immunogenotype maturity, probably due to the ongoing recombinatorial activity.
Summary and Conclusions: To date, some individual twin pairs have been reported with very limited screening for *IG/TCR* rearrangements. We have carried out a comprehensive screen for these rearrangements in a cohort of twin pairs. Our data suggests that the pre-leukemic clone spawned *in utero*, and shared by the twins, has the *IG* and *TCR* feature of a pro- or pre-B cells in 4 out of 5 twin pairs. In the fifth pair, the lack of any shared *IG/TCR* sequences is uninformative and could reflect either an origin in a cell prior to RAG1/2 expression or a pro-B/pre-B origin obscured by ongoing VDJH replacements. The most parsimonious interpretation of these results, also considering previous studies, is that *ETV6-RUNX1* fusion arises in a fetal progenitor or stem cell that lies upstream of B lineage-restricted RAG1/2 active precursors but is either permissive only for B-lineage differentiation or only has a proliferative fitness impact on early B cells. The pre-leukemic clone therefore arises and expands preferentially in the pro- or pre-B lineage compartment, initially in one twin and undergoes DJH and VDJH rearrangements. Clonally descendent cells with self-renewal stem cell activity are sustained in both twins after birth as independent targets for secondary genetic hits essential for clinical development of ALL.

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MER TYROSINE KINASE PROMOTES SURVIVAL OF T(1;19) POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN THE CENTRAL NERVOUS SYSTEM (CNS) NICHE

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Background: TAM-receptors (Tyro3/Axl/Mer) have been investigated as therapeutic targets in human cancers as they mediate interactions with the microenvironment and play a role in tumor cell proliferation and survival. ALL cells with t(1;19) translocation upregulate Mer and patients with this disease are prone to late CNS relapses.
Aims: To examine the functional role of Mer in the CNS niche in preclinical models and to examine its clinical relevance in a cohort of pediatric ALL patients with t(1;19) translocation.
Methods: 4 ALL (697, UoCB6, REH, SUP-B15) and 1 AML cell line (HL-60) were analyzed for Mer protein expression. Next, they were subjected to coculture with U343 human glioma cells. We examined cell cycle profiles, performed chemosensitivity assays and analyzed Mer regulation in ALL cells subjected to glioma coculture. Furthermore, we established an *in vivo* model in NSG-mice to analyse meningeal infiltration by ALL primary cells. 10 patient samples were grouped as Mer^{high} or Mer^{low} and their ability to infiltrate the murine CNS was assessed. Mice xenografted with a Mer^{high} patient sample were treated with the Mer-specific inhibitor UNC569 in order to prevent CNS infiltration. Finally, a cohort of 64 pediatric ALL patients with the t(1;19) translocation and a control cohort of 95 patients negative for t(1;19) were analyzed for Mer mRNA expression by qRT-PCR. Correlations with clinical parameters were performed.
Results: 697 cells were Mer^{high}, UoCB6 and REH cells Mer^{intermediate}, SUP-B15 cells Mer^{low} and HL-60 Mer^{negative}. Glioma coculture caused a quiescent phenotype in ALL cells, in a manner dependent on their Mer expression levels. A Mer^{high} phenotype correlated with a profound growth arrest in G0/G1, a 7-fold drop in S-Phase and chemotherapy resistance in the presence of glioma

cells. These responses could not be observed in Mer^{low} and Mer^{negative} cells. Glioma coculture upregulated Mer surface expression in Mer^{intermediate} cells but not in Mer^{high}, Mer^{low} and Mer^{negative} cells. *In vivo* experiments with primary cells from 10 ALL patients revealed that systemic disease was established in all xenografts. High xenograft CNS involvement was observed in 4/6 Mer^{high} and 0/4 Mer^{low} patients. Primary cells from Mer^{high} xenografts also showed increased *in vitro* survival in coculture with glioma cells. Mice xenografted with a Mer^{high} patient sample were treated with UNC569 which delayed leukemia onset and remarkably reduced CNS infiltration. Screening of patients for Mer expression by qRT-PCR revealed that Mer was upregulated in all 64 patients in the t(1;19) cohort. All control patients, regardless of lineage (B- or T-ALL) and cytogenetics, were Mer negative. We identified 33 Mer^{high} and 31 Mer^{low} patients in our t(1;19) cohort. We observed a significant correlation between high Mer expression and older age and a trend towards high Mer and a higher WBC count at presentation. Most importantly, high Mer expression was correlated with a positive CNS status at initial diagnosis.
Summary and Conclusions: Our data provide evidence that Mer is a survival marker for t(1;19) positive ALL cells in the CNS niche. Mer expressing ALL cells are growth-arrested and chemo-resistant in the presence of glioma cells. This suggests that dormancy of leukemic cells may be a reason for late relapses in the CNS. Targeting Mer can delay leukemic onset and diminish CNS infiltration. Altogether, our data indicate a potential role for Mer as a diagnostic marker and a therapeutic target to treat and prevent CNS disease in t(1;19) positive ALL.

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SMAC MIMETIC TREATMENT IS HIGHLY EFFECTIVE IN A PRECLINICAL PRIMOGRAFT MODEL OF HIGH-RISK ALL *IN VIVO*

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Background: In childhood acute lymphoblastic leukemia (ALL) major improvements in therapy have led to increased survival rates, however 20% of patients relapse. Because treatment failure is, at least in part, due to defects in apoptosis programs, novel therapeutic strategies that counter apoptosis resistance are required. Since "Inhibitor of Apoptosis" (IAP) proteins are expressed at high levels in acute leukemia and block apoptosis at a central point of the apoptotic machinery, molecules antagonizing these apoptosis inhibitors, so called SMAC mimetics, provide a promising strategy to overcome apoptosis deficiency and effectively treat high-risk ALL. Recently, we reported that B cell precursor- (BCP-) ALL cell lines show a heterogeneous sensitivity towards SMAC mimetics, which induce a TNF-alpha feed forward loop in sensitive cells.
Aims: In this study, we aimed to investigate the effect of the small molecule SMAC mimetic BV6 in a series of 42 primary BCP-ALL samples and in a preclinical primograft model of high-risk ALL *in vivo*. BV6 was kindly provided by Genentech.
Results: Intriguingly, upon treatment with nanomolar concentrations of BV6, induction of cell death was observed in 70% of all individual patient-derived leukemias. Interestingly, SMAC mimetic induced cell death in primary ALL samples was inhibited by the soluble TNF-alpha receptor Etanercept indicating the induction of a TNF-alpha feed forward loop in primary ALL. We previously described that deficient apoptosis signaling in ALL cells is an independent risk factor and indicative for early patient relapse. Importantly, also high-risk ALL with constitutive deficient apoptosis signaling showed increased cell death upon treatment with the SMAC mimetic BV6 and activation of the apoptosis pathway, demonstrating that SMAC mimetics enable intact apoptosis signaling in former apoptosis resistant primary ALL cells. Based on these findings, we further evaluated the *in vivo* efficacy of BV6 on high-risk ALL using our NOD/SCID/huALL primograft model in a preclinical setting. ALL bearing recipients were treated with either multiagent chemotherapy, the SMAC mimetic BV6 or the combination of both for a given time of two weeks. Most importantly, a profound reduction of tumor load and a significant delay of post-treatment leukemia reoccurrence were observed upon BV6 treatment alone, which was as effective as the multiagent chemotherapy regimen. The combination of BV6 with multiagent chemotherapy was even more effective in tumor load-reduction resulting in remission-induction and significantly prolonged survival of animals. Interestingly, concomitant *in vivo* therapy with Etanercept revoked the cell death inducing effect of BV6, indicating that BV6 induced cell death involves signaling via TNF-alpha *in vivo* and thereby provides a potential biomarker for the identification of patients who would benefit from SMAC mimetic treatment. The sensitizing effect of BV6 to multiagent chemotherapy *in vivo* however was dependent on TNF-alpha to a lower extent, demonstrating that sensitization to drug induced cell death by SMAC mimetics is mediated additionally via prevention of caspase inhibition thereby enabling efficient apoptosis signaling.
Summary and Conclusions: Taken together, we show that the small molecule SMAC mimetic BV6 induces cell death via a TNF-alpha loop *ex vivo* and *in vivo* in primary patient-derived ALL. Moreover, BV6 is able to overcome apoptosis deficiency of high- risk ALL leading to prolonged *in vivo* survival in a preclinical therapy model of patient-derived ALL primografts. Thus, induction of cell death by new generation small molecule SMAC mimetics provides a promising novel strategy for targeted therapy of high-risk acute lymphoblastic leukemia.

Hematopoiesis, stem cells and microenvironment

S686

RECONSTITUTION OF THE HUMAN HEMATOPOIETIC STEM CELL NICHE BY *IN VIVO* IMPLANTATION OF CARTILAGE PELLETS DIFFERENTIATED FROM HUMAN BONE MARROW- AND CORD BLOOD- DERIVED MESENCHYMAL STEM CELLS (MSCS)

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Background: The bone/bone marrow microenvironment that maintains the hematopoietic stem cell (HSC) state by controlling stem cell self-renewal and differentiation has been defined as the stem cell niche.
Aims: We conceived a novel model in which *in vitro* differentiated cartilage pellets from MSCs generated complete ossicles upon heterotopic implantation in the absence of exogenous scaffolds, reproducing the human HSC niche *in vivo*.
Methods: Human bone marrow (BM) and cord blood (CB) derived MSCs were cultured as micromasses for 21 days in the presence of TGFβ1 to differentiate into cartilage pellets. The generated pellets were implanted in the subcutaneous tissue of SCID-beige mice and harvested at 8 weeks to assess bone and bone marrow formation through histology and FACS analysis.
Results: Heterotopic ossicles, featuring human bone and bone marrow stroma, were regularly observed at 8 weeks. Of note, a near-perfect architecture of a miniature bone organ, including cortical bone, marrow cavity, donor-derived marrow stroma, host-derived sinusoidal circulation, and host-derived hematopoietic tissue developed in a timed fashion. Hematopoietic tissue within the ossicles included erythroid, myeloid and megakaryocytic lineages, as well as hematopoietic progenitor cells, which gave rise to CFU-M, CFU-G, CFU-GM, BFU-E, CFU-GEMM in a methylcellulose-based assay. BM-ossicles contained fewer LSK cells (Lin$^-$Sca-1$^+$c-kit$^+$) than those of BM (0.096±0.007% *vs.* 0.201±0.034%, N=3). Instead, the frequency of LSK in CB-ossicles was similar to that of BM (0.128±0.048%, N=3). Interestingly, the frequency of putative long-term HSC (LSK$^+$CD34$^-$Flk2$^-$), short-term HSC (LSK$^+$CD34$^+$Flk2$^-$) and multipotent progenitors (LSK$^+$CD34$^+$Flk2$^+$) displayed a comparable distribution as found in BM. Similarly, no major differences were observed in megakaryocyte-erythroid (MEP, LSK$^-$FcγR$^-$CD34$^-$), common myeloid (CMP, LSK$^-$FcγR$^-$CD34$^+$), and common granulocyte-macrophage progenitors (GMP, LSK$^-$FcγR$^+$CD34$^+$). Finally, we investigated whether human hematopoietic cells could stably engraft into the generated human niche. CD34+ cells isolated from human CB were injected into sublethally irradiated mice previously implanted with cartilage pellets. Human cell engraftment was in similar proportion in the ossicle marrow generated from both sources and in the BM of the ossicle-bearing mice. Also the multilineage reconstitution was similar in ossicle marrow and control BM, showing the presence of more than 90% of human CD19$^+$ B lymphocytes, with a small percentage of CD3$^+$ T cells, CD56$^+$ NK cells and CD33$^+$ myeloid cells. The majority of human B cells were CD10^{hi}/CD20$^-$ immature, with a small proportion of mature CD20$^+$ showing polyclonal expression of immunoglobulin kappa and lambda light chains on their surface.
Summary and Conclusions: We have shown that heterotopic ossicles that reproduce a functional bone/bone marrow microenvironment can be established, in the absence of any exogenous scaffolds, by human cartilage pellets generated *in vitro* from MSCs of different sources. In this system, blood borne HSCs are retained and seed the development of functional hematopoiesis. Following transplantation of human CB cells, the heterotopic niche is able to support human hematopoiesis, demonstrating that CD34+ HSCs can engraft and generate lineage-committed hematopoietic cells in the ossicles, similarly to BM.

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P19INK4D CONTROLS HEMATOPOIETIC STEM CELLS THROUGH MICROENVIRONMENT AT BASAL STATE AND THROUGH APOPTOSIS AT HEMATOPOIETIC STRESS

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Background: Hematopoietic stem cells (HSC) are characterized by self-renewal capacity and the ability to reconstitute the entire hematopoiesis. In the bone marrow (BM) the majority of HSC remains in quiescence. When a stress occurs, HSC rapidly enter the cell cycle to replenish the hematopoietic system. The maintenance of adult HSC in quiescence is mediated either by intrinsic or extrinsic factors. It has been previously shown that thrombopoietin, the main regulator of megakaryocyte (MK) differentiation, has been shown to be required for

the maintenance of adult HSC quiescence through induction of two cyclin-dependent kinase inhibitors (CDKI) $p57^{Kip2}$ and $p19^{INK4d}$, CDKI are divided into two groups, the INK4 ($p19^{INK4d}$, $p18^{INK4c}$, $p16^{INK4a}$, and $p15^{INK4b}$), and the Cip/Kip ($p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$) family. p57, p18 and p21 where described to regulate HSC in basal or stress conditions. p19 is expressed in diverse tissues were it plays an important role in cell differentiation, cell cycle, DNA repair and resistance to apoptosis in response to genotoxic stress. We also demonstrated the implication of p19 in megakaryopoiesis and its direct regulation by RUNX1, which negatively regulates the HSC number.

Aims: Although number of studies suggests implication of p19 in HSC biology, its precise role remains to be fully elucidated. Here we used a p19, KO mouse model to study its role in basal and stressed hematopoiesis.

Results: Our studies revealed that p19 KO mice displayed a slight decrease in absolute number of HSC, an enhanced progression from G0 to G1 phase without any increase in S/G2-M phase. Our *in vivo* study of HSC proliferation showed an increased apoptosis in absence of p19. The induction of replicative (5-FU) or genotoxic (5-FU, menadione) stress to quiescent HSC leads to an enhanced mortality of p19, KO mice associated with a marked increase in the exit of HSC from quiescence. Together with an increase in DNA-double strand breaks (DNA-DSB) and a massive apoptosis in S/G2-M phase, these results establish that $p19^{INK4d}$ is crucial to protect HSC from apoptosis especially under stress. Aging HSC is characterized by an amplification of myeloid-biased HSC and an accumulation of DNA damages probably at the origin of some malignant myeloid hemopathies in elder persons. In p19 KO mice, this amplification was not detected. Moreover, the p19 loss enhances accumulation of DNA-DSB during aging at basal state. Surprisingly WT HSC when transplanted in p19 KO, recipients exhibited a defect in their survival protective effect after 5-FU injection. In competitive transplantation assays in WT recipients, p19 KO, HSC display competitive advantage compare to control HSC. This strongly suggests that the HSC microenvironment is also defective in p19 KO, mice. By investigating the MK lineage, we detected an increase in progenitor and mature MK numbers in BM and spleen and a hyperproliferation of 2N/4N MK in BM of p19 KO, mice during aging. This phenotype is accompanied by an increased synthesis of TGFb1 in the extra fluids of BM and spleen of p19 KO mice. All these defects lead to a splenomegaly and a fibrosis in the BM and in the spleen at a higher extent.

Summary and Conclusions: In conclusion, we report here for a first time how a fibrosis could be induced by impairing a protein playing a crucial role in direct regulation of MK cell cycle. The present results also underscore the protective role of $p19^{INK4d}$ during a genotoxic stress and demonstrate that p19, plays an important role in hematopoiesis including during aging. It opens new avenues of research for delineating its precise function in HSC and their niche.

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HUMAN STEM CELL MODEL OF RUNX1 ALTERATIONS IN FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKEMIA (FPD/AML) DISEASE

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Background: FPD/AML is a constitutional disorder characterized by germline abnormalities in the hematopoietic transcription factor RUNX1, also called AML1. Mouse models have revealed the essential role of Runx1 during the first steps of hematopoiesis with a complete absence of definitive hematopoiesis in *runx1*$^{-/-}$ mouse. The discovery of induced pluripotent stem cells (iPSC) offers a new opportunity to precisely decipher the mechanisms of human pathologies *in vitro*.

Aims: We derived iPSC from FPD/AML patients to explore the disease pathogenesis and to study the first events at the origin of thrombocytopenia and/or leukemia occurring *in utero* due to constitutive abnormalities of *RUNX1*. Our model also aims to explain a long standing observation that certain constitutive mutations of RUNX1 are associated with increased risk to develop leukemia while others lead only to a mild thrombocytopenia.

Methods: iPSC lines were obtained from fibroblasts of 2 FPD/AML pedigrees (2 patients with missense R174Q mutation, and 1 patient with *RUNX1* monoallelic deletion) and from 3 controls. We used a lentivirus, carrying a cassette encoding for the four reprogramming factors, and excisable by Cre-recombinase. To strengthen our model we also knocked down (KD) *RUNX1* in human embryonic stem cells (hESC) using a shRNA. To confirm that the phenotype we observed is due to the *RUNX1* mutation, we introduced a wild type (WT) copy of the gene by homologous recombination. This was achieved using zing finger nucleases specific for the AAVS1 genomic locus with the WT *RUNX1* under the control of the promoter of the pan-hematopoietic gene CD43. Hematopoiesis was induced by coculture on OP9 stromal cells in presence of VEGF, SCF, IL-3, TPO, and EPO.

Results: After genotypic and phenotypic characterization of iPSC lines, hematopoiesis was explored. Hematopoietic progenitors ($CD34^+CD43^+$) were studied in semi-solid and liquid cultures. Methylcellulose colony-forming assays revealed an increased production of granulo-monocytic progenitors by only R174Q FPD/AML patients and a profound decrease in generation of megakaryocytic and primitive erythroid progenitors in both FPD/AML pedigrees compared to controls. Accordingly, in liquid medium, a deep defect in production of erythroblasts and megakaryocytes was observed in both pedigrees and increased production of macrophages and granulocytes in the pedigree with the R174Q mutation. The decrease in RUNX1 level by 80% by using shRNA in the hESC led to the hematopoietic defects similar to those obtained with iPSC carrying the R174Q mutation. The proplatelet formation was affected in both pedigrees in a similar manner, while increased genetic instability was detected only in the presence of R174Q mutation. Finally, we investigated the expression of RUNX1 target genes and confirmed a diminution of *p19INK4D*, *NR4A3* and *GADD45A* expression in hematopoietic progenitors, and of *MYH9*, *MYL9*,and of *p19INK4D*, in megakaryocytes. The reintroduction of the WT *RUNX1* reverted the observed phenotype in the lines with both types of mutations.

Summary and Conclusions: In conclusion, the hematopoietic defects linked to the RUNX1 alterations in adults occur probably already during embryogenesis. The fact that the KD of RUNX1 in hESC recapitulates the phenotype of the R174Q mutant and that this phenotype is reversible by the reintroduction of WT RUNX1 suggests that the leukemia development risk is dependent on the gene dosage of WT RUNX1 in the cells. In summary, these results demonstrate that FPD/AML iPSC represent a valid model for studying this disorder, successfully reproducing the phenotype observed in patients. Moreover, it allows to explain the differences observed with different *RUNX1* mutations.

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EVI2B, A NOVEL C/EBPA TARGET GENE, IS REQUIRED FOR MURINE NEUTROPHILIC DIFFERENTIATION AND CONTROLS HEMATOPOIETIC STEM CELL MAINTANENCE

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Background: *Evi2b* encodes a type I transmembrane protein, which is ubiquitously expressed in hematopoietic cells. *Evi2b* was originally identified as a common virus integration site in murine retrovirally-induced leukemias, suggesting that *Evi2b* might be a tumor suppressor gene or a proto-oncogene. Interestingly, we recently identified *EVI2B* as one of the genes belonging to the C/EBPα signature, suggesting it plays a role in granulocytic development. Nevertheless, little is known about the role of Evi2b in hematopoiesis and leukemic transformation.

Aims: To define the function of Evi2b in hematopoiesis. In particular, we aimed to determine the role of Evi2b in granulocytic differentiation and hematopoietic stem cell (HSC) maintenance.

Methods: K562 cells stably transfected with an inducible C/EBPα-ER fusion protein and 32D/G-CSF-R myeloid progenitor cells were used as a model for human or murine granulocytic differentiation, respectively. Distinct murine bone marrow populations were sorted according to expression of cell surface markers (LKS: $lineage^-$ $ckit^+$ $sca1^+$, short-term (ST)-HSC: LKS $CD48^+$ $CD150^-$, long-term (LT)-HSC: LKS $CD48^-$, $CD150^+$). Gene expression was measured by quantitative RT-PCR. Binding of C/EBPα to human *EVI2B* promoter and transactivation were determined by ChIP-seq analysis and luciferase assays, respectively. Murine *Evi2b* silencing was mediated by 2 distinct lentiviral shRNA constructs. Colony cultures were performed using semi-solid Methocult medium. Bone marrow transplantation experiments were performed using C57Bl/NCrl congenic mice strains. LT-HSC proliferation was evaluated by single cell sorting into Terasaki plates with subsequent counting of divided cells. Cell cycle analysis was performed by Pyronin Y and Hoechst 33342 staining. Human *EVI2B* levels were determined using the expression data of 525 AML patient samples (GSE14468).

Results: We demonstrated that C/EBPα binds to and transactivates *EVI2B* promoter in a dose-dependent manner, and that activation of C/EBPα upregulates *EVI2B* expression. In line with these results, we observed that *EVI2B* expression is downregulated in acute myeloid leukemias characterized by *C/EBP*α promoter hypermethylation. Next, we showed that *Evi2b* expression positively correlates with C/EBPα upregulation during granulocytic differentiation, and that downregulation of Evi2b leads to a block of neutrophilic differentiation in 32D/G-CSF-R cells. Since we observed that *Evi2b* is also abundantly expressed in ST- and LT-HSC, we investigated the role of Evi2b in HSC maintenance. Downregulation of Evi2b in murine LKS cells impairs the ability of this population to form colonies in semi-solid cultures. Further, we showed that Evi2b-silenced LKS cells present reduced ST- and LT-HSC engraftment into lethally irradiated mice. We demonstrated reduced LT-HSC cell proliferation after Evi2b silencing, and accordingly, we showed that Evi2b knockdown in the LKS population increases the percentage of cells in the G_0 quiescent cell cycle phase.

Summary and Conclusions: We have identified *Evi2b* as a target gene of C/EBPα, a crucial transcription factor in granulocytic differentiation. We demonstrated that Evi2b is required for proper granulocytic differentiation in 32D/G-CSF-R cells, and that Evi2b plays a critical role in HSC regulation. Altogether, our data demonstrates that Evi2b is an essential regulator of granulocytic differentiation and HSC maintenance.

Presidental Symposium

Best abstracts

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TOLL-LIKE RECEPTOR 2 MARKS THE EMERGENCE OF EMBRYONIC HEMATOPOIETIC PRECURSORS

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Background: Toll like receptors (*Tlrs*) are critically important in the pathogen recognition and regulation of innate and adaptive immune responses. In addition, direct pathogen sensing of adult bone marrow hematopoietic stem cells and hematopoietic progenitors via *Tlrs* seem to play a crucial role in directing the hematopoietic cell fates towards enhanced myelopoiesis under inflammatory conditions. So far, the expression of *Tlrs* during early stages of embryonic development at the onset of hematopoiesis has not been addressed.
Aims: The aim of this study was to determine the lineage potential of *Tlr2* expressing embryonic hematopoietic progenitors.
Methods: Mouse embryos at different stages of development were dissected from time pregnant females, enzymatically dissociated by Dispase (1 mg/ml) and upon antibody staining analyzed by flow cytometry. For qRT-PCR analyses and *in vitro* assays distinct cellular populations were sorted using Influx cell sorter. Sorted lineage$^-$ CD45$^{+/-}$, c-kit$^{+/-}$, TLR2$^{+/-}$, cells were grown on OP-9 stroma along with myeloid cytokines (SCF, IL-3, M-CSF, GM-CSF) or TLR2 agonist Pam3CSK4. Alternatively, sorted cells were plated directly to semisolid media supplemented with erythroid and myeloid differentiation promoting cytokines (Methocult M3434). Day 3 colonies grown in semisolid media were subjected to single colony qRT-PCR to detect the presence of embryonic hemoglobin. For microscopic analyses embryonic day 7.5 (E7.5) embryos were fixed, stained with TLR2 antibody and visualized by Zeiss LSM 780 microscope equipped with two photon, argon and helium–neon lasers.
Results: Using a transgenic model to trace cells of embryonic origin we showed that *Tlrs* are expressed on embryonic myeloid cells as well as hematopoietic precursors. Together with the prototypic marker of hematopoietic progenitors, c-kit, TLR2 is specifically expressed on the surface of hematopoietic precursors in early gastrulation embryos. Our qRT-PCR analyses showed that E8.5 CD45$^-$, c-kit$^+$, TLR2$^+$, cells express markers of hematopoietic progenitors as well as endothelial cells and myeloid cells. E7.5-E8.5 TLR2$^+$ c-kit$^+$ cells express CD45 mRNA and gradually differentiate through an intermediate c-kit$^+$, CD45$^+$ stage to c-kit$^-$, CD45$^+$ myeloid cells. Upon TLR2 triggering, E8.5 TLR2$^+$ c-kit$^+$ cells proliferate and differentiate to CD45$^+$, CD11b$^+$ myeloid cells in a *MyD88* dependent manner. Pre-circulation E7.5 CD45$^-$, c-kit$^+$, TLR2$^+$, cells as well as E6.5 CD45$^-$, TLR2$^+$, cells differentiate to myeloid cells when cultured on OP-9 stroma with myeloid cytokine supplements. In addition E7.5 CD45$^-$, c-kit$^+$, TLR2$^+$, cells give rise not only to myeloid but also to primitive erythroid colonies when cultured in semisolid media supplemented with erythroid and myeloid differentiation promoting cytokines. Using flow cytometry and whole mount fluorescence microscopy we show that E7.5 TLR2$^+$ progenitors are predominantly detected in the yolk sac (YS) region. Nevertheless scarce TLR2$^+$ c-kit$^+$ cells with a distinct surface expression profile can be also found in the embryo proper (EP). This opens the question whether the emergence of hematopoietic precursors in EP is dependent or not on YS primitive hematopoiesis.
Summary and Conclusions: Our results indicate that the expression of TLR2 marks the emergence of a common embryonic hematopoietic progenitor of early erythro-myeloid lineage and endows embryonic hematopoietic progenitors with the capacity to boost the production of myeloid cells. These data suggest a functional link between embryonic hematopoiesis and pattern recognition receptors under inflammatory conditions.

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A SINGLE ONCOGENIC ENHANCER-REARRANGEMENT CAUSES CONCOMITANT EVI1 AND GATA2 DEREGULATION IN LEUKEMIA

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Background: Acute myeloid leukemia (AML) with chromosomal rearrangements inv(3) or t(3;3) is characterized by overexpression of the proto-oncogene *EVI1* and clinically by an extremely poor response to chemotherapy. The molecular basis of *EVI1* deregulation of this distinct AML subtype is not understood. In the current World Health Organization classification of myeloid malignancies it is speculated that regulatory elements of the housekeeping gene *RPN1* at 3q21 are juxtaposed to the *EVI1* locus at 3q26.2 [inv(3)/t(3;3); *RPN1-EVI1*]. However, this hypothesis has not been experimentally validated.
Aims: To identify regulatory elements involved in the chromosomal 3q21q26 rearrangements driving *EVI1* expression, and to uncover the genomic origin of candidate ectopic *EVI1* enhancers.
Methods: Targeted next generation sequencing of the entire 3q21q26 regions (3q-seq) and transcriptional profiling by RNA-seq were performed in 41 primary AML patient and cell line samples harboring inv(3)/t(3;3). Candidate enhancer elements were identified by combining ChIP-seq with chromosome conformation capture sequencing (4C-seq) and further characterized using *in vitro* reporter assays. Functional validation of the candidate enhancer was carried out by genome-editing experiments, using CRISPR/Cas9 nucleases, followed by qPCR, cytology, proliferation and apoptosis assays.
Results: High-resolution mapping of chromosomal breakpoints by 3q-seq revealed a breakpoint-free segment of 18 kb size downstream of *RPN1*, relocating to the *EVI1* locus in all analyzed cases. Thus, we hypothesized the existence of candidate *EVI1* enhancer elements in this minimal region of rearrangement. 4C-seq was carried out to scan the three-dimensional chromatin environment of the *EVI1* promoter for loop interactions with the 18 kb segment. A contact hotspot of 9 kb within this commonly rearranged segment was identified. ChIP-seq analysis for enhancer-associated histone modifications, as well as BRD4- and p300-binding confirmed exceptionally broad deposition of H3K27ac and BRD4 occupancy in the entire 18 kb segment, with a p300 binding peak in the center of the 9kb *EVI1*-interacting chromatin segment. This 1 kb enhancer element showed significant *EVI1*-promoter driven reporter-gene induction in luciferase assays. Surprisingly, analysis of control samples without 3q rearrangements and of the remaining normal allele in inv(3)/t(3;3) AML revealed that the identified enhancer element was a constituent of the *GATA2* regulatory domain located at 3q21, instead of *RPN1*. RNA-seq and qPCR analysis confirmed reduced and monoallelic *GATA2* expression only from the remaining normal chromosome 3 allele in inv(3)/t(3;3) AMLs (n=69) compared to controls (n=213 unselected AML cases). Genomic excision of the ectopic enhancer element in an inv(3) cell line model (MUTZ-3) abrogated *EVI1* transcription and led to a profound reduction in cell viability, higher rates of apoptosis, along with increased maturation of the AML cells toward a monocyte/macrophage phenotype. Furthermore, we demonstrate that pharmacologic BRD4-inhibition can partly block activity of the ectopic *EVI1* enhancer owing to unique disease-specific enhancer features characterized by BRD4-superloading of the rearranged *EVI1* enhancer.
Summary and Conclusions: Rewiring of a single enhancer deregulates two unrelated disease genes. Using genome-editing tools, we show that targeting the enhancer activity relieves the maturation block of inv(3) leukemia cells. The ectopic enhancer is targetable by BET-inhibitors, which may represent an adjunct to current therapies for this incurable disease.

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EFFICACY OF THALIDOMIDE IN THE TREATMENT OF SEVERE RECURRENT EPISTAXIS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA: RESULTS OF A PROSPECTIVE PHASE II CLINICAL TRIAL

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Background: Hereditary hemorrhagic telangiectasia (HHT) is a genetic disease that leads to multiregional angiodysplasia. Severe recurrent epistaxis is the most common presentation, frequently leading to severe anemia. Multiple therapeutic approaches have been tried, but they are largely palliative. Since angiogenesis is involved in the pathogenesis of HHT, anti-angiogenic agents may be effective in its treatment.
Aims: The aims of our open label, phase II, prospective, non-randomized, single-centre study (EudratCT 2011-004096-36, ClinicalTrials.gov Identifier: NCT01485224) were to assess the effectiveness of thalidomide (Thal) in reducing epistaxis and to identify the lowest effective dose in patients with HHT refractory to standard therapy; to evaluate specific biological and clinical parameters predictable for patients response and side effects profile; to assess the efficacy of Thal retreatment in relapsing patients.
Methods: HHT patients with at least one episode of overt bleeding/week requiring at least one blood transfusion during the last three months and refractory to mini-invasive surgical procedures were enrolled. Thal was administered at a starting dose of 50 mg/day orally (off-label use). In the event of no response, Thal dosage was increased by 50 mg/day every 4 weeks until complete or partial response to a maximum dose of 200 mg/day. After response achievement, patients were treated for 16 additional weeks. Monthly follow-up was based on the epistaxis severity score and transfusion need, with adverse events being reported. Response to treatment and side effects profile were correlated with the mutations responsible for HHT and the genetic polymorphisms of *CYP2C19*. Patients relapsing within 52 weeks of ending Thal could be treated again for 8 weeks at the same maximum dose employed during the induction. Thal courses could be repeated at most three times.
Results: Twenty-eight patients for whom informed consent was obtained, 17 M and 11 F, aged 44-84 years (median 64), with mutations in either *ACVRL1* (23 cases) or *ENG* gene (5 cases), were enrolled (median follow-up 62 weeks, range 3-117). Treatment was effective in all 26 evaluable patients. Sixteen cases (62%) responded within 4 weeks of starting the drug: cessation of nose bleeding was observed in 4 cases, and reduction in the severity of epistaxis in 12 cases. Ten patients (38%) achieved partial response after 8 weeks of treatment. Thal significantly increased hemoglobin levels (P=0.04), decreased the transfusion need and improved the quality of life. Slight changes of skin telangiectasias were recorded. Only nonserious, drug-related adverse effects were observed, including constipation and drowsiness. Twenty patients completed the treatment: with a median follow-up of 51 weeks, range 5-97, after the end of therapy, 9 cases remained stable without the loss of response, whereas 11 relapsed (median time to relapse 43 weeks). No correlation was found between genetic or clinical features and time to response or response duration. Four relapsed patients were retreated with Thal with favorable response and without noticeable side effects. The improvement was persistent at 8-30 weeks follow-up.
Summary and Conclusions: Low-dose Thal is safe and very effective for the therapy of epistaxis in HHT patients who did not benefit from other modalities of treatment, allowing for a rapid, often durable clinical improvement. However, the effect of Thal is not permanent and maintenance therapy might be required.
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RANDOMIZED COMPARISON OF IBRUTINIB *VERSUS* OFATUMUMAB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA / SMALL LYMPHOCYTIC LYMPHOMA: RESULTS FROM THE PHASE III PCYC-1112 RESONATE(TM) TRIAL

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Background: Treatment options for CLL/SLL patients (pts) who fail chemotherapy are limited. Novel targeted therapies, such as ibrutinib (ibr), a first in class covalent inhibitor of Bruton's tyrosine kinase (BTK), may provide an effective therapy in such patients. We report interim results from a phase III randomized study of ibr compared with the anti-CD20 antibody ofatumumab (ofa) in pts with relapsed/refractory (R/R) CLL/SLL (PCYC-1112: RESONATE™). The independent Data Monitoring Committee recommended the interim analysis be considered final because the primary endpoint and a key secondary endpoint were met.
Aims: To evaluate the efficacy and safety of ibrutinib compared with ofatumumab in patients with R/R CLL/SLL.
Methods: Patients with R/R CLL/SLL who failed ≥1 therapy were randomized 1:1 to receive 420 mg oral ibrutinib once daily until progression or IV ofatumumab 300/2000 mg for 12 doses. The primary endpoint was progression-free survival (PFS) assessed by an independent review committee (IRC). Secondary endpoints included overall survival (OS), IRC-assessed overall response rate (ORR), and safety.
Results: Of 391 pts enrolled (median age 67 years; 40% ≥70 years), 195 were randomized to ibr and 196 to ofa. Overall, 53% of pts had Binet Stage C disease, and 32% of those ≥65 years had a cumulative illness rating score >6. Approximately 32% had del17p, 31% del11q, 97% received prior cytotoxic chemotherapy and 45% had disease refractory to purine analogs. Patients in the ibr arm reported a median of 3 prior therapies compared with 2 for ofa. Median time on study was 9.4 months. Ibrutinib significantly lengthened PFS compared with ofa (median not reached *vs* 8.1 months; HR 0.215, CI 0.146–0.317, p<0.0001), representing a 78.5% reduction in risk of progressive disease or death. Ibrutinib significantly improved OS compared with ofa (median not reached for both arms; HR 0.434, CI 0.238–0.789, p=0.0049), representing a 56.6% reduction in the risk of death. ORR was significantly higher with ibrutinib (42.6% *vs* 4.1%, respectively; p<0.0001) and ORR+PR with lymphocytosis was 62.6% *vs* 4.1% for ibr compared with ofa. ORR by investigator assessment similarly favored ibr, and was generally higher than that reported by the IRC at 69.7% and 21.4% for ibr *vs*. ofa, respectively. Analysis of PFS, OS, and ORR in pts with del17p or those with disease refractory to purine analogs similarly favored ibr. The most frequently reported adverse events (AE) for ibr *vs* ofa were diarrhea (47.7% *vs* 17.8%), fatigue (27.7% *vs* 29.8%), and nausea (26.2% *vs* 18.3%). Atrial fibrillation was more frequent with ibr (5.1% *vs* 0.5%). Bleeding events, including petechiae (13.8%) and contusions (10.8%), occurred more frequently with ibr, whereas major hemorrhages were reported at a similar frequency in both treatment arms (1.0% ibr *vs* 1.6% ofa). Two pts in each arm had confirmed Richter's transformation. Rate of AE as the primary reason for study drug discontinuation was similar in the ibr and ofa groups: 4.1% and 3.6%, respectively. At the time of analysis, 86.2% of ibrutinib pts were continuing treatment, and 57 pts randomized to ofa with confirmed progressive disease had initiated ibr in cross-over.
Summary and Conclusions: Compared with ofatumumab, ibrutinib significantly improved PFS, OS and ORR in patients with relapsed/refractory CLL/SLL. The safety profile was comparable to that previously reported (Byrd NEJM 2013). These results support ibrutinib as a beneficial therapy for R/R CLL/SLL patients irrespective of del17p or purine analog refractory disease.

S694

THE KRÜPPEL-LIKE FACTOR 2 (KLF2) TRANSCRIPTION FACTOR IS RECURRENTLY MUTATED IN SPLENIC MARGINAL ZONE LYMPHOMA

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Background: Next generation sequencing has revealed that the coding genome of splenic marginal zone lymphoma (SMZL) is characterized by mutations of genes involved in the physiological differentiation of marginal zone (MZ) B-cells.
Aims: To identify additional molecular mechanisms responsible of the patho-

genesis of SMZL, we applied a candidate gene approach to genes that are well established regulators of MZ B-cells differentiation, but did not emerge from previous genomic studies.
Methods: Seventeen genes were investigated by mutational analysis. Copy number abnormalities were assessed by SNP array (Affymetrix 6.0) and/or FISH. Expression was assessed by biochemical assays and immunohistochemistry. Wild type and mutants were conditionally expresses in HEK-293T, Jurkat, OCI-Ly8 and VL51 cells.
Results: The zinc finger transcription factor *KLF2* was somatically mutated in 20% (19/96) SMZL, thus representing one of the most frequently altered genes in this lymphoma, along with *NOTCH2* (20%) and *MLL2* (15%). Beside mutations, *KLF2* was deleted in 11% (11/96) SMZL, including one focal loss of 174 kb encompassing the sole KLF2 locus. By combining mutations and deletions, 30% (30/96) SMZL harbored *KLF2* lesions. To investigate the full complement of *KLF2* mutations in the spectrum of lymphoid neoplasia, we extended the analysis to 547 mature B-cell tumors. In addition to SMZL, *KLF2* was selectively mutated in post-GC neoplasia, including 16% (4/24) hairy cell leukemia, 15% (12/77) non-germinal center (GC) diffuse large B-cell lymphoma (DLBCL), 9% (5/56) nodal marginal zone lymphoma and 8% (5/61) extranodal marginal zone lymphoma. Conversely, *KLF2* mutations were rare or absent in GCB-DLBCL, follicular lymphoma, Burkitt lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia, and multiple myeloma. The somatic origin of mutations was confirmed by analysis of paired normal DNA. Most (62%) of *KLF2* mutations disrupted its nuclear localization signal (NLS) and/or affected codons required for the interaction between KLF2 and DNA. In normal MZ B-cells of the spleen, KLF2 was expressed with a preferential pattern of nuclear localization. In cell lines and primary tumor cells harboring mutations that truncated or changed the amino acid composition of the NLS, KLF2 was aberrantly displaced into the cytoplasm. Consistently, transient transfection of HEK-293T and OCI-Ly8 cells showed a predominantly nuclear localization of the wild type KLF2, while NLS mutants were dislocated into the cytoplasm. The *CDKN1A* promoter, a known KLF2 direct target, was strongly induced by the wild type KLF2 in luciferase assays. In contrast, KLF2 mutants failed to efficiently up-regulate the reporter. To further investigate the consequences of these mutations, we overexpressed the wild type and mutant KLF2 alleles in lymphoid cell lines. Trunsduction of wild-type KLF2 upregulated the expression of the endogenous CDKN1A mRNA and protein, activated the apoptotic cascade, and enhanced cell death. On the contrary, cells infected with vectors expressing transactivation defective mutants were unable to induce the expression of the endogenous CDKN1A and did not show apoptotic activation.
Summary and Conclusions: These data indicate that: i) *KLF2* is a putative novel tumor-suppressor gene recurrently disrupted in 30% SMZL and, to a lesser extent, in other tumors originating from post-GC B-cells; and ii) *KLF2* mutations promote cell survival by causing the cytoplasmic delocalization of KLF2 and the impairment of its transcriptional function.

S695

RUNX1, A NOVEL V(D)J RECOMBINATION COFACTOR IN HUMANS

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Background: The regulation of TCR rearrangements, particularly *TCR*δ and *TCR*β, plays a decisive role in lymphoid differentiation and oncogenic transformation. Unlike *TCR*γ and *TCR*α, the *TCR*δ and *TCR*β loci contain D segments. Productive recombination at the *TCR*β locus is tightly ordered (D-J before V-DJ), with c-Fos acting as a recombinase co-factor. The early stages of *TCR*δ recombination are poorly described and it is generally considered that they are not ordered in mice, whereas it has been suggested that they could be in humans.
Aims: To characterize the control of V(D)J recombination at the human *TCR*δ locus.
Methods: (i) chromatin immunoprecipitation (ChIP), (ii) protein co-immunoprecipitation (Co-IP), (iii) Proximity Ligation Assay (PLA), (iv) testing of RAG enzymatic activity against extrachromosomal substrates and (v) *in vitro* T-cell differentiation of human CD34+ cord blood cells on an OP9-DL1 stromal support, with or without shRNA-mediated inactivation of RUNX1 by lentiviral transduction.
Results: We show that the rearrangements of *TCR*δ are strictly ordered in humans. Within the human *TCR*α/δ locus, the first rearrangement (Dd2-Dd3) occurs at a very immature thymic ETP (Early T-cell Precursor) $CD34^{+}/CD1a^{-}CD7^{+dim}$ stage and systematically precedes Dd2(Dd3)-Jd1 rearrangement. *In silico* analysis identified a full consensus binding site for the RUNX1 transcription factor spanning the human *Dδ2-23RSS* heptamer and *Dδ2* coding sequence. This site is bound directly by RUNX1 in human $CD34^{+}/CD3^{-}$ thymocytes. The RUNX1 binding site is not conserved in the mouse *Dδ1* (which is homologous to human *Dδ2*), and as expected, RUNX1 binding to *Dδ1-23RSS* was not observed in mouse DN RAG2-/- thymocytes. The RUNX1 protein interacts with RAG1, and specifically increases the binding of RAG1 to the *Dδ2-23RSS*. Inactivation of RUNX1 abolished *TCR*δ rearrangements in human CD34+ cord blood cells, thereby inhibiting further T-cell differentiation. Conversely, stable transfection of RUNX1 in a non-lymphoid cell line (BOSC23) that lacked *TCR*δ rearrangement specifically induced Dd2-Dd3 recombination.
Summary and Conclusions: With respect to physiological lymphoid development, the minor population of TCRγδ expressing T-cells (γδ T-cells) has been conserved throughout vertebrate evolution. Our data show that RUNX1 imposes the use of two Dδ gene segments in all rearranged *TCR*δ chains during human, but not murine, *TCR*δ locus recombination. This is an evolutionary advantage for human TCRγδ lymphocytes, which have potentially longer and more diversified TCRδ CDR3 segments than those seen in mice. From an oncological point of view, the majority of *TCR*δ translocations occur during Dδ2-Dδ3 recombination, suggesting a potential role for RUNX1 in their pathogenesis. RUNX1 loss of function or dominant negative fusion proteins could induce maturation arrest due to a failure to initiate *TCR*δ rearrangement. This could, for example, explain the very immature maturation arrest observed in AML FAB M0 or ETP T-ALL with RUNX1 somatic mutations. Furthermore, our results suggest that RUNX1 mediates distinct roles in early lymphoid differentiation in humans and mice, with obvious consequences for extrapolation of murine models to human hematopoiesis. We have identified a novel function of RUNX1 as a cofactor of V(D)J recombination, providing novel insight into its role in normal lymphopoiesis and lymphoid oncogenesis.

S696

VEMURAFENIB IS SAFE AND HIGHLY ACTIVE IN HAIRY CELL LEUKEMIA PATIENTS REFRACTORY TO OR RELAPSED AFTER PURINE ANALOGS: A PHASE-2 ITALIAN CLINICAL TRIAL

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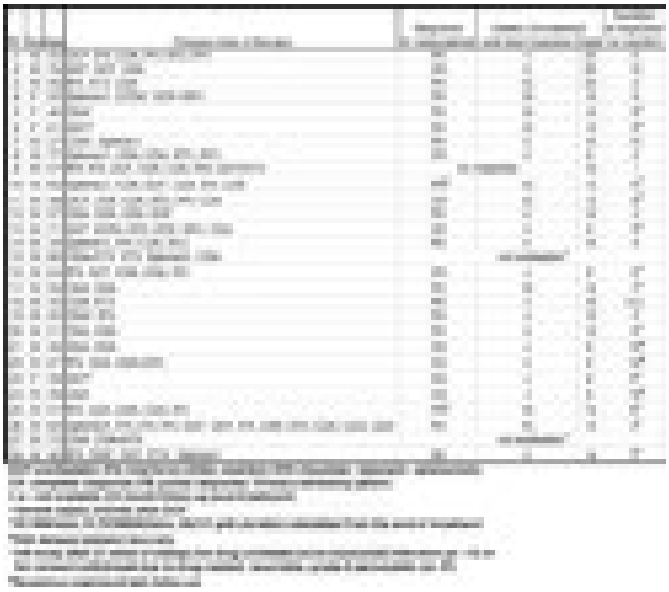
Background: Purine analogs (PAs) are highly effective in hairy cell leukemia (HCL), but ~40% of patients relapse and become less responsive to PAs, which are also myelotoxic and immune-suppressive. We recently discovered BRAF-V600E as the mutation underlying HCL (Tiacci *et al.*, NEJM 2011;364:2305), just at the same time when the oral BRAF inhibitor Vemurafenib proved effective in BRAF-mutated metastatic melanoma. Thus, we designed the first clinical trial to test the concept of BRAF inhibition in refractory/relapsed HCL.
Aims: To assess the efficacy and safety of Vemurafenib in HCL patients refractory to or relapsed after PAs by an academic, phase-2, single-arm, italian, multi-center (n=8) clinical trial (HCL-PG01; EudraCT 2011-005487-13).
Methods: Vemurafenib (960 mg bid) was given for a median of 16 weeks on an outpatient basis to 28 BRAF-V600E+ HCL patients requiring therapy because of cytopenia(s), including (see Table 1): **i)** 6 patients primary refractory to a PA; **ii)** 21 patients who relapsed early and/or repeatedly after PAs and had undergone a median of 4 previous therapies, with 5/21 (24%) and 9/21 (43%) patients not responding to the last PA and last therapy, respectively; in the remaining 16/21 and 12/21 patients, the median response duration was 27 and 26 months after the last PA and last therapy, respectively; **iii)** a 81-year old patient with severe myelotoxicicity after a PA that precluded its further use. Therapies other than PAs included interferon, rituximab and splenectomy in 12, 14 and 8 patients, respectively. Enrollment was completed in 11 months. Complete response (CR) required normal blood counts (N≥1500/mmc, PLT≥100000/mmc, Hb≥11 g/dl), morphological absence of hairy cells in the bone marrow biopsy and blood smear, and no splenomegaly. Partial response (PR) required normal blood counts, and a ≥50% reduction of splenomegaly and of marrow and blood HCL involvement by immunophenotyping. Two patients, who went off-study after ≤1 week of treatment, were not evaluable (see Table 1).

Results: Vemurafenib was well tolerated: drug-related adverse events (mainly arthralgias, various types of skin toxicity, pancreatitis) occurred in all patients, but were always reversible and mostly grade 1-2, with only 6 (23%) patients having grade 3 events and none grade 4. Notably, no myelosuppression was recorded. No keratoacanthomas nor cutaneous squamous cell carcinomas (as reported in melanoma patients) developed, but we observed two basaliomas and one superficial melanoma (all treated with a simple excision) in 3 patients. Strikingly, overall responses (CRs+PRs) were 96% (25/26 patients), with 9/26 (34.6%) CRs and 16/26 (61.4%) PRs and with a median time to response of 8 and 9 weeks, respectively. CR and PR patients included 1 and 5 primary refractory ones, respectively. In all CR patients immunohistochemistry documented minimal residual disease (≤10%) at the end of treatment. Seven of 9 (78%) CR patients still maintain normal blood counts at a median of 9 (range 6-12) months post-treatment. In the other 2 CR patients (pts. 2 and 9), a mild neutropenia (N~1000/mmc) developed 12 and 5 months post-treatment, respectively. Pt. 2 still does not require therapy 15 months post-treatment; pt. 8 required therapy 9 months post-treatment due to a worsening neutropenia. Among the 15/16 PR patients with >1 month follow-up, 8 (53%) maintain normal blood counts at a median of 6 (range 3-9) months post-treatment. The other 7 (47%) patients developed cytopenia(s) at a median of 5 (range 2-10) months post-treatment.

Table 1.



Summary and Conclusions: A brief, oral monotherapy with Vemurafenib proved safe and highly active in heavily pre-treated HCL patients poorly responsive to all conventional therapies. Strategies to further increase CRs and response duration include: combining a BRAF inhibitor with a MEK inhibitor or a B-cell targeting antibody or a kinase inhibitor of the pathways downstream of the B-cell receptor; and/or maintenance therapy with lower, pulsed Vemurafenib doses.

SIMULTANEOUS SESSION II

Myeloma and other monoclonal gammopathies - Biology

S697

HDAC8 INHIBITS MYELOMA CELLULAR GROWTH THROUGH REGULATION OF HOMOLOGOUS RECOMBINATION AND CYTOSKELETON INTEGRITY

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Background: To date, both clinical and preclinical studies have confirmed that MM is vulnerable to epigenetic intervention, with histone deacetylase enzymes (HDAC) emerging as the most promising epigenetic targets. However, little is known about the role of HDAC8, a member of Class I HDAC isoenzymes in MM.
Aims: To investigate the function of HDAC8 in MM biology and to evaluate its potency as therapeutic target.
Methods: Lentiviral-shRNA delivery system was used for knockdown of HDAC8 in OPM2 and U266 cells. The HDAC8 inhibitor PCI-34051 was used as chemical inhibitor. A panel of 15 antibodies (HDAC8, γH2Ax, H2Ax, pATM, pATR, pBRCA1, pBRCA2, pCHK2, pCHK1, ku70, RPA70, 53BP1, DNA-PKs, pP53, Rad51) and nucleolin, GAPDH, were used in immunoblot analysis. Immunofluorescence staining was performed with HDAC8, actin, p53 and Smc1 and Smc3 antibodies and the images were analyzed with confocal microscopy. Singe cell electrophoresis under neutral conditions (comet-assay) was performed in OPM2-HDAC8 knockdown cells with or without exposure to γ irradiation (IR), and in OPM2 and U266 treated and untreated with HDAC8 inhibitor in combination with IR. Co-immunoprecipitation assay was performed for investigation of interactions of HDAC8 after induction of DNA damage. DNA Double-Strand Break (DSB) repair occurring via homologous recombination (HR) pathway was assessed using a transient direct repeat DsRED-GFP/I-SceI plasmid-based system. Expression of DNA damage and repair pathway (DDR) genes was evaluted using a high-throughput PCR assay. Cellular senescence was assessed with SA-β-Galactosidase staining.
Results: We evaluated the expression of HDAC8 in 172 newly-diagnosed MM patients from the IFM myeloma dataset and observed HDAC8 overexpression as well as its significant correlation with poor survival outcome ($p<0.0015$). We further evaluated the expression of HDAC8 in HMCLs (probe ID_223909-s_at, 223345_at) and confirmed the high expression and its cytoplasm and nuclear localization in all six MM cells lines studied (MM1S, OPM2, RPMI8226, U266, MOLP8 and NCI-H929). The HDAC8 depletion in two MM cells lines (OPM2 and U266) resulted in significant inhibition of proliferation of MM cells at 1 week as measured by 3[H]-thymidine assay, and decrease in colony formation as evaluated after 3 weeks post transfection ($p<.001$). Interestingly, the combination of HDAC8 inhibitor with melphalan or bendamustine enhanced the anti-MM effects of the genotoxic agents (all<0.01) and was confirmed to be synergistic using Calcusyn software. Interstingly, U266 cells with HDAC8 depletion exhibited increased levels of markers of DNA damage including γH2Ax, H2Ax, pATR, pBRCA2, pCHK1, RPA70, 53BP1, DNA-PKs, Rad51 and pP53. Moreover, in consistence with this observation HDAC8 knock down led to decreased HR activity as measured by a plasmid based assay and decreased repair of DSBs after IR measured by Singe cell electrophoresis under neutral conditions with or without exposure to γ irradiation (IR). Similar observation were also confirmed following treatment of the cells with HDAC8 inhibitor. The HDAC8 protein co-localized and co-immunoprecipitated with p53 after IR, and with Scm3, member of cohesin. Finally, the depletion of HDAC8 resulted in the higher prevalence of senescence associated with β-Gal-positive cells 3 weeks post transduction.
Summary and Conclusions: Our results demonstrate a mechanistic connection of HDAC8 and DNA damage repair pathway, and provide insights into the effect of HDAC8 on cytoskeleton, that may have therapeutic implications in MM.

S698

ADDITIONAL PROGNOSTIC VALUE OF MULTIPARAMETER FLOW CYTOMETRY (MFC) MINIMAL RESIDUAL DISEASE (MRD) MONITORING OVER COMPLETE RESPONSE (CR) ACROSS THE CLINICAL COURSE OF MULTIPLE MYELOMA (MM) PATIENTS

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Background: The continuously increasing rates of CR as well as therapeutic options in MM brought the debate on whether the CR criteria could be refined to further improve prediction of outcome, and particularly to identify patients in CR with shorter TTP and OS. Consequently, the role of MRD monitoring is now under evaluation and published data based on different clinical trials has shown positive results. Interestingly though, it is perhaps outside of clinical trials (thereby without planned regimens) where MRD monitoring may be more valuable to improve prediction of outcomes and help on therapeutic decisions.

Aims: To specifically address the clinical relevance of MRD investigations among patients in CR throughout the whole spectrum of MM patients' clinical course.

Methods: A total of 113 patients in CR as defined by the IMWG criteria were prospectively screened for MRD levels at the University Hospital of Salamanca, between 2000 and 2013 and outside the setting of a specific clinical trial. Elderly (>65y) patients (n=30) were studied after induction therapy (50% being treated without novel agents), whereas transplant-eligible cases (n=51) were monitored after HDT/ASCT (70% receiving previous induction without novel agents). In 22/51 transplant-eligible patients, MRD monitoring was also performed in stem cell harvests. Follow-up MRD studies were performed on 31 patients in CR after up-front treatment (both elderly and transplant-eligible). A total of 43 patients in CR after salvage therapy were also screened for MRD (11 of them overlapping with up-front studies). Overall, a total of 155 bone marrow samples and 22 harvests were analyzed by 4-color MFC, and flow-CR was defined as <0.01% phenotypically aberrant clonal plasma cells. Median follow-up was 6, 5 and 1.5 years for elderly, transplant-eligible and relapsed patients. TTP and OS were measured from the moment of MRD assessment.

Results: Among the 30 elderly patients in CR after induction, 19 (63%) also attained flow-CR, which translated into significantly superior TTP (medians of 39 *vs* 19 months; P=.02) and OS (63m *vs* 24m; P=.03). Similarly, among the 51 patients in CR after HDT/ASCT, 33 (65%) were in flow-CR and again the absence of detectable MRD resulted in significantly prolonged TTP (85m *vs* 13m; P<.001) and a trend for OS (98m *vs* 52m; P=.18). Noteworthy, 73% and 94% of elderly and transplant-eligible patients in CR, but with persistent MRD, had relapsed when the study was closed for analysis. Another interesting finding was the prognostic information derived from MRD investigations on stem cell harvests; MRD was detected in 6 of 22 (27%) harvests (from patients in CR after HDT/ASCT), which translated into significantly shorter TTP (median of 10m *vs.* 85m; P=.001). Regarding those cases in CR after salvage therapy (63% after auto or allo SCT), it was surprising to see that also in the relapse setting approximately half of the patients (46%) could achieve flow-CR, which doubled TTP as compared to cases MRD-positive (25m *vs.* 13m; P=.035), without significant differences for OS (NR *vs* 38m; P=.22). Finally, we took advantage of available longitudinal MRD monitoring after up-front treatment to investigate if MRD recurrence predicted clinical relapse. For this purpose, we focused on 31 patients in conventional plus flow-CR (either after induction or HDT/ASCT), and observed that those cases turning into MRD-positive (42%) had a significantly higher probability of relapse compared to cases with undetectable MRD during follow-up analysis (85% *vs.* 39%; P=.011).

Summary and Conclusions: We specifically showed among patients in conventional CR, that MRD monitoring by MFC is a powerful prognostic biomarker that consistently identifies those patients at higher risk of unsustained CR, and could potentially help on therapeutic decisions throughout MM patients' clinical course.

S699

INHIBITION OF THE STK4 KINASE RESTORES THE HIPPO CO-FACTOR YAP1 AND INDUCES DNA-DAMAGE MEDIATED APOPTOSIS IN MULTIPLE MYELOMA CELLS

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Background: Multiple myeloma (MM) is a clonal proliferation of malignant plasma cells characterized by complex genomic abnormalities. We and others have shown that MM cells have ongoing DNA damage and DNA damage response (DDR), in contrast to normal plasma cells (NPCs) and peripheral blood mononuclear cells (PBMCs) (Walters, Leukemia, 2011).

Aims: Normally ongoing DNA damage leads to elimination of aberrant cells. We investigated the mechanisms which prevent DNA-damage mediated apoptosis in MM, and how we could modulate these pathways to provide novel therapeutic options.

Methods: MM cells were cultured in supplemented RPMI-1640. Lentiviral delivery systems or AMAXA electroporation were used for expression and knockdown of YAP1 and STK4 in MM cells. MM samples and PBMCs were obtained after informed consent and subjected to Ficoll-Paque density sedimentation, followed by CD138-positive selection for MM cells.

Results: MM patient cells and cell lines have ongoing DNA damage, which triggers ATM-mediated DDR but surprisingly no apoptosis. In normal cells, ATM can cause apoptosis not only by activating P53, but also by promoting re-localization of the kinase ABL1 into the nucleus where it phosphorylates YAP1, thereby promoting stabilization of the tumor suppressor p73 and activation of pro-apoptotic p73-target genes. Remarkably, ABL1 has consistent nuclear localization in the majority of MM cells and patient samples tested. Its cellular localization is modulated by DNA damage: ATM inhibitors re-localize ABL1 to the cytosol, while doxorubicin increases ABL1 nuclear localization, promoting apoptosis in MM cells. Conversely, ABL1 is not basally present in the nucleus in normal PBMCs, nor does it shuttle to the nucleus or cause apoptosis upon treatment with similar doses of doxorubicin. A known target of ABL1 is YAP1, a modulator of the Hippo pathway. aCGH data show that YAP1 genomic locus (chr. 11q22) is deleted, alongside with BIRC2 and BIRC3, in up to 11% of MM patients and in two MM cell lines (KMS-18 and KMS-20). Moreover, while PBMCs show variable YAP1 expression levels, the majority of MM patients and cell lines have low levels of YAP1, suggesting a tight regulation of YAP1 levels in these tumor cells. To define the relevance of YAP1 silencing in MM cells, we re-expressed YAP1 in MM cell lines with either YAP1 deletion or low YAP1 expression. These experiments revealed reduced growth and increased apoptosis in cells in which YAP1 had been re-expressed, whereas enhanced growth was observed upon YAP1 silencing in UTMC-2 cells. We showed that YAP1 stabilizes p73 protein in MM cells, thereby promoting transcription of p73-target genes (BAX, PUMA and p21) and apoptosis. ABL1 is crucial to YAP1 apoptotic phenotype, since imatinib treatment precludes cell death by blocking YAP1 phosphorylation and preventing YAP1/p73 complex formation. Moreover, no growth inhibitory effects were identified when a mutant lacking the WW-p73 interaction domains (dWW-YAP1) was re-expressed in MM cells devoid of YAP1. Importantly, STK4 silencing increases YAP1 levels associated with inhibition of MM growth in both *in vitro* and *in vivo* MM models; no similar effects are noted in YAP1-MM deleted cell lines.

Summary and Conclusions: MM cells with ongoing DNA damage avoid cell death due to reduced YAP1 levels. STK4 kinase controls YAP1 levels and cellular localization, and indeed STK4 and YAP1 levels inversely correlate in MM patients. Functional inhibition of STK4 promotes YAP1 upregulation and related MM growth inhibition and cytotoxicity. STK4 kinase inhibitors therefore represent a potential novel therapeutic approach in MM.

S700

IN VIVO TARGETING OF STROMAL-DERIVED FACTOR-1 AS A STRATEGY TO PREVENT MYELOMA CELL BONE TO BONE DISSEMINATION

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Background: Multiple myeloma (MM) patients present with multiple lytic lesions at diagnosis, indicating the presence of continuous dissemination of MM cells from the primary site of tumor development to multiple distant bone marrow (BM) niches. We hypothesized that stromal-derived factor-1 (SDF1) may represent a target for preventing transition from MGUS (micrometastatic-stage) to active-MM (macrometastatic-stage). We therefore evaluated SDF1 expression in the BM of patients with MGUS, MM, compared to healthy individuals; and tested the effect of SDF1 neutralization withing the bone marrow niches as a strategu to precent MM cell homing and disease progression. AIMS. 1) To evaluated to level of SDF-1 in bone marrow niches colonized by MM cells 2) To evaluate the effect of neutralizing SDF-1 in modulating the bone marrow niches and its effect in preventing MM cell colonization and growth.

Aims: 1) To evaluate the level of SDF-1 in MM cell-colonized bone marrow niches. 2) To evaluate the effect of SDF-1 neutralization on modulating the bone marrow niches and its effect on preventing MM cell colonization and growth.

Methods: SDF1 levels were evaluated by immunohistochemistry on BM specimens of patients with MGUS, active-MM, or healthy individuals; and confirmed by ELISA, using conditioned-medium of BM-mesenchymal stromal cells (BM-MSCs) from MGUS, active-MM and healthy individuals. BM metastatic lesions from primary epithelial tumors were also evaluated, including medullary thyroid-, gastric-, renal-carcinoma, lung adenocarcinoma. Co-localization of SDF1 with MM cell (MM.1S-GFP+)-enriched BM niches was evaluated using *in vivo* confocal microscopy. Effect of NOX-A12 on modulating MM cell dissemination was tested *in vivo*, by using an *in vivo* MM metastasis model. *In vivo* homing and *in vivo* tumor growth of MM cells (MM.1S-GFP+/luc+) was assessed by using *in vivo* confocal microscopy and bioluminescence, in mice treated with 1) vehicle; 2) NOX-A12; 3) bortezomib; 4) NOX-A12+bortezomib. Effects of drug combination on dissemination of MM cells to distant BM niches was evaluated *ex vivo* by immunofluorescence on explanted femurs. DNA synthesis and adhesion of MM cells in the context of NOX-A12 treated primary MM BM-MSCs in presence or absence of bortezomib were tested by thymidine uptake and adhesion *in vitro* assay. Synergism was calculated by using CalcuSyn software.

Results: Patients with active-MM present with higher BM SDF1 expression *vs* MGUS patients and healthy individuals. Similarly, BM presenting with metas-

tasis from epithelial primary tumors had higher SDF1 levels compared to healthy subjects, thus suggesting the importance of SDF1 in favoring tumor cell metastasis to BM niches. SDF1 co-localized at BM level with MM tumor cells *in vivo*. I*n vitro,* NOX-A12 induced a dose-dependent de-adhesion of MM cells from the BMSCs supported by inhibition of BM-MSC-mediated phosphorylation of ERK1/2 and cofilin. Importantly, NOX-A12 induced MM cell mobilization from the BM to the peripheral blood (PB) as shown *ex vivo* by reduction of MM cells in the BM and increased number of MM cells within the PB compared to control mice (P<0.05). This was supported by inhibited homing and dissemination of MM cells to the BM of those mice pre-treated with NOX-A12. Similarly, NOX-A12-pre-treatred mice presented with inhibited dissemination of MM cells from bone to bone, as shown by using an *in vivo* model of MM metastasis (P<0.05). NOX-A12 enhanced MM cell sensitivity to bortezomib, *in vivo*: tumor burden was similar between NOX-A12- and control mice whereas bortezomib-treated mice showed significant reduction in tumor growth *vs.* control (P<0.05); importantly, significant reduction of tumor burden in those mice treated with sequential administration of NOX-A12 and bortezomib was observed, compared to bortezomib-treated mice (P <0.05). Similarly, NOX-A12+bortezomib combination induced significant inhibition of MM cell homing, as shown by *in vivo* confocal microscopy. *In vitro* studies confirmed synergism between NOX-A12 and bortezomib in modulating MM cell survival and adhesion to BM-MSCs.
Summary and Conclusions: SDF-1 represents a valid target for inhibiting MM cell dissemination to distant BM niches, thus providing the rationale for using the SDF1 inhibiting spiegelmer NOX-A12 to target MM cells at the stage of micrometastasis (MGUS), thus preventing development of macrometastatic MM.

S701

CROSS-TALK BETWEEN SPHINGOSINE 1-PHOSPHATE (S1P) AND CXCR4/CXCL12 REGULATES VIABILITY, SUPPORTS CXCR4-DEPENDENT CELL MOTILITY AND DEFINES THE SENSITIVITY TO FTY720 IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a malignancy of plasma cells that home and accumulate in bone marrow (BM) microenvironment. CXCR4/CXCL12 chemokine axis has been implicated in MM trafficking and retention in BM. Sphingosine 1-phosphate (S1P) and S1P receptors (S1P1-S1P5) control lymphocyte and plasma cell egress from lymph nodes and support CXCR4-dependent migration and BM homing of hematopoietic stem cells. Sphingosine kinase (SPHK) is a key enzyme in the sphingolipid pathway that produces S1P.
Aims: We therefore asked whether crosstalk between the S1P and CXCR4 pathways is important for MM cell survival and motility *in vitro* using MM cell lines and human MM samples and in xenograft mice MM tumor model *in vivo*.
Results: Expression profile of S1P(1-5) receptors and SPHK1 in MM cell lines and primary MM samples was evaluated by RT-PCR. S1P1, S1P2, S1P4 and SPHK1 were found to be expressed by different MM cell lines (n=7). In addition, primary samples from BM of MM patients (n=24) were analyzed for S1P(1-3) receptors and SPHK1 expression. S1P1 receptor and SPHK1 were found to be was co-expressed in 12 human MM samples. Next, to evaluate the effect of S1P axis modulation on MM viability, cells were treated *in vitro* with FTY720, S1P analogue, which binds to four out of five S1P receptors, and with the SKI-II, SPHK1 inhibitor. FTY720, but not SKI-II, treatment induced cell death of MM cell lines and primary human CD138+ MM cells in a dose-dependent manner (IC50 2-3.5 μM). FTY720-triggered cell death by inducing apoptosis manifested by an increase in Annexin V staining, caspase 3 cleavage and cytochrome C release. FTY720-induced MM cell death was accompanied by reduction in BCL-XL, but not in MCL-1 levels, indicating that FTY720-promoted mitochondrial destabilization with BCL-XL involvement. In addition, treatment of MM cells in the presence of protective bone marrow stromal cells (BMSCs) with FTY720 sensitized the MM cells to the cytotoxic effect of lenalidomide. Exogenous CXCL12 treatment and co-incubation with BMSCs partially protected the MM cells from the FTY720-induced apoptosis, indicating the involvement of CXCR4/CXCL12 pathway in S1P signaling. However, higher doses of FTY720 effectively overcame this protective effect. Oppositely, FTY720 treatment completely abrogated CXCR4-dependent migration of MM cells in response to elevated concentrations of CXCL12 (p<0.001). Mechanistically, pre-treatment with FTY720, but not with SKI-II, fully blocked CXCL12-induced phosphorylation of AKT and Erk1/2 kinases in MM cells. Of note, enforced expression of CXCR4 in MM cells promoted resistance to FTY720-induced apoptosis, whereas silencing of endogenous CXCL12 in MM cells resulted in significantly increased sensitivity to FTY720 treatment (p<0.01). Finally, in xenograft model of systemic MM with BM involvement *in vivo* FTY720 effectively reduced tumor burden in 4 out of 6 treated mice, decreasing the number of MM cells in BM and levels of M protein in blood.
Summary and Conclusions: Taken together, our novel findings demonstrate the activating interaction between S1P and CXCR4/CXCL12 signaling pathways that may be important for MM cell survival and localization of the MM cells in CXCL12-expressing protective niches. Furthermore, S1P targeting with FTY720 limits tumor-promoting activities of S1P and CXCR4/CXCL12 axes and thus may serve a therapeutic target in MM.

Chronic lymphocytic leukemia and related disorders - Clinical 1

S702

ABT-199 (GDC-0199) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA AND SMALL LYMPHOCYTIC LYMPHOMA: HIGH RESPONSE RATES AMONG PATIENTS WITH HIGH RISK DISEASE FEATURES INCLUDING UNMUTATED IGHV

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Background: Overexpression of BCL-2 in relapsed/refractory (R/R) Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Lymphoma (SLL) is associated with resistance to both chemotherapy and apoptosis. ABT-199 is an orally bioavailable, selective BCL-2 inhibitor that triggers apoptosis of CLL cells *in vitro* and *in vivo*, independent of TP53 functional status, making it a promising agent for the treatment of patients (pts) with CLL/SLL, including those with high-risk disease features.
Aims: The primary objectives of this phase I study were to evaluate the safety and pharmacokinetics (PK) and to determine the maximum tolerated dose and a recommended phase 2 dose (RP2D) of ABT-199 monotherapy. A secondary objective was to assess preliminary efficacy.
Methods: Pts received daily oral ABT-199 until progressive disease (PD), intolerance or withdrawal. Following events of tumor lysis syndrome (TLS) early in the trial, a schedule with weekly dose increases to the target cohort dose (150-1200 mg) was implemented. Pts are now being enrolled in the safety expansion (SE) cohort incorporating a weekly ramp-up period from 20, 50, 100, 200 mg to the final RP2D of 400 mg.
Results: As of January 17, 2014, 93 pts (37 in the SE) were enrolled with a median time on study of 6.1 (range, 0 - 27) months. Twenty-three (24%) pts had del(17p), 55 (59%) had fludarabine (F)-refractory CLL, 32 of the 42 pts with available status have unmutated *IGHV*. The median number of prior therapies was 4 (range 1-11). The most common treatment-emergent AEs (>25% pts) of any grade were neutropenia (39%), diarrhea (36%), nausea (35%), upper respiratory tract infection (31%), and fatigue (27%). Grade 3/4 AEs (>5% pts) were neutropenia (36%), anemia (10%), TLS (8%, including 1 Gr5), thrombocytopenia (7%), hyperglycemia (7%), and febrile neutropenia and hypokalemia (6% each). Thirty-one pts discontinued treatment: 20 for progressive disease, 9 for AEs, and 2 for other reasons. Preliminary efficacy results are detailed in the Table 1.

Table 1.

	Total N=93	*IGHV* Unmutated n=32 (42 assessed)	Del (17p) n=23	F-Refractory n=55
Gender M/F	68/25	22/10	18/5	40/14
Age (median)	66	64	66	66
Median Baseline Lymphocyte Count (x 10^9/L)	5.9	5.9	7.0	5.9
Responses				
Total Evaluable*	70	23	21	39
CR/CRi, n (%)	14 (20)	4 (17)	3 (14)	6 (15)
PR, n (%)	39 (56)	13 (57)	12 (57)	23 (59)
SD, n (%)	11 (16)	4 (17)	4 (19)	8 (21)
PD, n (%)	2 (3)	1 (4)	1 (5)	1 (3)
D/C, n (%)	4 (6)	1 (4)	1 (5)	1 (3)
Response Rate (ORR, CR+PR)	76%	74%	71%	74%

**Evaluable pts exclude those with unconfirmed PR and those not yet evaluable. D/C: Discontinued prior to week 6 assessment. The median duration of response (DOR) was 20.5 months [95% CI; 13.8, -] for the 53 responding pts. At 12 months, 91% of CR and 67% of PR pts remain progression-free. Of the 14 CR/CRi pts, 9 were evaluated in local labs by flow cytometry for minimal residual disease (MRD); 5 were MRD negative and of these, 2 were IGHV unmutated, 4 were F-refractory, and 1 had del(17p). Summary and Conclusions: ABT-199 induces a high rate of response in pts with R/R CLL, including those with unmutated IGHV, del(17p), and F-refractory disease. Responses appear to be durable, especially in the 21% who achieved CR/CRi. Several of these high-risk pts achieved MRD negativity. Enrollment continues in the safety expansion cohort to further define safety and efficacy.*

S703

ABT-199 (GDC-0199) COMBINED WITH RITUXIMAB (R) IN PATIENTS (PTS) WITH RELAPSED / REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): INTERIM RESULTS OF A PHASE 1B STUDY

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Background: ABT-199 is a selective, orally bioavailable BCL-2 antagonist that induces rapid apoptosis of CLL cells and an approximately 80% response rate as monotherapy in pts with R/R CLL. As with DNA damaging chemotherapy, R synergizes with ABT-199 in preclinical models of CD20+ lymphoid cancers.
Aims: Objectives were to evaluate the safety, pharmacokinetics (PK), preliminary efficacy and to determine a maximum tolerated dose (MTD) / recommended phase 2 dose (RPTD) for ABT-199+R.
Methods: Pts began daily ABT-199 at 50 mg (modified to 20 mg) with weekly increases to a final cohort dose (200-600mg). R was then administered at a starting dose of 375 mg/m^2 then 500mg/m^2 monthly for total of 6 doses (cohort 1-2 had 2 additional weekly doses in month 1) with continuation of daily ABT-199 until progressive disease (PD). The MTD/RPTD for the combination will be determined by the Bayesian Continual Reassessment Method. Pts were assessed by CT scan at month 3 (followed by confirmation CT for responders per IWCLL 2008) and by CT scan and bone marrow (BM) biopsy after the end of combination therapy.
Results: As of Jan 17, 2014, 37 pts were enrolled in 5 cohorts (median age 68, 14/23 F/M) with a median time on study: 4.8 (range 0 - 15.2) months, median number of prior therapies: 2 (range 1 - 5); 9 pts were fludarabine-refractory, 9 R-refractory, and 9 had del(17p). Six pts discontinued: 4 due to PD (3 Richter's transformation, 1 progressive CLL), 1 withdrew consent, 1 due to fatal hyperkalemia in the setting of tumor lysis syndrome (TLS) at 1st dose (50 mg) prior to dose modification. The most common treatment-emergent adverse events (AEs, >25% pts) were neutropenia (43%), nausea (38%), diarrhea (30%). The most common grade (G) 3/4 AEs were neutropenia (43%), thrombocytopenia (16%), and anemia (11%). Nine pts had G4 AEs with 14 events (9 neutropenia, 4 thrombocytopenia, 1 hemophagocytic syndrome), of these, 6 pts had interruptions or reductions. All but 1, maintained at a reduced dose of 200 mg, resumed treatment at the final cohort dose. Two G4 events were dose limiting toxicities occurring on combination therapy (in cohort 2): thrombocytopenia (300mg/375mg/m^2) and hemophagocytic syndrome (300mg/500mg/m^2). Preliminary PK data suggest a negligible effect of R on ABT-199 exposure. Of 18 pts who have completed combination therapy or discontinued prior to completion, 7 (39%) achieved complete remission (CR/CRi) and 7 (39%) partial remission (PR). Minimal residual disease (MRD) was quantified by local laboratory in 6/7 CR pts: 4 pts are MRD negative in BM (3 at 10^{-4} and 1 at 10^{-3} sensitivity) and 1 in peripheral blood (10^{-3} sensitivity). Additional results are included in the Table 1 below:

Table 1.

Cohort	Dose	N	Months on Study (median)	Assessment	Responses
Pts evaluable for CR (≥6 mo or PD)					
1	200	6	14	CT & BM	2 CR/CRi, 4 PR
2	300	10	10	CT & BM	5 CR/CRi, 3 PR, 2 PD
3	400	1	-	-	
1 DC					
Pts not yet evaluable for CR (<6 mo)					
3	400	7	5	CT	2 PR, 4 uPR, 1 DC
4	500	6	3	CT	2 PR, 4 uPR

**uPR: unconfirmed partial remission; DC: discontinued*

Summary and Conclusions: To date, the RPTD of R+ABT-199 has not been determined and dose escalation continues. No additional toxicities have been identified when compared to ABT-199 monotherapy. The combination is active in R/R CLL, with a number of patients achieving CR with MRD negativity. A fatal episode of TLS occurred during the ABT-199 lead-in period (prior to R). Following dosing and monitoring modifications, no further TLS events were reported.

S704

SECOND INTERIM ANALYSIS OF A PHASE 3 STUDY EVALUATING IDELALISIB AND RITUXIMAB FOR RELAPSED CLL

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Background: Idelalisib (IDELA), an oral inhibitor of PI3Kδ, is highly active in heavily pretreated patients with CLL as a single agent or combined with rituximab (R) as demonstrated in Phase 1 trials.
Aims: This report presents the results from the second interim analysis of a Phase 3, randomized, double-blind, placebo-controlled study of IDELA+R *vs.* placebo (PBO)+R.
Methods: Patients with CLL requiring therapy after progression <24 mos since completion of last therapy and considered unfit to receive cytotoxic therapy were enrolled. Primary endpoint PFS was assessed by IRC and standard criteria (Hallek 2008, Hallek 2012, Cheson 2012). After progression, patients could enroll into a blinded extension study to receive IDELA at 150 mg BID (prior PBO+R) or 300 mg BID (prior IDELA+R). The first interim analysis (Furman *et al.*, NEJM 2014) led to the decision of early termination due to overwhelming efficacy.
Results: A total of 220 patients (110 patients on each arm) with a median age of 71 yrs (78% ≥65 yrs), a median time since diagnosis of 8.5 yrs, and a median number of 3 prior therapies (range: 1-12) were randomized. 44% of patients had del(17p)/*TP53* mutation, 84% had unmutated *IGHV*. The Table 1 summarizes efficacy and safety.

Table 1.

Summary and Conclusions: Similar to the first interim analysis, IDELA+R demonstrated significant improvement in progression-free survival, overall response rate, and lymph node response rate, compared to control, with acceptable safety. The overall survival of patients on IDELA+R remained superior, including patients that crossed over into the extension study.

S705

A PHASE I STUDY OF THE ORAL BTK INHIBITOR ONO-4059 IN PATIENTS WITH RELAPSED/REFRACTORY AND HIGH RISK CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background: Bruton's tyrosine kinase (BTK) is a critical kinase involved in B-cell receptor signal transduction. ONO-4059, a highly potent and selective oral BTK inhibitor has demonstrated anti-tumour activity in pre-clinical models (Yasuhiro *et al.*, AACR2013) and in the clinic in both NHL and CLL patients (Salles *et al.*, ASH 2013; Rule *et al.*, ASH 2013). Here, we present data from CLL patients enrolled in the ongoing Phase I study ONO-4059POE001.
Aims: Twenty five CLL patients were administered ONO-4059 as monotherapy, given once daily (QD) to determine safety, pharmacokinetics and pharmacodynamics, and preliminary efficacy. Patients can receive ONO-4059 for up to a maximum of 2 years and upon completion of the first 6 months of treatment, intra-patient dose escalation is permitted. Here, we present data for 25 evaluable patients treated with ONO-4059 at doses ranging from 20-600mg (8 cohorts). Patients had a median age 67 yrs [range 40-79], median baseline tumour burden 5,125 mm^2 [461-19,750 mm^2], median of 4 prior therapies [2-8], including fludarabine (23/25) and rituximab-containing therapy (22/25). Baseline median haematology parameters included hemoglobin 11.1 g/dL [8.5-15.2], WBC 41.2x10^9/L [2.1-394.8], platelets 97x10^9/L [23-185], lymphocytes 37.4x10^9/L [0.5-391], neutrophils 2.39x10^9/L [0.5-11.1]. 8/22 patients were 17p deleted [36%] and 6/22 had the 11q deletion [27%], 19/22 were IgVH unmutated status [86%], with 12/22 [55%] displaying TP53 mutation.
Results: ONO-4059 was found to be well tolerated. A total of 44 ONO-4059-related Grade 1 (27) and 2 (17) adverse events were reported in 18 out of 25 patients. Eleven ONO-4059-related G3 or G4 haematological toxicities were reported in 6 patients and were infrequent and independent of dose; with neutropenia in 5 patients, [G3 x5, G4 x3]), febrile neutropenia in 1 patient [G3] and leucopenia in 2 patients [G3 x2]. Only 3 Grade 3 ONO-4059-related non-haematological events were reported (pyrexiax2, back painx1). Seven ONO-4059-related SAEs were reported in 4 patients (febrile neutropenia [G3] and pyrexia [G3 x2] at 20 mg; rash [G2] at 80 mg, neutropenia [G4] at 320 mg, purpura [G2] and back pain [G3] at 400 mg). All patients experienced rapid reductions in lymphadenopathy observed early in treatment, between D1-D28 of the first cycle, accompanied by an increase in absolute lymphocyte count in some (67%), but not all patients. Best overall response rate according to IWCLL criteria (including modified PR with lymphocytosis) was 90% [based on CT-scan and P/E for 21/25 evaluable patients with 14 PR, 5 PR with lymphocytosis (for 19 responding patients, median reduction of lymph nodes was 79% [Range 52-100]), 1 SD and 1 PD]. Improvements in hemoglobin and platelets were observed from 3 months onwards. To date, 22 of 25 patients remain on treatment with a median duration of treatment of 270 days [Range 1-540].
Summary and Conclusions: In conclusion, ONO-4059 is a highly potent and selective oral Btk inhibitor with a favourable safety profile along with promising efficacy in this heavily pre-treated population, with responses observed in relapsed, refractory and 17p deleted patients and/or TP53 mutated patients.

S706

THE ADDITION OF CD20 MONOCLONAL ANTIBODY TO LENALIDOMIDE IMPROVES RESPONSE RATE AND SURVIVAL IN RELAPSED/REFRACTORY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Lenalidomide is an immunomodulatory drug active in CLL. The benefit of combination therapy with CD20 mAb compared to Lenalidomide monotherapy (Len) in patients (pts) with CLL is unknown.
Aims: Compare the experience with Len and Len+CD20 mAb in relapsed/refractory (R/R) pts with CLL.
Methods: We studied 139 R/R pts treated with Len *vs.* Len+CD20 mAb at MDACC from 2005-2011; 44 received Len, 59 Len+rituximab (LR) and 36 Len+ofatumumab (LO). We analyzed baseline characteristics associated with response, time-to-treatment failure (TTF), overall survival (OS) and toxicity requiring study cessation.
Results: Groups were similar (Table 1); Len pts were more heavily pre-treated. The following were associated with higher ORR: treatment with Len+CD20 mAb, mutated *IGHV* gene, 73% *vs.* 50%, p=0.03 and B2M <4.0 mg/l, 64% *vs.* 46%, p=0.04. On multivariable analysis (MVA), receiving Len+CD20 mAb was the only independent characteristic associated with higher ORR, [OR 3.8 (1.6-8.4), p=0.002]. Median time to CR was 12mo (3-31), without significant difference between groups. Median TTF was 35 *vs* 28 *vs* 17mo for CR *vs.* nPR *vs* PR, p=0.001. Achieving a response correlated with OS; median OS was 17mo in non-responders *vs.* not reached (NR) at a median follow-up of 43mo in responders, p=0.008. Baseline characteristics associated with shorter OS were: unmutated *IGHV* (37mo *vs.* NR, p=0.01), B2M ≥4.0 (23 *vs.* 71mo, p<0.001), del17p (25 *vs.* 62mo, p=0.005) and fludarabine-refractory CLL (17 *vs.* 62mo, p=0.004). In MVA, the following were independently associated with OS: treat-

ment with Len+CD20 mAb [HR 0.46 (0.27-0.80), p=0.005]. B2M ≥4.0 [HR 2.64 (1.56-4.50), p<0.001], unmutated *IGHV* [HR 2.68 (1.11-6.45), p=0.01], del17p [HR 1.61 (1.0-2.57), p=0.001] and fludarabine-refractory disease [HR 1.91 (1.11-3.29), p=0.02]. Treatment was discontinued due to toxicity in 11 pts (9 in LO and 2 in LR); most common reasons for discontinuation were prolonged cytopenia in 4 cases and infection in 2. One case of tumor flare reaction requiring treatment cessation was seen in the LO group. Age >70 was associated with more frequent cessation for toxicity (22 *vs*. 5%), p=0.007 (Figure 1).

Table 1.

Characteristic	Len (n=44), n(%)	LR (n=59), n(%)	LO (n=34), n(%)	P value
Age (median, range)	63 (49-86)	62 (42-83)	63 (34-82)	0.29
IGHV Unmutated	35 (80)	43 (73)	23 (64)	0.177
N. prior therapies (Median, range)	4 (1-15)	2 (1-9)	2 (1-8)	0.001
B2M ≥4.0 (mg/l)	24 (60)	24 (40)	21 (62)	0.14
17p-	8/42 (19)	15/57 (26)	9/28 (32)	0.45
Fludarabine-refractory	13 (30)	13 (22)	7 (21)	0.58
Rai III/IV	23 (52)	28 (47)	21 (62)	0.52
ORR	14 (32)	39 (66)	23 (68)	0.001
CRR	9	12	9 (26.5)	0.07
TTF in responding pts (mo)	17	25	23	0.26
OS (mo), median	20	NR at 50mo	NR at 39mo	0.008

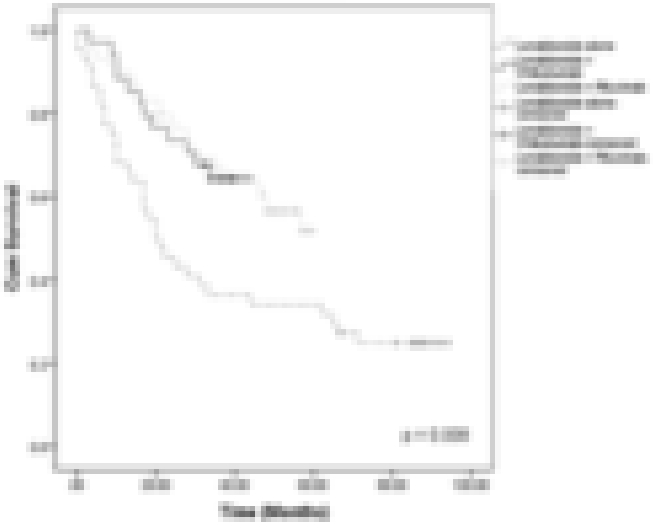

Figure 1. Overall survival according to treatment group.

Summary and Conclusions: Within the limitations of comparing outcomes across studies, addition of CD20 mAb to Len in R/R pts appears to improve response and survival. Achieving CR was associated with longer response duration. B2M ≥4.0, unmutated *IGHV* gene, del17p and fludarabine-refractory disease are all independently associated with worse outcomes, including OS. Prospective confirmation of these findings is warranted.

Myeloproliferative neoplasms - Biology 1

S707

RECURRENT ETNK1 KINASE DOMAIN MUTATIONS IN SYSTEMIC MASTOCYTOSIS WITH ASSOCIATED EOSINOPHILIA

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Background: While systemic mastocytosis (SM) in adult patients is virtually universally characterized by the *KIT*D816V mutation, there is considerable clinical heterogeneity, suggesting additional genetic or epigenetic events. Studying somatic genetic changes in mast cells is challenging due to their relative inaccessibility in peripheral blood (PB) and bone marrow samples. Up to 30% of SM patients exhibit PB eosinophilia (Lim *et al.* Blood 2009). Further, eosinophils are relatively easily isolated (≥97% purity) and represent the neoplastic clone in such patients (Pardanani *et al.* Leuk Res 2003).
Aims: To identify somatic genetic changes in purified eosinophils from a patient with SM with eosinophilia, using whole exome sequencing.
Methods: The study was approved by our institutional review board. Eosinophils (tumor) were isolated as CD16- cells from the PB granulocyte fraction and T-lymphocytes (non-tumor) were isolated from the PB mononuclear cell fraction by CD3-antibody labeled magnetic-bead separation. Matched genomic libraries were prepared and submitted for exome sequencing. Data were analyzed using the GeneSifter® Software (Perkin Elmer). Post-processed alignments from eosinophils were compared to alignments from the T-lymphocytes to make calls for somatic variants. We initially focused on identifying exonic non-synonymous single nucleotide variants (SNVs). Candidate mutations were validated by Sanger sequencing.
Results: Our experimental design was validated by the observation that heterozygous *KIT*D816V was present in eosinophils (69 reads, 46.4% mutant), but not in T-lymphocytes. Other somatic mutations of interest were: *EZH2*H694R, chr7 position 148506431 (32 reads, 100% mutant), *STAT5*αV707E, chr17 position 40461400 (9 reads, 67% mutant) and *ETNK1*N244S, chr12 position 22811995 (37 reads, 49% mutant). All 3 mutations were validated by targeted resequencing. We conducted targeted sequencing of *STAT5*α and *ETNK1* in 50 patients with SM (23 with eosinophilia), and 25 patients each with myelofibrosis, primary eosinophilia and chronic myelomonocytic leukemia (CMML). No *STAT5*α mutations were identified; however, 9 additional patients carried *ETNK1* mutations (10 total), all within the evolutionarily conserved choline kinase domain. *ETNK1* mutation prevalence was 10% in SM (22% and 0% with and without eosinophilia, respectively; N244S=3, G245A=2), 16% in CMML (all without eosinophilia; N244S=2, N244K/T=1 each), and 4% in primary eosinophilia (N244S=1). *ETNK1* (ethanolamine kinase 1; *EKI1*) catalyzes the rate-controlling step in phosphatidylethanolamine biosynthesis. Ethanolamine is a major membrane phospholipid, in the form of glycerophosphoethanolamine and is also a component of the glycosylphosphatidylinositol (GPI) anchor, which is necessary for cell-surface protein attachment. Computational tools for predicting the functional consequence of non-synonymous SN*Vs* suggested that the aforementioned *ETNK1* mutations were functionally deleterious (http://snps.biofold.org/ phd-snp/phd-snp.html; http://genetics.bwh.harvard.edu/pph2/index.shtml).
Summary and Conclusions: We identified recurrent somatic mutations targeting the kinase domain of *ETNK1*. The mutations appeared to cluster in patients with SM with eosinophilia or CMML. The COSMIC database lists only 2 missense *ETNK1* mutations in hematologic malignancies (one case each with H243Y in atypical chronic myeloid leukemia and N244S in myelodysplastic syndrome). These data suggest that *ETNK1* mutations have pathogenetic significance in myeloid malignancies, particularly SM with eosinophilia. We have cloned full length wild type and mutant *ETNK1* into expression vectors and are pursuing functional studies for confirmation.

S708

DOWN-REGULATION OF THE RIBOSOMAL PROTEIN S14 PATHWAY CONTRIBUTES TO IMPAIRED MEGAKARYOPOIESIS IN PRIMARY MYELOFIBROSIS

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Background: Primary myelofibrosis (PMF) is a clonal hematologic malignancy resulting from the transformation of a pluripotent hematopoietic progenitor cell. A major consequence of this transformation is increased hematopoiesis and an overproduction of abnormal blood cells. PMF is associated with bone marrow fibrosis, extramedullary hematopoiesis, increased numbers of circulating $CD34^+$ cells, splenomegaly, and a propensity to evolve to AML. Patients also display anemia and thrombocytopenia and harbor abnormal, immature megakaryocytes (Mks) in their bone marrow and spleen. PMF patients can present well known mutations including JAK2V617F (65%), MPL (10%), TET2 (17%), CBL (6%), IDH

(4%,), which are not specific to the disease. Recently it has been revealed that most of patients not mutated for JAK2 carry a CALR mutation.
Aims: We hypothesize that the genetic events associated with PMF contribute to defects in Mk maturation, but that additional changes are needed to explain the striking abnormalities seen in PMF relative to the other myeloproliferative diseases. We chose to examine the changes that occur in gene expression of Mks as a way to better understand their abnormal differentiation and to determine their contribution to the disease.
Methods: Primary $CD34^+$ cells from PMF patients and healthy donors were cultivated for 9 days in media without serum and supplemented with thrombopoeitin. Cells were then analysed for their phenotype and their gene expression.
Results: Primary $CD34^+$ cells from PMF patients and healthy donors were cultivated for 9 days. We found that PMF specimens gave rise to a lower percentage of mature ($CD41^+CD42^+$) Mks as compared to healthy donors associated with a lower ploidy level and a greater proliferation. These observations are consistent with the clinical observations that PMF bone marrow is characterized by an increased number of immature, dysplastic Mks. $CD41^+CD42^+$ mature MKs derived from healthy and PMF $CD34^+$ were sorted by flow cytometry. Then, RNA was extracted and whole genome microarray analysis was performed with Illumina Human HT12-v4 arrays. In PMF samples, myeloid transcription factors including CEBP, GFI1, and SPI1 (PU.1), are strikingly and significantly overexpressed. Moreover, c-myb, which regulates the erythroid/Mk cell fate decision, FOG-1 and AML1, are also overexpressed in PMF Mks. This aberrant myeloid gene expression program in PMF Mks is reminiscent of a similar defect we observed in Mks with reduced expression of GATA-1 and GATA-2. Gene Set Enrichment Analysis (GSEA) analysis revealed down-regulation of the RPS14 (Ribosomal protein S14) pathway, which was first described in myelodysplastic syndrome 5q- where this protein is absent. Using two different shRNAs directed against *RPS14*, we show that its down-regulation in primary $CD34^+$ leads to a strong reduction of Mk differentiation, reproducing the phenotype observed with patient samples. Conversely, over-expression of RPS14 in PMF $CD34^+$ cells partially restores their differentiation. As expected, down-regulation of the RPS14 pathway is accompanied with p53 pathway activation. Moreover in wild-type megakaryocytes, down-regulation of RPS14 leads to a reduction of GATA-1 protein level. As GATA-1 has previously been shown to be reduced in PMF Mks, we over-expressed GATA-1 in primary culture of PMF $CD34^+$ with a lentivirus and followed their differentiation. In this condition we observed a greater percentage of $CD41^+CD42^+$ cells and a higher ploidy.
Summary and Conclusions: In conclusion, we show here for the first time the implication of ribosomal pathway in PMF pathogenesis. Since the over-expression of RPS14 or GATA1 partially restore Mk differentiation of PMF patients, these novel findings open new avenue for therapeutic options in PMF.

S709

GENETIC STUDIES REVEAL JAK2 IS NOT ESSENTIAL FOR PLATELET PRODUCTION

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Background: The intensity of JAK kinase inhibitor treatment has been limited by cytopenias, which are thought to be due to on-target JAK2 inhibition. However, the role of JAK2 signaling in megakaryopoiesis has not been evaluated.
Aims: We studied the significance of JAK2 for platelet formation.
Methods: We conditionally deleted *Jak2* in thrombopoiesis through Cre-recombinase-mediated deletion under the control of the Pf4 promoter. We evaluated the impact of *Jak2* deletion in platelets on blood counts, stem/progenitor frequencies, and Jak-Stat signaling.
Results: Pf4-Cre-mediated *Jak2* deletion resulted in abrogation of Jak2 mRNA expression in platelets and in a modest, but significant reduction in megakaryocyte progenitors (MkP, mean reduction 33%, p=0.029). Jak2 mRNA expression was unaffected in Pf4+ $Jak2^{f/f}$ meg-erythroid progenitors (Pre-MegE, p=0.618) and $Lin^-Sca1^+Kit^+$, cells (LSK, p=0.802). Pf4-Cre mediated *Jak2* deletion abolished Jak2 protein expression in Pf4+ $Jak2^{f/f}$ platelets while platelet expression of the thrombopoietin receptor Mpl was not significantly affected. In response to thrombopoietin (Tpo) stimulation, Jak2, Tyk2, Stat3 and Stat5 were not phosphorylated in Pf4+ $Jak2^{f/f}$ platelets in contrast to littermate controls with intact Jak2. Platelet-specific *Jak2* deletion *in vivo* did not result in any reduction of platelet counts, but in significant and progressive thrombocytosis. Average platelet counts in Pf4+ $Jak2^{f/f}$ mice were 4720 G/l (3436-6460 G/l, p<0.0001 *vs.* controls) at 2 months, 3965 G/l (2820-4920 G/l, p<0.0001) at 3 months, 6966 G/l (5008-7884 G/l, p<0.0001) at 4 months and 5966 G/l (3204-7892 G/l, p<0.0001) at 5 months of age. Pf4+ $Jak2^{f/f}$ mice also showed moderate leukocytosis with a mean white blood cell count of 23.7 G/l (compared to 11.9 G/l in controls, p=0.005) suggesting cell non-autonomous effects of Jak2 loss in platelets on megakaryocyte progenitors and hematopoietic stem/progenitor cells with intact Jak2. Pre-MegE were 2-fold (p<0.0001) and MkP 9-fold (p=0.0001) expanded in Pf4+ $Jak2^{f/f}$ mice consistent with expansion of non-*Jak2* deleted progenitors. We observed increased megakaryocyte formation from Pf4+ $Jak2^{f/f}$ bone marrow or from sorted Pf4+ $Jak2^{f/f}$ Pre-MegE or MkP. Platelet-specific Jak2 loss affected hematopoietic stem/progenitor cells *in vivo* with 5- to 6-fold expansion of LSK cells (p<0.0001) which formed higher numbers of megakaryocytic colonies on a per-cell basis than LSK from controls. The proportion of $c\text{-}Kit^{hi}Lin^-Sca1^+Kit^+CD150^+CD48^-$, hematopoietic stem cells in Pf4+ $Jak2^{f/f}$ mice was increased, suggesting LSK expansion was due in part to expansion of megakaryocyte-biased $c\text{-}Kit^{hi}$ stem cells. Serum Tpo levels remained elevated in Pf4+ $Jak2^{f/f}$, mice at a 6.9-fold higher level (Tpo 2524 +/- 230 pg/ml, p<0.0001) as compared to MPL W515L mice with similarly increased platelet counts but markedly reduced serum Tpo levels as compared to control mice (Tpo 230 +/- 37 pg/ml in MPL W515L *vs.* 2787 +/- 204 pg/ml in controls, p<0.0001) Surface expression of Mpl in Pf4+ $Jak2^{f/f}$ platelets was modestly decreased consistent with reduced thrombopoietin internalization/turnover by Jak2-deficient platelets.
Summary and Conclusions: These data demonstrate that JAK2 is dispensable for the production of platelets. Jak2 loss in platelets leads to altered regulation of serum thrombopoietin and cell non-autonomous expansion of stem/progenitor cells. These data suggest the effects of JAK inhibitors on platelet counts are not due to JAK2 inhibition in platelets, but likely due to inhibition of other targets or as a consequence of JAK2 kinase inhibition in stem/progenitor cells.

S710

THE COMBINATION OF JAK INHIBITOR RUXOLITINIB, PAN-PIM INHIBITOR LGH447, AND CDK4/6 INHIBITOR LEE011 IN A PRECLINICAL MOUSE MODEL OF MYELOPROLIFERATIVE NEOPLASIA

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Background: The JAK/STAT axis is a critical component downstream of multiple cytokine and growth factor receptor signaling pathways. The genetic aberration of *JAK2* V617F and the associated activation of STAT in myeloproliferative neoplasia (MPN) is one example of the involvement of this pathway in human cancer. Activated JAKs phosphorylate STAT proteins, which then upregulate the transcription of STAT target genes, such as *PIM1*. Pim kinases are involved in the regulation of the cell-cycle and proliferation. The inhibition of JAK1/2 by the JAK inhibitor ruxolitinib results in suppression of the JAK/STAT pathway and promotes significant clinical benefit in patients with myelofibrosis. Our previous preclinical studies in a Ba/F3-*JAK2* V617F–driven MPN model demonstrate that the combination of ruxolitinib and the pan-PIM inhibitor LGH447 exhibits greater inhibition of spleen weight increase and some reduction of *JAK2* V617F allele burden compared with ruxolitinib monotherapy. Mutant *JAK2* V617F has been shown to increase CDC25A transcription through activated STAT5. It also regulates p27 at both the gene expression and phosphorylation levels. The activation of CDC25A and modulation of p27 have been postulated to trigger the activation of cyclin-dependent kinases (CDK), such as CDK4, to initiate cell-cycle progression. Additionally, activated STATs and Pims have been shown to activate D cyclins that are upstream of CDK4/6.
Aims: Here, we explored the hypothesis that CDK4/6 inhibition, in conjunction with JAK and PIM inhibition, would enhance therapeutic efficacy against MPN.
Results: LEE011 is a potent and selective inhibitor of CDK4/6. The combination of ruxolitinib and LEE011 was tested in an MPN model with Ba/F3 cells harboring EPOR-*JAK2* V617F. While ruxolitinib monotherapy reduced spleen weight and total tumor burden by more than 50%, it had a marginal effect on *JAK2* V617F allele burden in this model. The combination of ruxolitinib and LEE011 resulted in an additional 2- to 3-fold reduction in spleen weight and total tumor burden. Yet no clear modulation of *JAK2* V617F allele burden was observed. To further enhance the antitumor activity, we tested the triple combination of ruxolitinib, LGH447, and LEE011 in this MPN model. This triple combination resulted in a >99% reduction in total tumor burden and an ≈ 96% reduction in spleen weight. Furthermore, the triple combination of ruxolitinib, LGH447, and LEE011 significantly downmodulated *JAK2* V617F allele burden by >80%.
Summary and Conclusions: Our preclinical data indicate that the triple combination of ruxolitinib, CDK4/6 inhibitor LEE011, and pan-PIM inhibitor LGH447 may preferentially impact the *JAK2* V617F–mutant MPN clones. This combination also achieved greater reductions in tumor burden and spleen weight in our preclinical MPN model. Therefore, potentially greater therapeutic benefit in subgroups of MPN patients may be achieved with the triple combination of ruxolitinib, LGH447, and LEE011.
Note: M Pinzon-Ortiz and X Rong contributed equally to this abstract.

S711

THE HISTONE METHYLTRANSFERASES MLL2 AND MLL4 CONSTITUTE NOVEL NF-E2 TARGET GENES

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Background: Expression of the transcription factor and epigenetic modulator "nuclear factor erythroid-2" (NF-E2) is aberrantly elevated in patients with Myeloproliferative Neoplasms (MPN). We have shown that NF-E2 overexpression in a murine model causes a phenotype similar to MPN in humans. This includes thrombocytosis, leukocytosis, expansion of the stem- and progenitor cell compartments as well as spontaneous transformation to acute leukemia. Recently, we have identified in-del mutations in the NF-E2 gene in MPN patients. These alterations lead to the formation of truncated proteins, which, despite having lost the ability to bind DNA, nonetheless increase the activity of wild type NF-E2. However, as few target genes of NF-E2 are known, the downstream pathways by which this augmented transcription factor activity exerts its effects remain unknown.
Aims: We therefore sought to identify novel NF-E2 target genes in order to both understand how increased NF-E2 activity contributes to MPN pathophysiology as well as to identify novel drug targets in these etiologies.
Methods: We used lentivirally driven NF-E2 overexpression as well as RNAi mediated NF-E2 silencing in CD34-positive cells to identify novel NF-E2 targets.
Results: Here, we report that the histone methyltransferases MLL2 and MLL4 constitute novel NF-E2 target genes. *In silico* analysis revealed NF-E2 binding to distinct sites in both the MLL2 and MLL4 genes *in vivo* by chromatin immunoprecipitation sequence analysis (ChIP-seq). We confirmed these *in silico* data by ChIP analysis. In HEL cells, NF-E2 binds a site located at bp -1000 in the MLL2 promoter as well as three distinct sites located at -500bp, -20kb and -46kb of the MLL4 promoter. In reporter gene assays, co-transfection of NF-E2 and its obligate heterodimer, MafG, significantly increased MLL2 driven luciferase activity, demonstrating that NF-E2 transactivates transcription of this gene. Most importantly, quantitative RT-PCR showed an increase in MLL2 and MLL4 mRNA in patients with polycythemia vera (PV) compared to healthy controls. Hence, the expression of these epigenetically active enzymes is augmented in PV patients. The MLL4 protein (in the literature also referred to as MLL2) has been previously shown to interact with NF-E2. NF-E2 is required for recruitment of MLL2 to the β-globin locus and for chromatin remodeling and establishment of H3K4me3 and H3K79me3 histone marks at these sites. Our data establish a novel interaction network where NF-E2 both regulates the expression levels of MLL4 while at the same time modulating its epigenetic activity at NF-E2 target genes.
Summary and Conclusions: We therefore conclude that the histone methyltransferases MLL2 and MLL4 are novel NF-E2 target genes and propose that elevated NF-E2 levels in MPN patients contribute to disease pathophysiology in part by affecting histone methylation. These data provide a molecular basis for pre-clinical investigation into the effects of histone methyltransferase inhibitors on MPN cell biology.

Allogeneic stem cell transplantation

S712

HAPLOIDENTICAL STEM CELL TRANSPLANTATION OUTCOME IS NOT INFERIOR TO MATCHED RELATED AND UNRELATED DONOR TRANSPLANTATION: AN INTENTION-TO-TREAT ANALYSIS OF 611 PATIENTS

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Background: Allogeneic transplantation of haemopoietic stem cells (HCT) from an HLA-matched related (MRD) or unrelated donor (MUD) is a curative option for patients (pts) affected by high-risk hematological diseases. Recently, protocols of HCT from family haploidentical donors based on unmanipulated graft have potentially extended to 100% of candidate patients the access to HCT as the best chance of cure.
Aims: We offered a haploidentical HCT to adult pts lacking a matched donor in the appropriate time according to clinical indications (www.leukemianet.org, www.ebmt.org) as part of tratment algorithm for primary disease.
Methods: Here we are reporting the intention-to-treat (ITT) analysis of HCT in all consecutive HRHD pts referred to our Institution between January 2004 and June 2012.
Results: Indication to HCT was given to 611 pts (median age 49 y, range 10-76; male 394), 120 pts (20%) received a transplant from a MRD; 187 pts (30%) activated a MUD search; 118 pts (19% of total pts, 63% of MUD searching) received a MUD transplant; 12 pts (2%) received a umbilical cord blood unit. Overall, 237 pts received a haplo-HCT (49%). The median time from indication to HCT was 87 days. The median time from indication to activation was 12 days and the median time from activation to HCT was 114 days (range 22-824). Lack of donor was not a limiting factor. Age was not a limiting variable to HCT execution: 107 patients (out of 487 SCT – 22%) were in the 60-69 range of age. The overall survival (OS) analysis in ITT for the entire population was 43% at 2 years, with a median time of survival of 767 days. The 2yOS in pts transplanted in CR was 60%, in pts transplanted in PD is 25% (p <0.001). The OS according to donor source (MRD *vs* MUD *vs* haplo-HCT) was comparable (p=ns) in pts transplanted in CR. The 2-year OS in pts affected by acute myeloid leukemia was 66%, 60% and 13% for patients transplanted in CR1, >CR1 and PD, respectively. The outcome analysis (OS, relapse incidence, transplant related mortality) per donor source was comparable (p=ns) within CR setting.
Summary and Conclusions: For many pts with HRHD, HCT from a MRD provides the best chance for long-term survival. However, only approximately 30% of individuals have a MRD. In our experience, haplo-HCT offers a concrete option of cure to HRHD pts: outcome results are superimposable to MRD and MUD in pts in CR at HCT. In ITT analysis, 80% of candidate pts received an HCT as a potential immunotherapy. All patients affected by HRHD, for whom an HCT is part of their therapeutic program, should proceed with a well-timed HCT whatever the stem cell source, and in the absence of a fully HLA-compatible donor the option of a haploidentical donor should be simultaneously considered.

S713

SUPERIOR SURVIVAL OF UNMANIPULATED HAPLOIDENTICAL HSCT COMPARED WITH CHEMOTHERAPY ALONE USED AS POST-REMISSION THERAPY IN ADULT STANDARD-RISK ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST COMPLETE REMISSION

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Background: 40% - 50% adult patients with standard-risk ALL in CR1 still develope relapse. And, once relapse, overall survival (OS) at 5 years after relapse is only 15 - 25%. Studies have also shown that the outcome of adult standard-risk ALL in CR1 treated with HLA-identical sibling HSCT is better than treated with chemotherapy alone. However, lack of HLA-identical sibling has restricted the application of allo-HSCT in adult ALL patients. During last decade, the efficacy and safety of haploidentical HSCT have been confirmed to be comparable with HLA-identical sibling HSCT.
Aims: This study was performed to compare the efficacy of haploidentical HSCT with chemotherapy alone used as post-remission treatment of adult standard-risk ALL in CR1
Methods: Consecutive 163 adult patients with standard-risk ALL in CR1 in Peking University Institute of Hematology from January 1, 2000 to May 30, 2013 were retrospectively investigated. Out of these patients, 75 received chemotherapy alone (Group A) and 88 received unmanipulated haploidentical HSCT (Group B).
Results: Cumulative incidence of relapse at 5-year in Group A was significantly higher than those in Group B (68.8% *vs.* 26.7%, P<0.0001). The incidence of treatment-related mortality (TRM) at 5-year was 12.2% in Group A and 19.2%

in Group B (P=0.055). Besides, the OS and DFS in Group A were also significantly inferior to those in Group B (P<0.0001 and P<0.0001). Besides, multivariate analysis demonstrated that CNS leukemia (P=0.039, HR=4.832), T-cell immunophenotype (P=0.017, HR=5.493), expression of E2A-PBX1 (P=0.048, HR=10.312) and minimal residual disease (MRD)-positive before 2nd cycle of consolidation chemotherapy (P=0.008, HR=5.412) were significantly correlated with relapse. Based these risk factors, patients with one of four risk factors were assigned to high-risk group. Otherwise, patients without risk factors were assigned to low-risk group. In low-risk patients, even though the incidence of relapse at 5-year was higher in chemotherapy group than HSCT group (35.9% *vs.* 19.7%, P=0.026), the TRM at 5-year in chemotherapy group was lower than HSCT group (4.8% *vs.* 24.0%, P=0.123).Therefore, finally there were no differences in the OS and DFS between chemotherapy and HSCT (OS: 74.7% *vs.* 69.4%, P=0.861 and DFS: 59.3% *vs.* 56.3%, P=0.421). However, in high-risk patients, the incidence of relapse in chemotherapy group were statistically higher than HSCT group (81.0% *vs.* 34.8%, P<0.0001). And the OS and DFS at 3-year in chemotherapy group were only 5.8% and 5.0%; but the OS and DFS at 5-year in HSCT group were 66.5% and 51.7% (P<0.0001 and P<0.0001). In addition, the incidence of relapse, TRM, OS and DFS at 5-year in patients receiving HSCT in high-risk group were all comparable with patients receiving chemotherapy in low-risk group (relapse: 34.8% *vs.* 39.0%, P=0.351; TRM: 13.5% *vs.* 4.8%, P=0.413; OS: 66.5% *vs.* 74.7%, P=0.964; DFS: 51.7% *vs.* 59.3%, P=0.630). Figure 1. Relapse, treatment-related mortality, overall survival (OS) and disease-free survival (DFS) in all patients (n=163). (A) Cumulative incidence of relapse. (B) Cumulative incidence of treatment-related mortality. (C) OS. (D) DFS. One hundred and sixty three patients were finally enrolled in this study. Out of them, 75 patients received chemotherapy alone (Group A) and other 88 patients received haploidentical HSCT (Group B).

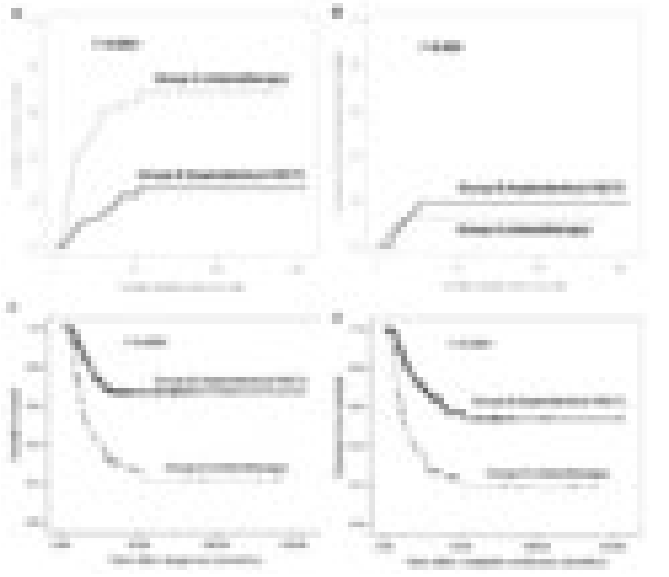

Figure 1.

Summary and Conclusions: This study is the first one to demonstrate the compared with chemotherapy alone, haploidentical HSCT is a better post-remission therapy in adult standard-risk ALL in CR1. Besides, based on the four risk factors, the establishment of risk-stratification could identify the subgroup of patients with higher risk of relapse in adult standard-risk ALL in CR1. Furthermore, risk-stratification directed post-remission therapies using haploidentical HSCT or chemotherapy alone not only reduce relapse rate, but also avoid unnecessary TRM, and finally improve survival.

S714

TREATMENT OF STEROID RESISTANT GRADE II TO IV ACUTE GVHD BY INFUSION OF MESENCHYMAL

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Background: Despite major improvements in the last decade in the field of HSCT, acute graft *versus* host disease (aGVHD) remains a life-threatening complication and substantially reduces efficacy of HSCT. In particular, the outcome of patients with severe steroid-resistant aGVHD continues to be poor. Therefore, the search for new therapeutic strategies for the treatment of aGVHD remains of vital importance.

Aims: To test whether treatment of steroid refractory aGVHD patients with Mesenchymal Stromal Cells (MSC) is safe and feasible.
Methods: We expanded MSC using platelet lysate (PL) and infused them in patients with steroidrefractory GVHD. Furthermore immunological changes after infusion of MSC were characterized *in vitro*. Anti-viral and antileukemia responses of reactive T-cells were tested and phenotypical changes in immune cells were followed up as were cytokines implicated in GVHD. MSC from bone marrow of third party healthy volunteers were isolated via plastic adherence using the CellStack system of Macopharma, expanded with PL for up to three passages (P3), harvested using TripLE and stored. In an open-label, non-randomized prospective phase I/II study patients with steroid-refractory GVHD grade II to IV were treated with hPPL-MSC. 50 patients were included and received up to four infusions. Response rates, TRM and other adverse events were assessed for up to 12 months. In addition, a comprehensive phenotypical and functional analysis was performed with PBMCs and serum isolated from all patients before, during, and after infusion of MSC.
Results: The production of MSC using PL in CellStacks takes±22 days to expand from bone marrow to P3, resulting in a mean number of $59x10^5$ MSC per double layer CellStack. All batches fulfilled the release criteria. Between January 2009 and July 2012, 48 out of the 50 patients included were eligible for analysis, 7 children and 41 adults. Mean age was 44,9 years (range 1,3-68,9). Organs involved in aGVHD were the skin (52%), the GI tract (88%) and the liver (35%). Overall GVHD grade was II for 12 (25%), III for 33 (69%), and IV for 3 (6%) patients. Mean number of infusions were 3 (1–4). No severe side effects were observed upon infusions. Median follow up was 5,0 months (range 0,3-46,5). Complete overall response of aGVHD was observed in 24 patients (50%) after a median of 53 days (range 3–116 days). Overall survival was significantly improved in responders when compared to non-responders (p <0.001). Patients who relapsed with GVHD of the gut were again sensitive to steroids, except one patient who then responded well to a second cycle of MSC. Immunological monitoring shows that anti-viral and anti-leukemia reactive T-cells are well preserved in all patients who responded to MSC treatment. In addition, we identified a combination of biomarkers that already 2 weeks after initiation of treatment predicts a complete resolution of GVHD, whilst this usually only became clinically apparent after months.
Summary and Conclusions: Generation and infusion of MSC in patients suffering from steroid-resistant aGVHD grade II- IV is feasible, safe and appears to be effective. Infusion of MSC did not impair anti-virus or anti-leukemia reactive T-cells. Identified biomarkers predict early a usually late clinical resolution of GVHD and thus might be useful to early guide clinical decision making.

S715

USE OF HIGH-THROUGHPUT SEQUENCING TO MONITOR T CELL RECONSTITUTION FOLLOWING CORD BLOOD TRANSPLANT

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Background: Cord blood transplant (CBT) recipients are at increased risk of transplant related mortality, in part due to delayed hematopoietic and immune system recovery resulting in increased infectious complications. However, without direct clinical measures of immunocompetence, the specific role of delayed immunity on CBT outcomes is not easily determined and intervention studies not possible.
Aims: We apply high-throughput sequencing of rearranged T cell receptor genes to monitor cellular adaptive immune reconstitution after myeloablative cord blood transplant. We compare our results to other available methods of assessing T cell reconstitution (TREC, CD3+ counts, spectratyping), and correlate our results with clinical outcomes.
Methods: We collected peripheral blood at several time-points (pre-transplant and at days 28, 56, 100, 180 and 365 post-transplant) in 34 consecutive patients undergoing myeloablative cord blood transplant for treatment of hematologic malignancies at Fred Hutchinson Cancer Research Center. All samples were subjected to multiplex amplification and high-throughput sequencing of somatically-rearranged T cell receptor loci. From these data, clonal presence and abundance of hundreds of thousands of clones were tracked for each time-point and total T cell diversity was estimated. Basic clinical outcomes for all patients were also determined, including GvHD, overall survival, disease free survival, regimen related toxicities and infectious complications.
Results: The results from high-throughput T cell receptor sequencing were generally concordant with spectratyping results when assessing patient T cell repertoire clonality post-transplant. However, the ability to track individual T cell clones demonstrated tremendous oscillation after cord blood transplant, with an almost entirely new T cell repertoire appearing at least monthly in CBT recipients. Furthermore, in contrast to healthy controls whose blood was sampled on a similar time-course, where the most frequent T cell clone at one time point remains dominant at subsequent time points, the largest clones observed early post-transplant in CBT recipients subsequently dropped below detection within weeks of direct measurement. Of the 34 patients studied, 19 died within one year of transplant. Despite the fact that most mortality was observed

≥100 days post-transplant, our estimated total T cell repertoire diversity values for these six patients were lower than those of the remaining patients by 100 days post-transplant (p-value=0.019 by permutation, see Figure 1). Neither TREC values nor absolute peripheral CD3+ counts showed a significant difference between outcome groups at 100 days post-transplant.

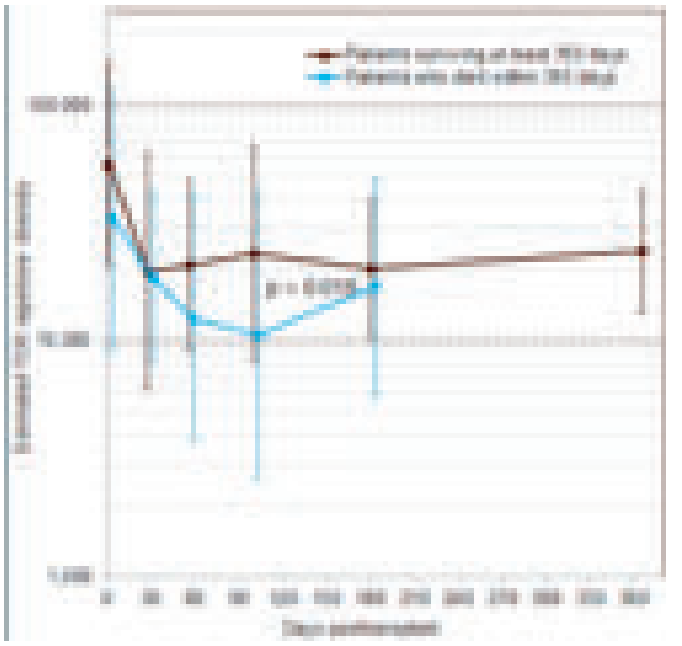

Figure 1.

Summary and Conclusions: The peripheral T cell repertoire is exceptionally dynamic following myeloablative cord blood transplant, with many T cell clones rising to high frequency and then receding to an undetectably low level in a matter of weeks. By three months after transplant, T cell diversity was correlated with clinical outcomes in this patient cohort, suggesting that diversity of the T cell repertoire may be useful in guiding clinical decision making in patients undergoing cord blood transplant.

S716

PROPHYLACTIC CD8 DEPLETED DLI OR PREEMPTIVE CD3POS DLI? – ON THE WAY TO AN INDIVIDUALIZED IMMUNOTHERAPY AFTER ALLOGENEIC HSCT

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Background: The combination of reduced-intensity conditioning (RIC) and *in vivo* T-cell depletion by Alemtuzumab prior to hematopoietic stem cell transplantation (HSCT) has demonstrated efficient engraftment and reduced graft-*versus*-host disease (GVHD). However, this regimen is associated with slow lymphocyte recovery leading to a delayed anti-infectious and anti-malignant immunity. DLI can be used to improve immune reconstitution.
Aims: Here we investigate on the impact of prophylactic use of CD8-depleted *vs* preemptive use of unmanipulated (CD3pos) DLI in these patients.
Methods: 188 patients with different hematologic malignancies were planned for treatment with DLI after allogeneic HSCT with a reduced intensity conditioning (Fludarabin 30mg/m² d-7 till d-3, Melphalan 140mg/m² d-2, Alemtuzumab20mg d-8 till -4). The calcineurin-inhibitor used for GVHD-prophylaxis was intended to be tapered until day+50 post HSCT. 134 patients should receive CD8depl DLI prophylactically after day +60 (Group A), 54 patients were planned for CD3pos DLI in a preemptive setting after day +100 in case of mixed donor chimerism or minimal residual disease (MRD). Both groups received DLI in escalating doses every 60 to 90 days. DLI application was stopped when GVHD occurs (Group A and B) or a full donor chimerism was achieved (in Group B). Both patient-groups did not differ in median age (A:56 years, B:57 years). The majority of patients either suffered from acute leukemia/MDS, lymphoma or myeloma.
Results: Of 134 patients (Group A) 41% received CD8depl DLI prophylactically, of 54 patients (Group B) 31% received preemptive CD3pos DLI. Overall survival (OS) significantly increased in all patients after DLI (74% with DLI, 45% without DLI) and relapse rate was reduced after DLI (17% after DLI, 37% without DLI). CD8depl prophylactic DLI did not induce less GVHD than CD3pos preemptive DLI (64% after CD8depl DLI *vs* 67% in Group B). After 2 years OS (50% *vs* 54%), relapse rate (33% *vs* 26%) and non-relapse mortality (NRM 34% *vs* 37%) did not differ between both groups. Analyzing distinct disease groups, there are significant differences in the outcome of lymphoma and acute leukemia patients for group A and B: With an OS of100% (*vs.* 56% Group A), relapse rate 0% (*vs.* 30%) and NRM 0% (*vs* 31%) the results for lymphoma patients are better in the preemptive DLI setting (Group B). Patients suffering from acute leukemia benefit from prophylactic CD8 depleted DLI (Group A): OS after 2 years was 59% in Group A *vs* 41% in group B (not stat. significant), progression free survival 54% *vs* 34%, NRM 27% *vs* 54%.
Summary and Conclusions: In summary, the application of DLI (prophylactic CD8depl and preemptive CD3pos DLI) after RIC in combination with Alemtuzumab is useful in regard to OS and disease control.
The differences in OS, NRM and disease control for acute leukemia and lymphoma patients concerning the different DLI modalities is remarkable. Our data strongly support a randomized trial, comparing prophylactic *vs.* preemptive/therapeutic DLI application for different disease groups in the context of T-cell depleted HSCT.

Acute myeloid leukemia - Biology 1

S717

FLT3-ITD INDUCED MYELOPROLIFERATION CAUSES A CELL EXTRINSIC DEPLETION OF HAEMATOPOIETIC STEM CELLS

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Background: Constitutively activating internal tandem duplications (ITDs) of FLT3 frequently occur in acute myeloid leukaemia (AML) and are associated with a high relapse risk. Thus, understanding which cells might propagate Flt3-ITD associated myeloid disease is of considerable importance. However, the ability of Flt3-ITDs to transform progenitor cells and impact of mutant Flt3 within the haematopoietic stem cell (HSC) compartment is unclear.
Aims: 1. To investigate whether Flt3-ITDs confer aberrant self-renewal properties to progenitor cells.
2. To determine the expression pattern of Flt3 and impact of Flt3-ITDs on the HSC compartment.
Methods: Using a murine knock-in model of Flt3-ITD myeloproliferation, we employed phenotypic and functional stem cell assays as well as single-cell gene expression analysis of over 1000 haematopoietic stem/progenitor cells to understand the expression pattern and impact of this mutation on HSCs.
Results: Phenotypic analysis demonstrated that numbers of HSCs (LSKCD48-CD150+) were markedly reduced in adult Flt3-ITD mice (x0.24; P<0.001). The loss of HSCs occurred in parallel to the development of myeloproliferation, beginning from 2 weeks of age, with normal numbers of HSCs in Flt3-ITD fetal livers. Competitive transplantation of 1 million BM cells confirmed a marked loss of functional HSCs in adult Flt3-ITD mice. Furthermore, purified multipotent and granulocyte macrophage progenitors failed to sustain long-term engraftment of Flt3-ITD myeloproliferation. In order to explore whether Flt3-ITDs cell-intrinsically impacted on HSCs, we employed single cell gene expression analysis of wild-type and Flt3-ITD murine HSCs, which do not express cell-surface Flt3, to explore heterogeneity of *Flt3* expression. This analysis demonstrated that in two-thirds of wild-type murine HSCs, *Flt3* mRNA transcript was not detected, even using nanofluidic cell capture (Fluidigm C1 Autoprep System) with sensitivity to the single molecule level. These *Flt3*-negative HSCs more frequently expressed stem cell associated genes such as *Vwf* (Sanjuan-Pla *et al.*, Nature, 2013), *Slamf1* and *Bmi1* than Flt3-positive counterparts (P<0.001). Single HSCs from Flt3-ITD mice did not show evidence of aberrant Flt3 signalling but did show less frequent expression of stem-cell associated genes. Analysis of human HSCs revealed lack of *FLT3* expression in one half of phenotypic HSCs at the single cell level with a similar negative correlation between *FLT3* and HSC-associated gene expression. Competitive transplantation using Flt3-ITD fetal liver cells, with wild-type vWF-EGFP competitor cells, resulted in Flt3-ITD myeloproliferation and an associated, marked cell-extrinsic suppression of wild-type competitor vWF-EGFP +ve HSCs (x0.05; P<0.01). To explore a possible mechanistic basis for this, we analysed key components of the BM niche. Flt3-ITD mice showed loss of endothelial cells (x0.28; P<0.05) and mesenchymal stem cells (x0.26; P<0.01) both of which displayed aberrant gene expression. Immunohistochemistry confirmed disruption of these niche elements, together providing a putative mechanism for the extrinsic suppression of HSCs by Flt3-ITDs.
Summary and Conclusions: These data provide insight into the mechanism by which Flt3-ITDs might exert a clonal advantage over normal haematopoietic cells, by expanding aberrant myeloid-biased multipotent progenitors (Mead *et al.*, Cell Reports, 2013) which drive myeloid disease, whilst causing an extrinsic suppression of non-malignant, Flt3-negative HSCs through disruption of the niche. That Flt3-ITD myeloid disease can be propagated by rare HSCs, which do not express the mutation, raises a new challenge for efforts to therapeutically target this poor prognostic mutation.

S718

NPM1 AND FLT3-ITD MUTATIONS COOPERATE TO IMPAIR HEMATOPOIETIC STEM/PROGENITOR CELLS DIFFERENTIATION AND DEREGULATE GATA1 IN A MOUSE MODEL OF AML

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Background: Nucleophosmin (NPM1) and FMS-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) mutations frequently occur together in human acute myeloid leukemia (AML) with normal cytogenetics suggesting cooperative leukemogenesis. Recently, compound NPM1/FLT3-ITD mutations were reported to induce leukemia in mice, however the molecular and cellular mechanisms of mutation cooperation remain unclear.
Aims: We generated an AML model using knock-in mouse strains to (1) study the mechanisms of NPM1 and FLT3-ITD mutations cooperation, (2) characterize the cellular origin of leukemia and (3) identify novel molecular targets of NPM1/FLT3-ITD mutations-driven leukemia.
Methods: We crossed NPM1 mutant (Sportoletti *et al.*, Blood 2013) and FLT3-ITD mice (Lee *et al.*, Cancer Cell 2007) and used: 1) FACS analysis to study the hematopoietic stem/progenitor cell compartment in bone marrow (BM); 2) real-time PCR and Western blot analysis to measure GATA1 mRNA and protein expression; 3) the FLT3 inhibitor AC220 for *in vitro* and *in vivo* treatment experiments.
Results: Concomitant expressions of NPM1 and FLT3-ITD mutant proteins in hematopoietic cells led to leukemia development with massive splenomegaly, leukocytosis, anemia and thrombocytopenia. Leukemia penetrance and latency depended on mutation dosage: NPM1/FLT3-ITD double heterozygous mice die after 1 year, while mice with two NPM1 mutant alleles and heterozygous for FLT3-ITD displayed a median survival of 3.5 months. Prior to the leukemia onset, NPM1/FLT3-ITD double heterozygous mice exhibited leukocytosis (WBC $20.8\pm18x10^9$/L; N=20) due to increased neutrophils (29.34%±6.5) and macrocytosis (MCV 58.74±5.6 fl). Notably, flow cytometry revealed significant alteration in the hematopoietic stem/progenitor cell compartment in NPM1/FLT3-ITD mutant mice. In particular, pre-leukemic mice display a significant reduction of long term hematopoietic stem cells (LT-HSC) (0.017%±0.009 *vs* 0.094%±0.02 *vs* 0.12%±0.04 *vs* 0.11%±0.07) and a significant increase in frequency of multipotent progenitor (MPP) cells (0.51%±0.21 *vs* 0.25%±0.03 *vs* 0.26%±0.06 *vs* 0.1%±0.009) compared to mice bearing NPM1 or FLT3-ITD mutations in heterozygosity and wild type controls. Common myeloid progenitor (CMP) and granulocyte-macrophage progenitor (GMP) compartments were expanded more than two-fold in NPM1/FLT3-ITD mice while the immature megakaryocytic and erythroid compartments (including MEP, preMegE, MKPs, pre-CFU-E, CFU-E) were significantly reduced compared to other genotypes. These results prompted us to analyze the status of GATA1, the master regulator of erythroid and megakaryocytic differentiation. Strikingly, cellular changes were accompanied by complete loss of GATA1 expression in the BM both at the mRNA and protein levels (Figure 1). Additional gene expression analysis revealed deregulation of different genes involved in megakaryocytic and erythroid development. The mechanism of GATA1 deregulation is currently under investigation. We then explored the effects of FLT3 inhibition on the cellular and molecular changes of NPM1/FLT3-ITD mice. *In vitro* treatment with the FLT3 inhibitor AC220 significantly reduced viability of BM cells isolated from NPM1/FLT3-ITD preleukemic or leukemic mice. *In vivo*, AC220 induced a reduction of WBC counts accompanied by a partial GATA1 re-expression in NPM1/FLT3-ITD mice peripheral blood cells.

Figure 1.

Summary and Conclusions: We found that NPM1 and FLT3-ITD mutations cooperate to AML development *in vivo* by inducing a "preleukemic state" characterized by leukocytosis and macrocytosis due to the expansion of immature granulocytes and reduction of megakaryocytic and erythroid progenitors. We correlated this cellular effect to GATA1 loss, which could be manipulated using a novel FLT3 inhibitor. These findings will allow for further exploration of novel potential therapeutic targets in AML.

S719

NPM1 HAPLOINSUFFICIENCY IS SUFFICIENT TO INDUCE AML IN COLLABORATION WITH MEIS1 IN MICE

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Background: Nucleophosmin-1 (*NPM1)* mutations are among the most frequent mutations in AML, occurring in up to 50% of CN-AML patients. Several different mutations in the *NPM1* gene exist, all of which lead to aberrant cytoplasmic localization of the nuclear shuttling protein (*NPM1*c+) and a reduction of its presence in the nucleus. Thus, the mutation of *NPM1* induces a) a haploinsufficiency for the wildtype NPM1 and b) expression of the mutated protein. So far it is not clear to which extent this haploinsufficiency of NPM1 contributes to AML development. Experimental models have shown that Npm1c+ alone only generates leukemia after a long latency. In this report we now demonstrate that *Npm1* haploinsufficiency is sufficient to rapidly cause AML with high pen-

etrance in collaboration with the homeobox gene *Meis1*, whose aberrant expression is one of the molecular hallmarks of NPM1c+ human AML.
Aims: To dissect the functional relevance of NPM1 haploinsufficiency in the leukemogenesis of NPM1c+ AML, and to understand the role of MEIS1 in this process.
Methods: 5-FU mobilized murine stem and progenitor cells isolated from Npm1$^{+/+}$ wildtype, Npm1$^{+/-}$, haploinsufficient and Npm1$^{+/c+}$ humanized knock-in mice were retrovirally engineered to express *Meis1* or YFP (control) and transplanted in lethally irradiated Npm1$^{+/+}$ recipients. OCI-AML3 cells were used to test the effects of MEIS1 knockdown or drug treatment on human cells. Taqman PCR, and RNA-Seq expression data from the TCGA Acute Myeloid Leukemia dataset was used for gene expression analysis.
Results: Analysis of RNA-Seq expression data from NPM1c+ AML patient samples demonstrated high expression of *MEIS1*, which has not been previously identified as a collaborating partner for NPM1c+ in leukemogenesis. To test our hypothesis that NPM1 haploinsufficiency is an essential driver in NPM1c+ AML we transplanted Meis1 overexpressing murine stem and progenitor cells from Npm1$^{+/+}$, Npm1$^{+/-}$, and Npm1$^{+/c+}$ bone marrow into recipient mice: Overexpression of Meis1 in Npm1$^{+/+}$ cells was not able to cause disease. In contrast, mice transplanted with Npm1$^{+/c+}$ cells transduced with Meis1 developed AML with a median latency of 98 days, demonstrating that Meis1 acts as a strong collaborator in NPM1c+ AML. Surprisingly, Npm1$^{+/-}$ cells overexpressing Meis1 also induced AML with a median latency of 111 days, illustrating that haploinsufficiency was sufficient for AML development in this model. In the control arms, only cells from Npm1$^{+/c+}$ transduced with the YFP vector were able induce AML in transplanted animals, with a delayed median latency 184 days. To further test the dependency of NPM1c+ AML on MEIS1, we knocked down MEIS1 in the NPM1 mutated human cell line OCI-AML3, which led to a significant decrease in colony formation and cell growth. Meis1 induced an overexpression of cKit and Flt3 in the murine Npm1 models, and expression of *MEIS1* and *FLT3* highly correlated in human NPM1c+ FLT3-ITD- AML samples (r=0.63, p<0.05 Pearson test). In line with this, treatment of OCI-AML3 cells with midostaurin, which targets FLT3, led to a 24 and 75% reduction in cell growth at 50 and 100 nM concentrations.
Summary and Conclusions: Together this data demonstrates that Npm1 haploinsufficiency is sufficient, in collaboration with Meis1, to cause AML in mice, emphasizing that depletion of the nuclear Npm1 wildtype pool by loss of one wildtype *Npm1* allele or by the cytoplasmic shift of the Npm1 protein is a driving factor in this AML genotype. It furthermore highlights the key role of MEIS1 overexpression in NPM1c+ AML, at least partly by inducing FLT3 expression.

S720

IDENTIFICATION OF A POTENTIALLY NOVEL TUMOR SUPPRESSOR IN AML

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Background: In acute myeloid leukemia (AML) genetic alterations that deregulate cellular proliferation, apoptosis and differentiation contribute to disease progression. Chromosomal deletions are often observed in AML; however, in many instances, we lack a full understanding of how specific genes within these deleted regions impact the pathobiology of the disease. For example, loss of the 9q21.32 locus is commonly observed in a subset of AML patients, suggesting a tumor suppressor must reside within this region. Genetic studies have mapped only several candidate genes to the minimally deleted region (MDR) of 9q21.32. However, these studies have been unable to definitively identify the putative tumor suppressor. Currently, the most intriguing candidate in the MDR is heterogeneous nuclear ribonucleoprotein K (hnRNP K). hnRNP K is a DNA and RNA binding protein thought to control cellular proliferation and differentiation programs through its transcriptional and translational activities. In support of the notion that hnRNP K may be the tumor suppressor at 9q21.32, a recent TCGA study showed that when hnRNP K is mutated it has the potential to drive AML progression.
Aims: To precisely determine the genetic alteration responsible for 9q21.32-dependent AML an *in vivo* model is needed. To this end, we generated a novel *hnRNP K* knockout mouse model that will allow us to understand how hnRNP K directly influences hematopoietic homeostasis and leukemic progression. This model potentially mimics the haploinsufficiency observed in AML patients with 9q21.32 deletions.
Methods: We are currently using mouse models to examine how aberrant hnRNP K expression impacts tumorigenesis, hematopoiesis, and the interaction between hnRNP K and the p53/p21- and C/EBP- pathways. Using AML patient sample carrying a 9q21.32 deletion, we are evaluating the impact of 9q21.32 deletions on hnRNP K expression and interrogating changes in the hnRNP K allele.
Results: Haploinsufficient *hnRNP K* mice (*hnRNP K*$^{+/-}$) have a significant reduction in survival, a highly penetrant malignant phenotype, and a significant expansion of the myeloid compartment. Our data also suggest these *hnRNP K*$^{+/-}$-dependent phenotypes result from its inability to activate p53-dependent p21 expression (proliferation program) and properly regulate expression of C/EBPβ and δ (differentiation program). Likewise, our preliminary clinical data suggest patients with 9q-deletions have reduced hnRNP K expression. These results suggest that hnRNP K is a critical regulator of the p53- and C/EBP- pathways and may be the illusive tumor suppressor located at the 9q21.32 locus.
Summary and Conclusions: These studies will allow us to determine whether hnRNP K is in fact the tumor suppressor residing at the 9q21.32 locus and will advance our understanding of how hnRNP K controls cellular differentiation and proliferation programs. These findings will also assist us in determining if hnRNP K loss can serve as a prognostic biomarker and a possible therapeutic target.

S721

ACUTE MYELOID LEUKEMIA WITH RUNX1 MUTATIONS CONSTITUTE A DISTINCT ENTITY ASSOCIATED WITH CHARACTERISTIC CLINICAL AND GENETIC FEATURES AND POOR OUTCOME. A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background: In the WHO 2008 classification of acute myeloid leukemia (AML), a large proportion of AML is now classified according to their underlying genetic abnormalities. For the first time, two new provisional entities defined by the presence of gene mutations were added, "AML with mutated *NPM1*" and "AML with mutated *CEBPA*". With the advent of the new sequencing technologies, the genomic landscape has now been more or less deciphered and the most frequently mutated genes in AML are known. With this increased knowledge important questions arise, for example, are there specific mutations that define unique subgroups that may be incorporated in the WHO classification or in the genetic stratification of the European LeukemiaNet (ELN). Mutations in the *RUNX1* gene (*RUNX1*^{mut}) have been identified as recurrent mutations in myeloid malignancies, such as myelodysplastic syndrome (MDS) and AML, and they have been associated with distinct clinical characteristics and poor outcome.
Aims: To evaluate the genetic and clinical characteristics of AML with *RUNX1*^{mut} in a large cohort of adult AML patients (pts; 16-84 years of age) who were entered on four consecutive AMLSG treatment protocols [AML HD98A (n=804; NCT00146120), AMLSG 07-04 (n=885; NCT00151242), AML HD98B (n=307), AMLSG 06-04 (n=443)].
Methods: *RUNX1*^{mut} screening was performed in 2.439 AML pts (*de novo* AML, n=2.114; secondary AML [s-AML], n=157; therapy-related AML [t-AML], n=143; unknown, n=25) using a DNA-based PCR assay covering exons 1 to 8 followed by direct sequencing.
Results: Overall, 280 *RUNX1*^{mut}, were found in 245 of the 2.439 pts (10.1%). Mutations clustered in exon 4 and 8, but were also found in other exons. Clinically, *RUNX1*^{mut} were associated with higher age (p<0.0001), male gender (p=0.015), s-AML (p<0.0001), higher platelet counts (p=0.007), lower LDH levels (p<0.0001), and FAB morphology (FAB M0, p=0.046). *RUNX1*^{mut} were almost mutually exclusive of the primary recurrent genetic abnormalities as listed in the 2008 WHO classification. *RUNX1*^{mut} were mainly found in the European LeukemiaNet (ELN) intermediate-I (47.6%) and intermediate-II (30.7%) genetic groups; specific cytogenetic associations were found with +13 (p=0.001) and del(7q) (p=0.041). With respect to cooperating mutations, *RUNX1*^{mut} significantly co-occurred with mutations in *ASXL1* (p<0.0001), *MLL* (partial tandem duplications [PTD]; p<0.001), and *IDH2* (p=0.023). Following induction therapy, AML with *RUNX1*^{mut} were associated with a lower complete remission rate (48.4% *vs* 68.1%; p<0.0001) due to a higher rate of refractory disease (40.6% *vs* 23.4%; p<0.0001) as compared to AML with *RUNX1*^{wt}. Univariable analysis revealed *RUNX1*^{mut} to be associated with inferior event-free (EFS, p<0.001), relapse-free (RFS, p<0.001), and overall survival (OS, p<0.001); in general, this negative prognostic impact was seen for pts 18-60 years of age (OS, p=0.001; RFS, p=0.007; EFS, p<0.001) and for pts above 60 years (EFS, p<0.001; OS, p=0.09). In multivariable analysis, *RUNX1*^{mut} was an independent prognostic marker for inferior EFS (p=0.001) and in trend for a worse OS (p=0.097).
Summary and Conclusions: *RUNX1*^{mut} are among the most frequent recurrent gene mutations in AML. They are mutually exclusive of the recurrent primary genetic abnormalities and cooperate with mutations in particular affecting epigenetic modifiers. *RUNX1*^{mut} are associated with distinct clinical features such as older age, prior MDS, and more immature phenotype, and poor outcome. Due to these unique characteristics, *RUNX1*^{mut} may be considered as a clinico-pathologic entity of AML.

Acute lymphoblastic leukemia - Clinical 1

S722

OPEN-LABEL, SINGLE-ARM, MULTICENTER CONFIRMATORY PHASE 2 STUDY OF THE BITE® ANTIBODY BLINATUMOMAB IN PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Blinatumomab is an investigational bispecific T-cell engager (BiTE®) antibody that directs T-cells to $CD19^+$ cells, resulting in serial lysis of B cells. An exploratory phase 2 study of blinatumomab in adult patients with relapsed/refractory B-precursor ALL (r/r ALL) previously demonstrated antileukemia activity (Topp MS, *et al.* Blood. 2012;120:abstract 670).
Aims: The objective of this large multicenter phase 2 study was to evaluate the efficacy and toxicity of blinatumomab as a single-agent treatment for adult patients with r/r ALL.
Methods: Eligible patients (≥18 years) had Ph-negative r/r ALL and any of the following characteristics: patients were primary refractory (induction failure), had experienced their first relapse within 12 months of first remission, had relapsed within 12 months of allogeneic hematopoietic stem cell transplantation (HSCT), or received blinatumomab as ≥2 salvage treatment. Patients with late first relapse (>12 months after first CR) were not eligible. Blinatumomab was administered by continuous IV infusion (4 weeks on/2 weeks off) for up to five cycles. During cycle 1, blinatumomab was administered at 9 µg/d on days 1 through 7, then at 28 µg/d on days 8 through 28. A constant dose of 28 µg/d was used for all subsequent cycles. The primary endpoint was complete remission (CR) or CR with partial hematologic recovery (CRh*) within the first two cycles.
Results: One hundred eighty-nine patients received blinatumomab; median (range) age was 39 (18–79) years. Median number of treatment cycles was 2 (1–5). Forty-three percent (95% CI, 36%–50%) of patients achieved CR/CRh*; 79% of these responses occurred within cycle 1. Within the first two cycles, 33% of patients achieved CR and 10% achieved CRh*. Among patients with prior allogeneic HSCT, 45% (29 of 64) achieved CR/CRh*. Among patients without prior allogeneic HSCT, CR/CRh* rates were 41% (12 of 29) for those who received blinatumomab as first early salvage, 49% (27 of 55) for those who received blinatumomab as second salvage, and 32% (13 of 41) for those who received blinatumomab as third or later salvage therapy. Median relapse-free survival was 5.9 months (95% CI, 4.8–8.3 months). Irrespective of causality, the most frequently occurring adverse events (AEs) were pyrexia (60%), headache (34%), and febrile neutropenia (28%). The most frequently occurring grade ≥3 AEs included febrile neutropenia (25%), neutropenia (16%), and anemia (14%). Grade ≥3 cytokine release syndrome occurred in 2% of patients. Headache (4%), encephalopathy (3%) and ataxia (2%) were the most frequently occurring grade ≥3 nervous system disorders. Three (2%) patients had grade 5 AEs (sepsis, n=2; candida infection, n=1) that were considered treatment-related.
Summary and Conclusions: This phase 2 study confirmed the antileukemia activity of blinatumomab as a single agent in an adult patient population with r/r ALL who had responded poorly to prior therapies.

S723

CLINICAL IMPACT OF ABL1 KINASE AND IKZF1 MUTATIONS IN ADULTS WITH PHILADELPHIA CHROMOSOME-POSITIVE (PH+) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): MOLECULAR ANALYSIS OF CALGB 10001 AND 9665 (ALLIANCE)

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Background: Despite improvement in outcomes with tyrosine kinase inhibitors (TKIs), a majority of adults with Ph+ ALL relapse and overall prognosis remains poor. Recent genomic studies in Ph+ ALL have identified oncogenic lesions that cooperate with *BCR-ABL1* to induce ALL as well as *ABL1* kinase domain mutations that confer resistance to TKIs. Here we sought to determine the incidence and clinical impact of these lesions in Ph+ ALL patients (pts) enrolled on CALGB 10001, a previously reported phase II study of imatinib and allogeneic (allo) or autologous (auto) stem cell transplantation (SCT), and companion Leukemia Tissue Bank protocol CALGB 9665.
Aims: One aim was to determine the incidence of *ABL1* kinase mutations at relapse and to monitor the kinetics of resistant clones. A second aim was to correlate oncogenic mutations in *IKZF1*, *CDKN2A/B*, and *PAX5* with clinical outcome and other biological variables, including *BCR/ABL1* fusion transcript (p190 *versus* p210).
Methods: Of the 58 pts enrolled on CALGB 10001, 22 pts relapsed, and 20 relapsed samples were evaluated for *ABL1* kinase mutations by direct sequencing and mutation-specific quantitative PCR (q-PCR) assays if a mutation was present. 28 pre-treatment samples from eligible pts were available for assessment of *IKZF1*, *CDKN2A/B*, and *PAX5* mutations using high-resolution SNP arrays and genomic DNA sequencing. Correlation with clinical outcome and other biological variables was estimated. Informed consent was obtained from all pts enrolled on CALGB 10001 and 9665.
Results: An *ABL1* kinase mutation was present at relapse in 13 out of 20 pts (65%) by direct sequencing. Using mutation-specific q-PCR, these mutations were detectable prior to relapse in 8 of 13 pts, and in 5 cases were present at diagnosis (Dx). All mutations were either P-loop (Y253H, E255K/V) or T315I, binding site mutations known to induce imatinib resistance. In 31 of 32 non-relapsed pts with available samples, no kinase mutations were detected at Dx using q-PCR. Kinase mutation at relapse was associated with worse disease-free survival (DFS; p=0.012; HR 4.54). In 28 available pre-treatment samples, 22 (78.6%) had an *IKZF1* deletion, 12 (42.9%) had a *CDKN2A/B* deletion, and 7 (25%) had a *PAX5* deletion. Among pts with *IKZF1* deletion, 17 of 22 (77.3%) had a p190 *BCR/ABL1* fusion transcript, which was associated with significantly better DFS compared to p210 in this subset (p<0.0001); see Figure 1. Adjusting for fusion transcript, *IKZF1* deletion was associated with worse DFS (p=0.011; HR 7.042). Median DFS in pts with *IKZF1* deletion was 13.2 months *versus* 53.1 months in pts without *IKZF1* deletion, although this difference did not reach statistical significance (p=0.18). In contrast to other reports, *CDKN2A/B* deletion did not affect survival in pts with *IKZF1* deletion. WBC level greater than 20,000 at Dx (p=0.021; HR 2.52) and minimal residual disease (MRD) at day 120 greater than 0.001 (p=0.013; HR 7.84) were associated with significantly worse DFS. No significant association was found between *IKZF1* deletion and presence of kinase mutation at relapse (p=0.18), *BCR/ABL1* fusion transcript type (p=0.31), WBC level at Dx (p=0.64), and MRD following induction (p=0.85) or at day 120 (p=0.77).

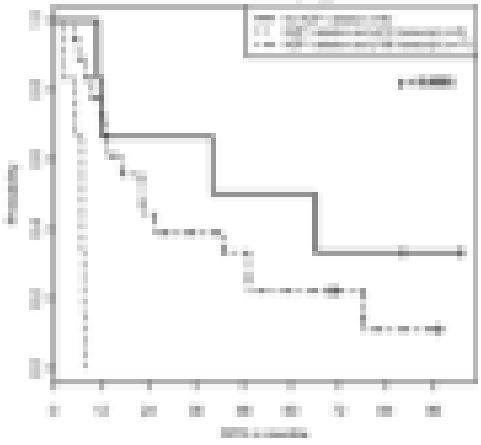

Figure 1. DFS stratified by IKZF1 deletion and BCR/ABL1 fusion transcript type.

Summary and Conclusions: *ABL1* kinase mutations conferring imatinib resistance were detectable at Dx or early during treatment in a majority (62%) of relapsed pts. Early identification using sensitive q-PCR may allow appropriate choice of TKI to avoid emergence of the resistant clone and relapse. *IKZF1* deletions, previously shown to have a pathogenic role in Ph+ ALL, were present in a majority of pts and associated with worse outcomes after adjusting for *BCR/ABL1* fusion transcript. Larger studies are needed to confirm these observations. Novel strategies to target *IKZF1* are being examined.

S724

PHASE II STUDY OF COMBINATION OF HYPERCVAD WITH PONATINIB IN FRONT LINE THERAPY OF PATIENTS (PTS) WITH PHILADELPHIA CHROMOSOME (PH) POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: Combination of cytotoxic chemotherapy with TKIs is effective in the treatment of Ph+ ALL. Ponatinib is a more potent BCR-ABL inhibitor. It also suppresses the T315I clones, a common cause of relapse in pts with Ph+ ALL.

Aims: The combination of chemotherapy and ponatinib may be associated with better response rates and higher likelihood of eradication of minimal residual disease (MRD) than those reported with imatinib or dasatinib and chemotherapy.

Methods: Pts with newly diagnosed Ph+ ALL received 8 cycles of hyperCVAD alternating with high dose MTX/cytarabine every 21 days. Ponatinib was given at 45 mg po daily for the first 14 days of cycle 1 then continuously for the subsequent cycles. Pts in CR received maintenance with ponatinib 45 mg po daily with vincristine/prednisone monthly for 2 yrs followed by ponatinib indefinitely. MRD monitoring was conducted.

Results: 34 pts with untreated Ph+ ALL and 3 pts previously treated (2 with 1 prior cycle of chemotherapy before Ph+/BCR-ABL status was known, not in CR, and 1 post HCVAD-dasatinib in CR) have received a median of 6 cycles (2-8) of therapy; 12 pts are receiving maintenance in CR. Median age was 51 years (27–75). Median WBC at diagnosis was $8.0x10^9$/L. All pts were in CR after cycle 1. 30 of the 32 pts (94%) with Ph+ metaphases by cytogenetic analysis at baseline achieved a CCyR after 1 cycle; 1 had a mCyR only and 1 had no cytogenetic analysis at CR, both of them achieved a CCyR after cycle 2; 5 had a diploid karyotype at the start of therapy (two in CCyR post previous chemotherapy and 3 diploid by standard G-banding technique and positive by FISH and PCR). To date, 35 pts (95%) have achieved a MMR, of whom 26 (70%) a CMR at a median of 10 weeks from initiation of treatment (2 -28). MRD assessment by flow cytometry is negative in 35/36 (97%) pts, in whom a sample was sent for MRD assessment, at a median of 3 weeks (3-14). Median time to neutrophil and platelet recovery for cycle 1 was 18 and 23 days, and 16 and 22 days for subsequent cycles, respectively. 8 pts have undergone allogeneic stem cell transplantation (ASCT) after a median of 4 courses (3-10). Grade ≥3 toxicity included infections during induction in 18 pts (49%), increased LFT's in 13 pts (35%), thrombotic events in 3 (8%, 1 renal vein thrombosis and 2 pulmonary emboli), myocardial infarction (MI) in 3 (8%, 2 unexplained, and 1 in the context of sepsis), skin rash in 4 (11%), and pancreatitis in 6 (16%). With a median follow up of 13 months (4-26), 31 pts are alive and in CR; 1 pt died in CR from an unrelated cardiac event after being taken off therapy and placed on imatinib, 1 pt died from MOF post sepsis (C2D13), 1 from non-ST elevation MI (NSTEMI) post cycle 2 (C2D41), 1 from potential MI at C4D42, 1 from head injury sustained after a fall at C4D13, and 1 from sepsis and MOF post ASCT. At the last follow-up, 7 pts (19%) are alive post ASCT; 15 pts (41%) are alive on ponatinib at 15 mg daily in 14 and 30 mg daily in 1; Of the other 9 alive pts, 7 were switched to dasatinib, one was switched to imatinib, and one is no longer receiving treatment. The 1-year PFS and OS rates were 100% and 86%, respectively.

Summary and Conclusions: The combination of hyperCVAD with ponatinib is highly effective in achieving molecular remissions in pts with Ph+ ALL. Due to the recent report of increased vascular events with ponatinib and occurrence of 2 MI on this trial, some pts switched to alternative TKI as noted above. In pts electing to stay on ponatinib, the dose was modified to 30 mg daily during consolidation with subsequent reduction to 15 mg in pts in CMR.

S725

ITALIAN GIMEMA 1308 PROTOCOL. TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN ADOLESCENTS AND YOUNG ADULT (AYA): INTENSIFICATION OF TREATMENT BASED ON THE PEDIATRIC AIEOP ALL 2000 PROTOCOL

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Background: Adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) have unique genetic, immunophenotypic and clinical features that differ from those of pediatric and older adults, and with outcomesthat fall somewhere between these two populations.In the last years, pediatric-inspired treatment approaches have improved the results in AYA.

Aims: The aim of the study is to test the efficacy and tolerability of a pediatric-inspired therapy in AYA and to compare the results with the historical GIMEMA ALL trials in patients with the same range of age. The study was registered at clinicaltrials.gov (NCT01156883).

Methods: The Italian GIMEMA 1308trial for the treatment of patients aged 18-35 years, with the exclusion of B-mature and Ph+ ALL, is derived from the childhood AIEOP ALL 2000 protocol. All patients receive a 7 day pre-phase with prednisone and 1 i.t. dose of Methotrexate (MTX), followed by induction IA and IB. From day 8, patients with T-ALL receive also dexamethasone.Minimal residual disease (MRD)by flow-cytometry (FC) and immunoglobulin/T-cell receptor gene rearrangement (Ig/TCR) is tested at time point 1 (TP1, end of IA) and2 (TP2, end of IB). Patients are stratified into two risk groups.Patients with at least one of the following criteria -t(4;11), prednisone-poor response (PPR),failure to achieve complete remission (CR) at TP1,Ig/TCR-MRD levels $\geq 10^{-3}$ at TP2 - are defined as high-risk (HR); all other are considered asstandard-risk (SR). Consolidation consists of 4 high-dose (HD)-MTX courses and of 3 blocks of non-cross resistant drugs for SR and HR, respectively. Reinduction II (IIA+IIB) follows consolidation for all patients.Standard maintenance isscheduled for a total of 24 months from diagnosis. Central nervous system (CNS)-directed therapy (i.t. MTX)is given during all treatment preceding cranial radiotherapy (CRT) for patients at HR and SR with T-ALL and a WBC>$100x10^9$/l or with CNS involvement at diagnosis. An allogeneic hematopoietic stem cell transplant (HSCT) from a matched family or unrelated donorisindicated for HR patients with t(4;11), not in CR at the end of IA or withIg/TCR-MRD levels$\geq 10^{-2}$at TP1 or $\geq 10^{-3}$ at TP2.

Results: All patients have given a signed informed consent.From September 2010 to January 2014, 66 patients have registered in the trial. Sixty-one are eligible and 54 are evaluable for steroid response: 42prednisone-good responders (PGR) and 12 PPR. Of the 50 patients evaluable for IA, 46 (92%) achieveda CR and 4 (8%) proved resistant; 46of the 47 patients (98%) evaluable for IB achieved a CR and 1 was resistant.Eleven severe adverse events (SAE) have been recorded: 8 with IA and 3 with IB.These include 6 Asparaginase-related adverse events and 5 infections.The median levels of FC-MRD at TP1 and TP2 are $10^{-4}$and 0; the median Ig/TCR-MRD levels at TP1 and TP2are 10^{-3} and 10^{-6}, respectively. Among the 46 patients in CR, 20 (43.5%) are classified as HR and 26 (56.5%) as SR. At 24 months, the overall survival (OS) for the entire group of patients is estimated to be 72.3% (95% CI 57.3–91). These results wereretrospectively compared with those achieved in AYA treated with the GIMEMA ALL 2000 and 0904trials.The CR rate (98%) is higher in the ongoing study compared to the previous ones(84% and 83%, respectively). At 24 months, the OS is 72.3, 61and 72%, in the 1308, 0904 and 2000 protocols.

Summary and Conclusions: The results presented suggest that the intensified protocol is effective and well tolerate in AYA; larger studies and a longer follow-up are necessary to conclusively confirm the role of intensified strategy on the clinical management of AYA.

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ENHANCED SURVIVAL IN ADULT PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH- ALL) WITH AN UPDATED PEDIATRIC-DERIVED MINIMAL RESIDUAL DISEASE (MRD)/RISK-SPECIFIC TREATMENT STRATEGY: NILG STUDY 10

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Background: The use of pediatric-derived therapeutic elements (PDT) in association with an MRD/risk-specific strategy could improve outcome of adult Ph-ALL.

Aims: To evaluate the results of a recent trial of the Northern Italy Leukemia Group (NILG 10/07).

Methods: PDT elements were added to a pilot study with an MRD/risk-based treatment design (ClinicalTrials.gov NCT-00795756). All patients signed an informed consent. PDT consisted of 3 modified BFM-type blocks and 3 lineage-targeted MTX blocks (LTM: B-precursor: 2.5 g/m^2; T-precursor: 5 g/m^2; age >55 years: 1.5 g/m^2) to obtain a MTX plasma concentration of ~35 and ~65 micromol/L in B- and T-ALL, respectively, according to St. Jude's Hospital studies. Central nervous system (CNS) prophylaxis compared standard intrathecal therapy with liposomal Ara-C, and MRD was evaluated molecularly at weeks (w) 4,10,16 and 22, to optimize risk stratification and indications for allogeneic stem cell transplantation (allo-SCT). The study sequence consisted of prephase (PDN/CY)/induction (IDA/VCR/L-Asp/DEX) and w4 MRD → PDT1 (CY/VCR/IDA/DEX/Ara-C/6MP) → LTM1/HD-Ara-C and w10 MRD → PDT2 → LTM2/L-Asp/6MP and w16 MRD → PDT3 → LTM3/HD-Ara-C and w22 MRD → reinduction (IDA/VCR/CY/PDN/DEX). Risk classes were standard (SR: non pro-B and WBC <30; cortical/CD1a+ T and WBC <100; CR cycle 1 and non-adverse cytogenetics) and high (HR). An early allo-SCT was prescribed to HR patients without molecular study, with highly adverse features (poor risk cytogenetics, WBC >100, early/mature T) or w10 MRD ≥10^{-4}; other MRD+ patients were eligible to post-consolidation allo-SCT or auto-SCT plus maintenance if allo-SCT was not feasible. Patients with w10 MRD <10^{-4} and negative w22

MRD were eligible to standard maintenance, and those lacking the MRD study were managed according to clinical risk class.
Results: Among 201 patients enroled from January '08 to August '12 (median age 41.4 years; range 17.8-67.7), 159 had Ph- ALL (117 B-ALL [SR 53%], 42 T-ALL [SR 26.2%]). CR rate was 97.6% in T- and 82.9% in B-ALL, due to greater toxicity in older patients; 65 patients eventually had a SCT (57 allo, 8 auto). Of 106 CR patients (76.8%) evaluable for MRD at w10 (as main prognostic timepoint), 77 reached a major MRD response $<10^{-4}$ (72%). With a maximum follow-up of 5+ years, projected overall survival (OS) at 4 years is 61% (101 survivors), 55% in B-ALL and 76% in T-ALL, respectively, and disease-free survival (DFS) 64% (89 survivors in CR1), 57% in B-ALL and 68% in T-ALL, respectively. DFS of 77 w10 MRD responders is 74% *vs.* 30.4% in 29 MRD-resistant (P<0.0001). Relapse rate was 26.8% (26/97) and 24.4% (10/41) in B- and T-ALL, respectively, whereas cumulative treatment mortality (TRM) was 17%, 4.7% (2/42) in T-ALL and 21.4% (25/117) in B-ALL (P=0.01), the latter correlating with age >60 (P=0.0001). Therefore, OS and DFS are 64.6% and 63.1% in patients aged 60 years and less *vs.* 35.5% and 41.6% in older ones (P=0.0001 and P=0.57).
Summary and Conclusions: This regimen yielded a CR rate close to 90% in unselected adults aged up to 65 years, obtaining an early MRD response of 72%. The associated toxicity mandated for a reduction of treatment intensity in older patients. Otherwise the PDT/LTM sequence proved feasible and the MRD/risk-oriented design achieved remarkable 4-year OS/DFS rates in patients aged 18-60 years, especially in T-ALL and w10 MRD responders, improving significantly over prior NILG data. Overall results remain suboptimal in MRD+ patients.

Bone marrow failure syndromes & PNH

S727

HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN SEVERE CONGENITAL NEUTROPENIA: DATA FROM THE EUROPEAN GROUP FOR BLOOD AND MARROW TRANSPLANTATION

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Background: The main indications to haematopoietic stem cell transplantation (HSCT) in patients affected with severe congenital neutropenia (SCN) are transformation to myelodysplasia or leukaemia (MDS/AL) and lack or partial response to Granulocyte Colony Stimulating Factor (G-CSF). Indication to HSCT in low or good responders to G-CSF is discussed because the experience in the field is limited.The EBMT data base offers an unique opportunity to address some unanswered questions in a large cohort.
Aims: To describe the outcome and risk factors of HSCT in a large cohort of SCN
Methods: All patients registered in the EBMT data base affected with SCN (early occurrence in life, block at promyelocyte stage, G-CSF dependence) were considered eligible to the study. Data regarding HSCT and outcome were derived from the "MED B EBMT form", while specific additional data regarding history before HSCT, were collected through a specific CRF (Med C) sent to all the participating centers.
Results: A total of 132 patients from 19 participating countries entered the study. Females were 49% of the cohort. Median age at diagnosis of neutropenia was 0.36 years (0-35-5yrs), while median age at first transplant was 4.5 years (range 0.2-43.1y). Eleven patients were affected with myelodysplasia (MDS) and acute leukemia(AL) at time of transplant. The cell source was bone marrow (BM) in 57%, peripheral blood (PB) in 25% and cord blood (CB) 18%. Fifty percent of patients were engrafted from a matched related and 50% from a matched unrelated donor. Conditioning regimen was myeloablative in 86% and reduced intensity (RIC) in 14% of the cohort. Engraftment was documented in 91% of subjects: 6% had primary graft failure and 3% lost the engraftment. Overall 24 patients (18%) died while the remaining 108 were alive (82%). Causes of death were: GVDH in 33% of patients, infections in 21%, organ failure in 17% and combination of previous causes in 21% and relapse/progression of the disease in 8%. Transplant related mortality occurred in 17% of the whole cohort. Acute GVDH grade 1-2 was documented in 31%, grade 3-4 in 15%. The 5-year OS and EFS (death,relapse,primary and secondary graft failure being the events) were 79% and 71% respectively. The 5-year OS according to donor type was 83% and 84% (p=0.76) for HLA identical family *vs* unrelated donor. Also the 5-year OS according to source of cells did not significantly differ (CB 92%, PB 65% and BM 82%p=0.12); the same held true also for 5-year EFS (CB 70% BM 79% and PB 54%p =0.068) even if it a worse trend was observed in PB recipients.The 5-years OS of patients affected with MDS/AL at HSCT, was not different to the one calculated in subjects without neoplastic transformation.Five year OS was significantly higher in patients transplanted before age

10 years (84% in age 0-5 yrs, 86% in age 5-10 yrs and 64% in age above 10 yrsp=0.035), The 5-years OS calculated according to time of HSCT was significantly better after year 2000(63% in patients transplanted before year 2000, 76% in subjects between 2001-2007 and 91% after year 2008 p=0.02).
Summary and Conclusions: This is the largest study ever conducted on a population of patients affected with SCN. It shows that the 5-year survival in transplanted SCN patients is close to 80% with no difference between matched related and unrelated donor. TRM, close to 17%, is still not negligible. Survival improved after year 2000 probably because of better donor typing techniques, but not for patients older than 10 years. Peripheral blood infusion seem to be less favourable than cord blood or marrow cells. MDS/AL at HSCT does not seem to influence the final outcome. Overall, this study provides the first solid evidence that HSCT in SCN, either from family or unrelated HLA matched donor, is a reasonably good rescue option in patients poorly responding to G-CSF or who transformed into MDS/AL.
On behalf of Severe Aplastic Anemia, Inborns Error and Pediatrics Diseases Working Parties.

S728

CHRONOLOGICAL ANALYSIS OF CLONAL EVOLUTION IN ACQUIRED APLASTIC ANEMIA

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Background: Acquired aplastic anemia (AA) is a prototype of idiopathic bone marrow failure, in which immune-mediated destruction of hematopoietic progenitors plays a central role but also characterized by frequent evolution to clonal myeloid disorders. Recently, we and other groups reported frequent somatic mutations in genes commonly mutated in myeloid malignancies, including *DNMT3A, ASXL1* and *BCOR*. However, the chronological behavior of the clonality and its link to the development of myeloid neoplasms has not been fully explored.
Aims: We aimed to define the clonality and its chronological behavior in acquired AA in terms of gene mutations and also investigate their pathogenetic link between myeloid malignancies.
Methods: We sought somatic mutations in a panel of 106 genes known mutational targets in myeloid neoplasms by whole exome sequencing (N=13) and/or targeted deep sequencing (N=178) of peripheral blood DNA from 188 patients with AA who had received immune-suppressive therapies (IST), using a SureSelect custom kit. Chronological alterations in variant allele frequencies of detected mutations were also investigated in 38 cases.
Results: In total, 84 high probability somatic mutations were detected in 33% (N=51) of 178 AA cases with varying variant allele frequencies, of which 10.8% of the cases harbored multiple mutations. The spectrum of mutations were substantially different from that observed in myeloid malignancies, such as acute myeloid leukemia (AML) and melodysplastic syndromes (MDS) and most frequently found in *DNMT3A* (12.1%), *BCOR* (4.3%), *PIGA* (3.3%), *ASXL1* (2.9%) and biased to nonsense (21%), frameshift (26%) and splice site changes (9%), supporting their driver roles. Mutations were associated with older age (p=0.0004), but not with response to treatment or progression to MDS/AML. Conspicuously, multiple independent mutations involving *DNMT3A,* and *ZRSR2* were identified in 2 cases, strongly suggesting their driver roles in clonal selection. Among 26 cases, in which serial samples are available for the targeted deep sequencing, 13 mutations were detected in 5 cases after IST. Nine out of the 13 mutations increased in their clone size and the remaining 4 maintained their clone size or decreased over time. None of the 5 cases developed MDS/AML at the time of the latest follow-up, even though mutations were found in common myeloid targets such as *TET2, ASXL1, DNMT3A, U2AF1, BCOR* and *SETBP1*. To further characterize clonal evolution in AA patients, we performed whole exome sequencing of multiple samples taken from 13 cases, for whom sequential samples (N=2~3) were available. The detected mutations were confirmed in all serial samples by deep sequencing. Somatic mutations were detected 8 out of the 13 cases at the time of diagnosis of AA and/or after IST with the mean number of mutations of 4.5 per specimen. Most of the mutations were present at the time of diagnosis and their allele frequencies increased during the clinical course, although some mutations in the diagnostic samples disappeared over time. Progression to MDS was confirmed in 5 cases, in which multiple round of acquisition of new mutations and subsequent clonal selection were indicated.
Summary and Conclusions: In AA, genes commonly mutated myeloid neoplasms were frequently mutated and were thought to be responsible for clonal evolution. The spectrum of mutations in AA was substantially different from that of MDS/AML, in which mutations in *PIGA, DNMT3A, ASXL1* and *BOCR* were predominant with rare mutations in *TET2, SF3B1* and other signaling pathway genes. Mutations were frequently detected prior to IST and increased their clone size over time after IST. Detection of clonality may or may not be associated with progression to MDS/AML, even though clonal evolution was evident, indicating complex pathogenesis of secondary MDS in AA.

S729

STRESS-INDUCED EXIT FROM QUIESCENCE INDUCES *DE NOVO* DNA DAMAGE IN HEMATOPOIETIC STEM CELLS AND RESULTS IN BONE MARROW FAILURE IN THE ABSENCE OF A FUNCTIONAL FANCONI ANEMIA SIGNALLING PATHWAY

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Background: Fanconi anemia (FA) patients harbour germ line inactivating mutations in an epistatic signalling pathway that mediates DNA repair, resulting in a highly penetrant bone marrow failure syndrome driven by an accelerated loss of hematopoietic stem cells (HSCs). Knockout mouse models of FA have exactly the same DNA repair defect as cells isolated from FA patients. However these mice never spontaneously develop aplastic anemia, leading us to speculate that laboratory mice are not subject to the same source of DNA damage as patients. Proliferative stress has been proposed to be a near universal source of DNA damage in somatic cells but HSC are known to predominantly reside in a state of long-term quiescence, which would preclude them from acquiring extensive DNA damage via this route. Recently, it has been recognised that murine HSC exit this quiescent state *in vivo* in response to stress stimuli such as infections or blood loss.
Aims: To test whether stress-induced exit from quiescence comprises a physiologic source of DNA damage in HSCs.
Methods: Wild type (WT) and *Fanca*$^{-/-}$ mice were injected with either the synthetic double stranded RNA mimetic polyI:polyC (pI:pC), to simulate viral infection and promote HSC proliferation, or a range of other pro-proliferative agonists (pI:pC; Interferon-α; G-CSF; TPO; and serial bleeding). DNA damage was evaluated by both enumeration of γ-H2AX foci and Comet assay.
Results: Treatment of WT mice with any of the individual stress-inducing agonists led to a robust induction of *in vivo* HSC cycle entry, resulting in a 3-5-fold induction of DNA damage in highly purified HSCs across all stimuli ($p<0.01$). On a mechanistic level, stress-induced HSC exit from quiescence resulted in elevated mitochondrial membrane potential (2-fold increase, $p<0.01$) and a 50% increase in 8-Oxo-dG lesions on DNA, suggesting that mitochondrial reactive oxygen species (ROS) directly precipitated DNA damage. Critically, retroviral overexpression of the ROS-detoxifying enzymes catalase and superoxide dismutase 2 completely rescued stress-induced DNA damage in HSCs *in vivo*. Analysis of the FA signalling pathway revealed a 4-fold induction in nuclear FANCD2 foci ($p<0.01$) in the HSCs of pI:pC-treated mice, consistent with this pathway being involved in the repair of stress-induced DNA damage. Indeed, treatment of *Fanca*$^{-/-}$ mice with pI:pC led to a 2-fold higher level of stress-induced DNA damage compared to WT HSCs ($p<0.05$). Four rounds of treatment with pI:pC led to a two- or four-fold depletion of transplantable HSC in WT or *Fanca*$^{-/-}$ mice, respectively ($p<0.01$). Further rounds of treatment led to the onset of severe aplastic anemia in all *Fanca*$^{-/-}$ mice but not in any of the WT pI:pC-treated controls nor the age matched PBS-treated *Fanca*$^{-/-}$ mice ($p<0.01$). Bone marrow failure (BMF) was characterized by profound neutropenia, thrombocytopenia and anemia and a >60% reduction in bone marrow cellularity corresponding to an almost complete depletion of all HSCs as well as multipotent and committed progenitors.
Summary and Conclusions: Our data strongly implicates stress-induced exit from quiescence as a cause of physiologic DNA damage in HSCs *in vivo* and provides a novel link between pro-inflammatory cytokines, DNA damage and BMF. Furthermore, we demonstrate that, contrary to previous suggestions, Fanconi knockout mouse models are likely to be useful for dissecting the etiology of this disease if they are subject to the same stress stimuli that FA patients are likely to encounter.

S730

TCIRG1 ASSOCIATED CONGENITAL NEUTROPENIA

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Background: Severe congenital neutropenia (SCN) is a rare hematopoietic disorder; the genetic basis for >30% of cases remains unknown. We have recently reported a five-generation SCN family of European descent with a novel sin-

gle nucleotide variant (SNV) R736S in *TCIRG1* which perfectly co-segregated between neutropenic and normal members. (Makaryan *et al.* Blood 2013;122:440)
Aims: To describe the clinical/ laboratory features of *TCIRG1* associated neutropenia and its underlying pathophysiological mechanisms.
Methods: We utilized clinical data, blood/ bone marrow samples, routine blood counts, histochemical staining, electron microscopy, immune-magnetic bead cell separation, FACS analysis, Western and Northern analysis for this study.
Results: The median blood neutrophil count (ANC) was $0.524x10^9$/L, range 0.074 to $1.1x10^9$/L for 13 affected members and $3.819x10^9$/L, range 2.343 to $6.5x10^9$/L, for 8 unaffected members. Classification by ANC levels showed a pattern of frequent and more severe infections and morbidity in members with ANC $<1.0x10^9$/L. Bone marrow aspirates from three affected members (none on G-CSF) showed only modest reductions in mature neutrophils. However, by electron microscopy there were major changes in cell cytoplasm (abnormal vacuoles), organelles (mitochondrial membrane disruptions) and cell membranes (loss of villous structures) consistent with apoptosis of myelocytes and more mature marrow neutrophils. Analysis by FACS showed increased annexin V staining of peripheral blood neutrophils and CD 33^+ marrow cells. *TCIRG1* encodes OC116, the vacuolar H+-ATPase a3 subunit, expressed in myeloid progenitors and essential for bone tissue homeostasis. Through alternative splicing and usage of an alternative initiation codon, this same gene also encodes another protein, TIRC7, which plays an important role for T-lymphocyte activation. Recent publications suggest additional splice variants expressed in various tissues with as yet poorly defined functions. To examine the molecular defect and its effect on cell functions, we used commercially available polyclonal antibodies and Western blot analysis of peripheral blood cells from affected and normal members to determine expression of TCIRG1 protein. We found that ~45kDa TCIRG1 product was down-regulated by 26%, 49%, and 35% in the 3 affected individuals, compared to 2 normal controls. An antibody against the TCIRG1 fragment 121aa-220aa, detects a ~120 kDa protein band with similar expression levels in affected individuals and healthy controls. Because of the known role of vacuolar H+-ATPase in acidification of intracellular organelles, we stained peripheral blood neutrophils and bone marrow myeloid progenitors with LysoSensor Green. There was no difference between the patients' and normal cells. We quantitatively examined the effect of mutant TCIRG1 on neutrophil oxidative burst using flow cytometry-based PMA stimulated DHR 123 assay. These studies show no abnormality.
Summary and Conclusions: We conclude that *TCIRG1* associated neutropenia is attributable to impaired neutrophil production due to apoptosis of developing neutrophils and their precursors in the bone marrow. Our findings also suggest that clinical problems or recurrent infection is due to defective production and not abnormal neutrophil function. Our current work is directed toward understanding the molecular and cellular mechanisms leading to intramedullary myeloid apoptosis and a search for molecularly directed therapies.

S731

CYTOGENETIC EVOLUTION AND CLONAL COMPOSITION: PROGNOSTIC IMPACT IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA FOLLOWING MDS

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Background: It is known for several years that the occurrence of cytogenetic evolution correlates with an adverse prognosis in patients with Myelodysplastic Syndromes (MDS). The phenomenon of clonal evolution can be observed either at diagnosis or at disease progression.
Aims: The main goal of the present study was to analyze the impact of clonal evolution and its modulation by clonal composition in patients with MDS and AML following MDS (s-AML).
Methods: 538 patients with MDS/s-AML and complete clinical data were included from a single institution. In 227 patients, sequential cytogenetic analyses were performed. Cytogenetic evolution, defined as gain of additional cytogenetic abnormalities, increase of the clone size or occurrence of additional clones was observed in 72 cases. Patients with clonal evolution were clustered according to the clonal composition (NN=no abnormal metaphases; NA: mosaic of normal and abnormal metaphases; AA: only abnormal metaphases). To allow a comparison, patients without cytogenetic evolution (n=466) were also classified according to these cytogenetic criteria. Both of the groups were analyzed in a univariate model regarding overall survival by the method of Kaplan- Meier.
Results: The median survival of patients in the group with cytogenetic evolution and NA and AA karyotypes was significantly reduced ($p<0.01$) in comparison to patients without cytogenetic evolution. Median survival for patients with cytogenetic evolution and NA- karyotype (n=43) was 18 months. The proof of AA-karyotypes and cytogenetic evolution is associated with a median survival of 6,6 (n=27) months only. Survival differences in pair-wise comparisons between patients with cytogenetic evolution with NA and AA cells and those without were also significant (Figure 1). Interestingly, patients with an early occurrence of clonal evolution (<365 days) show a considerably impaired prognosis (median survival 5.3 months) as compared to patients with a later appearance of the evolution (median survival 21.9 months; $p<0.01$).

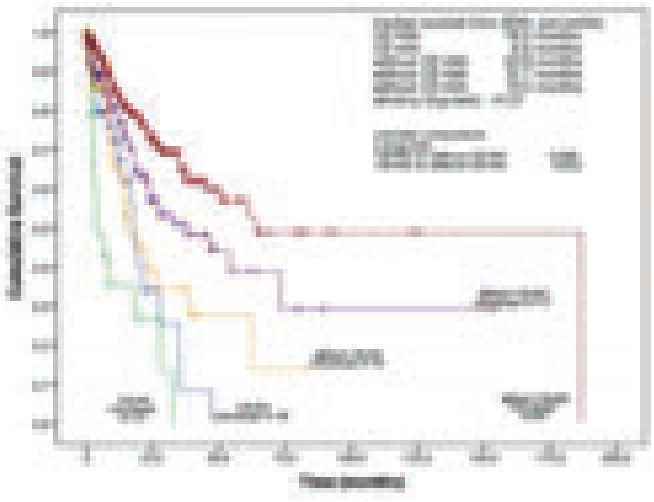

Figure 1.

Summary and Conclusions: Based on survival data of 538 patients, we present the prognostic impact of the clonal composition among patients with cytogenetic evolution and their worse outcome compared to those without cytogenetic evolution. Further data will be presented in detail.

Platelet disorders

S732

RESULTS FROM PETIT2 (TRA115450): A RANDOMIZED PLACEBO-CONTROLLED TRIAL OF ELTROMBOPAG TREATMENT IN PEDIATRIC PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: Eltrombopag (EPAG) is an oral non-peptide thrombopoietin-receptor agonist licensed for the treatment of thrombocytopenia in adults with chronic immune thrombocytopenia (cITP). In children with cITP, there is no agreed-upon standard treatment, and the risks of long-term consequences from current treatments are unacceptable to many families and physicians.
Aims: This phase III study was conducted to confirm the efficacy, safety, and tolerability of EPAG in children with cITP. The primary objective was to assess the efficacy of EPAG relative to placebo (PBO) in achieving a consistent platelet response defined as counts ≥50 Gi/L (without rescue) for ≥6 out of 8 weeks between weeks 5 and 12 when administered to pediatric subjects with insufficient response to prior ITP treatment.
Methods: Subjects were aged between 1 and <18 years with a confirmed diagnosis of cITP (duration >12 months) and platelet count <30 Gi/L at day 1. Informed consent (and assent as appropriate) was provided. Subjects were stratified as: 12-17 years (Cohort 1), 6-11 years (Cohort 2), and 1-5 years (Cohort 3). Part 1 was a 13-week, double-blind (DB), 2:1 randomization to EPAG or PBO. Subjects could continue stable baseline ITP medications. DB treatment was unblinded at week 13 then subjects began 24 weeks of open-label (OL) treatment with EPAG. Subjects aged 6-17 years weighing ≥27 kg started treatment at 50 mg, and those weighing <27 kg at 37.5 mg. Subjects aged 1-5 years started treatment at 1.2 mg/kg. Dosing at all ages was reduced for East Asian subjects. Dose was adjusted based on platelet counts to a maximum of 75 mg daily.

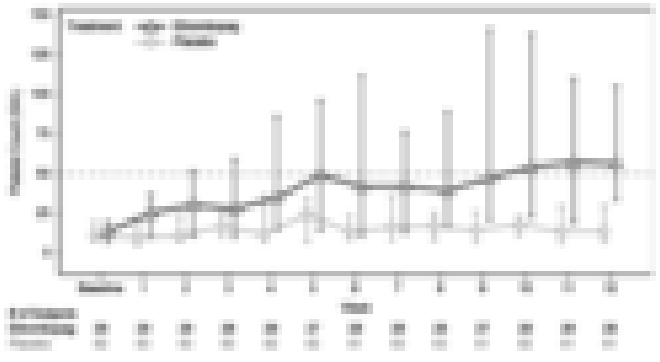

Figure 1. Median (IQR) platelet counts during double-blind treatment in part 1.

Results: 92 subjects were enrolled: 33 to Cohort 1, 39 Cohort 2, and 20 Cohort 3. 63 and 29 subjects were randomized to EPAG and PBO, respectively. 3.4% of subjects on PBO entered the study on ITP treatment compared to 20.6% on EPAG; 6.3% on EPAG had previously undergone splenectomy compared to none on PBO. Dosing regimens: During the DB phase, 63.5% of subjects on EPAG required ≥2 dose adjustments. The median daily dose during the OL phase was 67.7 mg for Cohort 1, 56.9 mg for Cohort 2, and 42.8 mg for Cohort 3. Efficacy: 39.7% of subjects on EPAG met the primary endpoint of consistent response compared to 3.4% on PBO (P<0.001). Response rates were similar across age cohorts at 39% for Cohort 1, 42% Cohort 2, and 36% Cohort 3. 74.6% of subjects on EPAG achieved platelet counts ≥50 Gi/L at least once during the first 12 weeks compared to 20.7% on PBO (P<0.001) (Figure 1). Clinically significant bleeding (World Health Organization grade 2-4) was present at baseline in 28.6% on EPAG and 13.8% on PBO compared to 4.8% at the end of DB phase on EPAG and 6.9% on PBO. In the OL phase, 80.5% of subjects achieved platelet counts ≥50 Gi/L at least once during 24 weeks. Safety: The most common adverse events (AEs) that occurred more frequently on EPAG than PBO included nasopharyngitis, rhinitis, cough, and upper respiratory tract infection. Grade 3/4 AEs occurred in 12.7% of EPAG and 10.3% of PBO subjects. Serious AEs were reported in 8% of subjects on EPAG compared to 14% on PBO. In Part 1, 2 subjects (3%) on EPAG discontinued treatment due to AEs of increased liver transaminases, compared to 1 subject (3%) on PBO who discontinued due to bleeding. Safety in the OL phase was consistent with that in the DB phase. No deaths were reported.
Summary and Conclusions: PETIT2 met its primary endpoint, demonstrating that a consistent response to EPAG can be achieved in children with cITP. There were no new safety concerns and few discontinuations due to AEs.

S733

ELTROMBOPAG TREATMENT OF CHILDHOOD PERSISTENT AND CHRONIC IMMUNE THROMBOCYTOPENIA: FINAL RESULTS OF THE PETIT STUDY (TRA108062), A PHASE 2, PLACEBO-CONTROLLED CLINICAL TRIAL

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Background: Eltrombopag (EPAG), an oral, non-peptide, thrombopoietin-receptor agonist, is approved for treating thrombocytopenia in adults with chronic immune thrombocytopenia (ITP) with insufficient response to prior therapy. However, there is no data describing the role of EPAG in pediatric ITP.
Aims: PETIT was a placebo-controlled, randomized, double-blind (DB) trial in 3 parts, designed to investigate the efficacy and safety of EPAG in subjects with persistent and chronic ITP and platelets <30 Gi/L. The primary objective was the proportion achieving platelet counts ≥50 Gi/L at least once (without rescue) between days 8 and 43 during the DB part of the study.
Methods: Subjects with ITP of >6 months duration and a platelet count <30 Gi/L who received ≥1 prior treatment were enrolled. Informed consent (and assent as appropriate) was provided. In Part 1, subjects were enrolled in an open-label (OL) dose-finding phase (Cohorts 1, 2, and 3 were aged 12-17, 6-11, and 1-5 years, respectively).[1] In Part 2, DB phase, subjects not participating in Part 1 were randomized 2:1 to EPAG:placebo (PBO) and stratified by age (Cohorts 1-3). After 7 weeks of treatment in Part 2, subjects entered Part 3 where EPAG and PBO subjects received 17 and 24 weeks of OL EPAG, respectively.[1]
Results: 45 and 22 subjects were randomized to EPAG and PBO, respectively. At baseline, 14.9% of subjects had persistent ITP and 85.1% had chronic ITP. 11.1% of EPAG and none of the PBO subjects were splenectomized. The primary endpoint was met by 62.2% and 31.8% of EPAG and PBO subjects, respectively (odds ratio 4.31; 95% CI: 1.4, 13.3; P=0.011). A higher proportion of EPAG-treated subjects responded at each week compared to PBO (Figure 1). Responses in individual cohorts were consistent with the overall response. Secondary endpoints achieved with EPAG and PBO, respectively, included reduced grade 2-4 bleeding on the World Health Organization scale (27% *vs* 59%) and less need for rescue (13.3% *vs* 50%). Platelet response in Part 3 was consistent with Part 2. Median dose in the OL phase was 64.25 mg, 59.05 mg, and 34.0 mg of EPAG daily for Cohorts 1, 2 and 3, respectively. The average of the maximum daily dosing in the OL phase was highest in the youngest cohort (Cohort 1, 1.2 mg/kg; Cohort 2, 1.9 mg/kg; Cohort 3, 2.9 mg/kg). The most common adverse events (AEs) in the EPAG *vs* PBO group, respectively, were headache (29.5% *vs* 42.9%), upper respiratory tract infection (15.9% *vs* 9.5%), and diarrhea (15.9% *vs* 4.8%). No subjects withdrew during Part 2 due to AEs. Grade 3/4 events occurred in 11% and 19% of EPAG and PBO subjects, respectively. Grade 4 events, occurring in similar proportions in each group (4.5% EPAG *vs* 4.7% PBO), included neutropenia and febrile neutropenia in 1 (2.3%) EPAG subject each, and abdominal pain in 1 (4.7%) PBO subject. 15.9% of EPAG subjects and 38.1% of PBO subjects had any bleeding event. Serious AEs (SAEs) were similar in both groups (9.1% EPAG and 9.5% PBO). No fatal AEs occurred. 65 subjects entered Part 3. The majority of AEs in Part 3 were consistent with Part 2. Two subjects withdrew due to Grade 3 increased alanine aminotransferase (ALT) (1 was a SAE), and 1 additional subject had ALT ≥3× ULN. These events resolved.

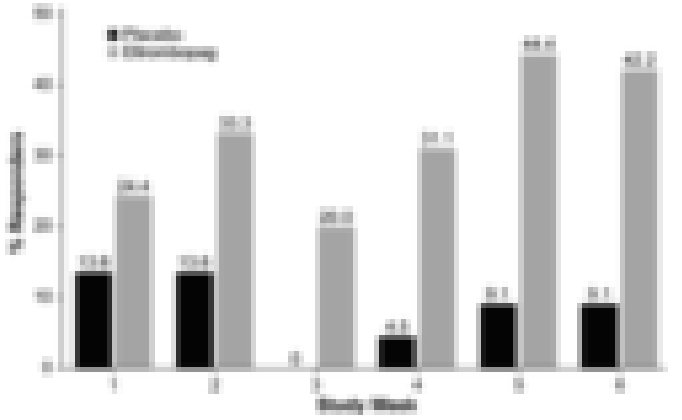

Figure 1. Summary of percent responders (platelet count ≥50K without rescue) during the double-blind period.

Summary and Conclusions: EPAG was an effective treatment in subjects with persistent and chronic ITP. Treatment with EPAG was well tolerated through the study as evidenced by the low incidence of SAEs, Grade 3/4 events, and withdrawals. No new safety concerns were identified.

Reference

1 Bussel JB *et al.* EHA 17th Congress. 2012: abs 501.

S734

GFI1B MUTATION CAUSES A NOVEL HUMAN PLATELET DEFECT WITH COMPLEX CHANGES OBSERVED IN ALPHA-GRANULES AND MITOCHONDRIA ASSOCIATED WITH ALTERED EXPRESSION OF PLATELET PROTEINS

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Background: Mutation of the human transcription factor GFI1B causes macrothrombocytopenia with variable platelet alpha-granule deficiency and red cell shape change.
Aims: Study of a four generation pedigree with an autosomal dominant bleeding disorder was conducted to identify the causative mutation and characterise the functional platelet defect.
Methods: Genetic linkage analysis followed by massively parallel sequencing was conducted to identify the mutation. Functional studies were then performed in primary human platelets and megakaryocytic cell lines.
Results: Bleeding scores for affected individuals were increased and most affected patients experienced excessive bleeding after surgery. PFA100 closure times were prolonged in affected family members and all affected individuals demonstrated markedly impaired platelet aggregation responses to collagen. Genotyping with a SNP array followed by massively parallel sequencing on telomeric chromosome 9 identified a single nucleotide insertion in exon 7 of GFI1B leading to a frameshift mutation. This mutation disrupts the DNA-binding region of the fifth zinc finger domain. The identified mutation in GFI1B alters the transcriptional function of the protein in a dominant negative manner with the introduction of mutant transcript de-repressing the promoter of the validated GFI1B target gene TGFBR3 and GFI1B itself in megakaryocytic cell lines as measured by a luciferase assay (TGFBR3 16.6 *vs* 23.8, P=0.03; GFI1B 0.73 *vs* 2.24, P<0.01). RNAseq performed on platelets from affected (n=3) and unaffected (n=3) members of this family showed transcriptional changes with 179 transcripts significantly increased and 84 transcripts significantly decreased (P<0.01). CD34 transcript was increased in affected platelets and this was associated with increased CD34 expression on platelets by flow cytometry. Transcripts related to mitochondrial function were significantly increased in the affected cohort and this was validated by morphological analysis by electron microscopy showing an absolute increase in mitochondrial number in GFI1B mutant platelets (3.0 *vs* 2.2, P<0.01). Marked reductions in expression of P-selectin and fibrinogen observed in mutant platelets were not associated with transcriptional changes as measured by RNAseq suggesting that these protein levels are altered via the relative deficiency of alpha-granules observed in GFI1B mutant platelets.
Summary and Conclusions: GFI1B is predicted to bind to more than 2000 gene promoters in haematopoietic cells. RNAseq predicts some protein changes observed in GFI1B mutant platelets whereas other platelet protein changes are secondary to the deficiency of alpha-granules. GFI1B mutation produces a novel human platelet defect with complex changes observed in granule and mitochondrial content associated with altered expression of platelet proteins.

S735

OUTCOMES OF 339 PREGNANCIES IN 181 WOMEN SUFFERING FROM 13 DIFFERENT FORMS OF INHERITED THROMBOCYTROPENIA: A RETROSPECTIVE AND MULTICENTRIC STUDY

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Background: Partum-related hemorrhage is the leading cause of morbidity and mortality among pregnant women resulting in more than a thousand deaths/year both in low and high resource countries. In women with inherited thrombocytopenias (ITs), bleedings are expected to be even more frequent and severe because of the association of thrombocytopenia and possible defects of platelet function. However, medical management of this condition cannot be based on evidence because of the lack of consistent information in the literature.
Aims: Gain information on maternal and neonatal bleeding risk in different forms of IT.
Methods: 181 women with 13 different forms of IT confirmed by genetic analysis were enrolled in this study carried out on behalf of the European Hematology Association-Scientific Working Group on Thrombocytopenias and Platelet Function Disorders: data from a total of 339 pregnancies and 156 IT newborns were collected from 45 institutions worldwide. Bleedings at delivery were defined as "excessive bleeding requiring blood transfusion" (EBBT), based on transfusion of platelets and/or red blood cells during or after delivery to treat bleeding, and "all excessive bleedings" (AEB) based on transfusion of blood products or the judgment of the treating physician that blood loss was larger than normal.
Results: Thrombocytopenia and bleeding tendency in the mothers did not worsen during pregnancy. The course of gestation was uneventful in 304 cases while miscarriages and preterm births occurred with a frequency similar to that of healthy women (10.1% and 9.9%, respectively). Comparison of platelet counts in newborns affected by IT with those in their mothers revealed that the degree of thrombocytopenia was similar. Only 5 IT newborns experienced minor bleeding diathesis, while 2 newborns, both born by vaginal delivery to *MYH9*-RD mothers, experienced a fatal cerebral hemorrhage. Prophylactic platelet transfusions were given in preparation for delivery in 46 of 301 evaluable cases, while other prophylactic treatments were given in 17 cases. One hundred and sixteen of 303 births were by cesarean section. General anesthesia was performed in 27.7% of cases and spinal or epidural anesthesia in 15.9%. No bleeding complications related to these procedures were reported. EBBT and AEB occurred in 6.8% of deliveries (CI 4.17-10.2) and 14.2% of deliveries (CI 10.4-18.7), respectively, with a frequency much higher than in general population. However, no women died from complications of childbirth or required hysterectomy to stop bleeding. EBBT was not less frequent in vaginal deliveries than in cesarean sections and was not reduced in women who

received prophylactic platelet transfusions prior to childbirth. The latter observation may be explained by the lower platelet count in women given platelet transfusions. EBBT at delivery correlated significantly with a history of grade 3 or 4 (OR 5.32, CI 1.22-23.11) and grade 4 (OR 24.50, CI 4.75-126.41) of WHO bleeding scale. ROC analysis identified the value of $50x10^9$ platelets/L as the optimal cut-off of platelet count for the identification of patients with a higher risk for EBBT (OR 7.61, CI 1.55-37.60). Similar results were obtained for AEB.
Summary and Conclusions: Delivery-related bleeding risk was higher in subjects with ITs than in healthy population for both the mothers and the affected newborns. However, childbirth was free from bleeding complications in the vast majority of mothers and neonates. The degree of thrombocytopenia and a history of severe bleeding tendency in the mother have been identified as useful parameters to predict the risk of delivery-related bleedings.

S736

PLATELET PHOSPHATIDYLSERINE EXTERNALIZATION MAY PLAY A CAUSATIVE ROLE IN IMMUNE THROMBOCYTOPENIA

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Background: Based on a mouse model, it has recently been shown that an intrinsic program for apoptosis controls platelet survival and dictates their lifespan. Specifically, pro-survival Bcl-xL counteracts the pro-apoptotic activity of Bak to maintain platelet survival, but as Bcl-xL degrades, aged platelets are primed for death. Thus, platelets are genetically programmed to die by apoptosis and the antagonistic balance between Bcl-xL and Bak constitutes a molecular clock that determines platelet life-span (Mason, 2007). In addition, data in a mouse model suggest that antibodies against platelet surface glycoproteins (GP) participate in the triggering of platelet apoptosis (Leytin, 2006). Interestingly, these mechanisms of regulation of platelet survival have never been characterized in patients with Immune Thrombocytopenia (ITP).
Aims: We aim to characterize whether in ITP: 1) abnormalities of the intrinsic pathway of apoptosis, specifically the Bcl-xL/Bak system, may play a pathogenetic role; 2) antiplatelet antibodies participate in the triggering of platelet apoptosis.
Methods: We studied 30 patients with active chronic ITP. Apoptosis together with the expression of Bcl-xL and Bak proteins and cytochrome-c release have been characterized at flow cytometry on freshly isolated and *in vitro* aged platelets of healthy subjects and ITP patients. A flow cytometry analysis of apoptosis induced by ABT-737, a selective Bcl-xL-inhibitor, was also investigated. Platelet apoptosis has also been characterized after incubation of normal platelets with platelet poor plasma (PPP) from antiplatelet antibodies-positive (Ab+) or antibodies-negative (Ab-) ITP patients or from healthy subjects. In addition, *in vitro* studies with monoclonal antibodies (MoAb) against platelet GPIIb (CD41) and GPIb (CD42b) have been also performed.
Results: We demonstrate that platelets from ITP patients, freshly isolated ($p<0.05$) and *in vitro* aged ($p<0.02$) in the presence of autologous plasma, show higher phosphatidylserine (PS) exposure in comparison to their normal counterparts. The MIF value of Bcl-xL protein in fresh platelets was similar between patients and controls. However, it was significantly reduced in *in vitro* aged platelets of patients as compared to that of healthy subjects ($p<0.04$). By contrast, we found no significant differences in Bak expression between patients and controls or between aged and fresh platelets. *In vitro* aged platelets from ITP patients showed significantly reduced expression of cytochrome-c ($p<0.02$). Pharmacological inhibition of Bcl-xL by ABT-737 induced a dose-dependent increase in PS exposure, which was significantly reduced in treated platelets from patients as compared to their normal counterparts ($p<0.02$). Healthy platelets incubated with PPP from antiplatelet Ab+ patients showed a significant increase in PS exposure compared with those incubated with PPP from antiplatelet Ab- patients or normal subjects ($p<0.03$). Same results were obtained when platelets from patients or healthy subjects were incubated *in vitro* with the MoAb anti-CD42b ($p<0.01$). At the opposite, PS exposure did not change significantly after incubation with the anti-CD41 MoAb.
Summary and Conclusions: Our results demonstrate that a deregulated intrinsically programmed cell death is the hallmark of *in vitro* aged platelets from ITP patients and suggest that it may play a role in the pathogenesis of the disease.

Hematopoietic disorders and transfusion

S737

25 YEAR-UPDATE ON THE LONG-TERM SAFETY OF TREATMENT WITH RECOMBINANT HUMAN GRANULOCYTE-COLONY STIMULATING FACTOR IN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIAS IN EUROPE

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Background: Congenital neutropenias (CN) include a heterogenous group of diseases characterized by a decrease in circulating neutrophils and different underlying germline gene mutations. Since 1988 recombinant human G-CSF (Filgrastim, Lenograstim) is available for the treatment of severe chronic neutropenia patients. In phase I/II/III studies in patients with severe congenital and cyclic neutropenia, treatment with G-CSF (Filgrastim) resulted in a rise in the absolute neutrophil counts (ANC) and a reduction in infections.
Aims: As an update on the long-term safety of subcutaneous G-CSF administration we now report on 443 neutropenia patients (congenital n=366, cyclic n=77) evaluated over the past 25 years by the European Branch of the Severe Chronic Neutropenia International Registry (SCNIR), Hannover, Germany, on behalf of the Local Liaison Physicians in 22 European countries.
Results: 368 of 443 patients (median age 13 years) receive long-term daily subcutaneous G-CSF treatment for a median duration of 7 years (maximum 24,7 years). Our cohort includes even 20 patients receiving G-CSF treatment for more than 20 years. A sustained ANC response was seen in the majority of severe congenital neutropenia and cyclic neutropenia patients documenting that there is no exhaustion of myelopoiesis in the bone marrow and no immune reaction with neutralizing antibody production. Eighteen patients completely failed to respond to G-CSF treatment and required immediate stem cell transplantation. Median G-CSF doses vary by neutropenia subtype and gene mutation. Up to now more than ten different germline mutations were identified (*ELANE, HAX1, G6PC3*, etc.) which can cause congenital neutropenias. Patients with congenital neutropenia revealing *ELANE* mutations require the highest G-CSF doses compared to other subtypes (median G-CSF dose 5 µg/kg/day in 88 patients). Four patients (including two patients with Shwachman-Diamond syndrome) developed pancytopenia under G-CSF and also received SCT. Significant adverse events noted which may or may not be related to therapy included: osteopenia, splenomegaly, vasculitis, glomerulonephritis, BM fibrosis and MDS/leukaemia. Independent of the genetic subtype patients with congenital neutropenia have a risk of more than 10% to develop leukemia which documents that CN is a preleukemic syndrome. There is an association of G-CSF dose with the relative hazard of MDS/AML documenting the correlation between the severity of the underlying disease as judged by the requirement of the G-CSF dose and the risk for malignant transformation. Recently we identified an unique pathway of leukemogenesis, namely that acquired *CSF3R* mutations followed by mutations in the gene for the transcription factor *RUNX1* are present in the majority of patients with secondary leukemias (Skokowa, *et al.*, Blood 2014). SCT is the only treatment of choice to achieve long-term remission in these patients. None of the 77 patients with cyclic neutropenia developed leukemia documenting that G-CSF treatment by itself does not cause leukemias.
Summary and Conclusions: In summary, a significant decrease in the incidence of severe infections and the need for intravenous antibiotics was noted in these patients. G-CSF treatment has been well tolerated in the majority of patients and resulted in a long-term improvement in their clinical status, prolonged survival, and improved the quality of life. As a proof of the benefit of G-CSF treatment a total 109 of the 363 congenital and 38 of the 77 cyclic neutropenia patients have meanwhile reached adulthood, including 16 women and 8 men who became parent. The long-term safety of G-CSF has been well documented by this 25-year follow-up study.

S738

LOSS-OF-FUNCTION COMPOUND HETEROZYGOUS MUTATIONS IN THE CSF3R GENE IN A PATIENT WITH SEVERE CONGENITAL NEUTROPENIA: SUCCESSFUL TREATMENT WITH GM-CSF

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Background: Previously we have presented a clinical case of 10 year old female severe congenital neutropenia patient who did not respond to the treatment with G-CSF (Filgrastim) in a dose up to 100 µg/kg/day. Since the birth this patient has severe neutropenia with reduced myelopoiesis in the bone marrow (BM) without maturation arrest at promyelocytes/myelocytes stage. No mutations in *ELANE*, *HAX1,* and *G6PC3* genes were detected and no involvement of other organs was documented. At age of 7 months treatment with GM-CSF (4 µg/kg/week) was applied and patient responded with ANC between 1000/µl and 2000/µl. At age of 6 years therapy was replaced by G-CSF with no response. CFU assay of BM MNC from this patient showed that G-CSF was not able to induce CFU-G, CFU-M and CFU-GM colonies due to diminished surface expression levels of G-CSFR and abrogated G-CSFR-triggered intracellular signaling pathways (e. g. phErk1/2, phSTAT5). In sharp contrast, bone marrow progenitors treated *in vitro* with 10ng/ml GM-CSF differentiated to all type of CFU colonies.
Aims: To search the reasons of the G-CSF unresponsiveness, we sequenced the exons of the *CSF3R* gene and identified inherited heterozygous stop-codon mutation p.W547* (NP_000751), C1641T (NM_000760) in the extracellular part of the G-CSFR protein within the fifth fibronectin type III domain. Since heterozygous *CSF3R* mutation does not explain completely unresponsiveness to G-CSF therapy, we aimed to identify additional abnormalities in G-CSFR by sequencing the whole *CSF3R* gene including introns and 3`- and 5`-UTRs by means of SOLID 5500xl. To evaluate the intracellular signaling pathways required for GM-CSF-triggered neutrophilic granulopoiesis in the absence of G-CSF signaling, we performed gene expression microarray analysis using RNA isolated from $CD33^+$ BM myeloid cells of affected individual in comparison to the cells of G-CSF- treated CN patients.
Methods: Next generationg sequencing, Sanger sequencing, PCR, RT-PCR, gene expression microarray analysis, real-time PCR, FACS, CFU assay.
Results: We identified a second mutation at the 3′ splice-acceptor site of intron 8 of the *CSF3R* gene. Sanger sequencing of DNA from the patient's parents confirmed that both *CSF3R* mutations are inherited: the non-sense mutation was inherited from the father, whereas the mutation at 3′ splice-acceptor site was detected in mother's DNA. Amplification of CSF3R RNA from patient's blood sample revealed the presence of the smaller abnormal *CSF3R* transcript additionally to the full-length transcript. The abnormal transcript appears presumably due to exon 8 skipping what is one of the most common consequences of the splice site mutations. We analyzed the microarray data by Ingenuity Systems Pathway Analysis (IPA). Intriguingly, neutrophil differentiation upon GM-CSF treatment was associated with highly upregulated expression (more than 3 fold) of neutrophil granule proteins (MPO, ELANE, CTSG, DEFA4, LCN2, OLFM4, etc.) and transcription factor C/EBPε in $CD33^+$ bone marrow myeloid cells, as compared to the cells of G-CSF-treated CN patients. However, in contrast to CN patients, we found no upregulation of the activators of emergency pathway responsible for neutrophilic granulopoiesis (NAMPT and C/EBPß).
Summary and Conclusions: Taken together, we reported here a patient with compound heterozygous mutations in the *CSF3R* gene leading to the complete "loss-of-function" of G-CSFR protein. Since the majority of studies on the *CSF3R* mutations are targeting the cytoplasmic part of the receptor only, we would suggest that in CN patients who do not respond to G-CSF whole *CSF3R* gene should be tested. Therapeutic application of granulopoietic cytokines other then G-CSF (*e.g.* GM-CSF) to activate alternative mechanisms of granulopoiesis (*e.g.* via C/EBPε) in this group of patients should be considered.

S739

ELUCIDATION OF THE INTRACELLULAR DEFECTS LEADING TO THE ABROGATED GM-CSF-TRIGGERED GRANULOPOIESIS IN CN PATIENTS

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Background: G-CSF is successfully used to treat patients with severe congenital neutropenia (CN) but GM-CSF failed to increase neutrophil numbers in the majority of these patients (Welte, el al, Blood 1990). GM-CSF induces monocytosis and eosinophilia in CN patients to the levels comparable to GM-CSF treated healthy individuals. This suggests isolated defects in the GM-CSF downstream signaling responsive for neutrophilic granulopoiesis in CN.
Aims: We aimed to evaluate why GM-CSF-triggered neutrophil granulopoiesis is abrogated.
Methods: We performed *in vitro* differentiation of CD34+ hematopoietic cells, stimulated hematopoietic cells with GM-CSF and further evaluated downstream signaling by ELISA, qRT-PCR and GeneExpression microarrays from Affymetrix.
Results: Recently, we demonstrated severely diminished expression levels of LEF-1 and C/EBPa transcription factors in CN myeloid cells leading to abrogated "steady-state" neutrophil granulopoiesis (Skokowa, *et al.*, Nat Med 2006). After inhibition of LEF-1 in $CD34^+$ cells from healthy individuals by shRNA GM-CSF was not able to induce neutrophil granulopoiesis, but activated monocytopoiesis. Previously we found that in CN patients G-CSF induces C/EBPß-triggered "emergency" granulocytic differentiation by activation of NAMPT/SIRT1 signaling (Skokowa, *et al.*, Nat Med 2009). NAMPT is important for GM-CSF-triggered neutrophilic granulopoiesis: (1) GM-CSF induces NAMPT, SIRT1 and C/EBPß in hematopoietic cells of healthy individuals; (2) GM-CSF failed to induce CFU-G after pre-treatment of hematopoietic cells from healthy individuals with the specific NAMPT inhibitor FK866. GM-CSF only slightly induced expression of NAMPT, SIRT1 and C/EBPß in CN hematopoietic cells, in comparison to the pronounced effects of G-CSF. We concluded, that GM-CSF is not able to induce neutrophilic granulopoiesis in CN patients due to a lack of LEF-1/C/EBPα and its inability to induce NAMPT/SIRT1/C/EBPß.

To better understand the intracellular signaling pathways activated by GM-CSF/NAMPT we performed microarray studies. We compared gene expression signature of GM-CSF treated CD33+ myeloid progenitor cells of CN patients and healthy individuals. In one group of CD33+ cells of healthy individuals we additionally blocked NAMPT signaling by treatment of cells with FK866, to mimic the "CN-like phenotype". We selected a list of genes that were up- or downregulated in GM-CSF- treated CD33+ cells of healthy individuals but were not modified by GM-CSF in NAMPT-deficient healthy controls and in cells of CN patients. We assumed that these genes are normally activated by GM-CSF/NAMPT and could not be regulated by GM-CSF in CN patients due to a lack of NAMPT activation. We applied the obtained gene list for the Ingenuity Pathway Analysis (IPA) and found that the IL-13R signaling pathway was most significantly affected (activation z-score=0,333; p-value of overlap=0,0000546). GM-CSF regulated the components of IL-13R signaling (incl. ADAM28, ARNTL2, CD209, CLEC4A, CXCL5, CYBB, IL1B, IL1RN, SLC16A6) in healthy individuals, but failed to do it after inhibition of NAMPT and in cells of CN patients. To prove the link between NAMPT and IL-13 signaling, we activated NAMPT by treatment of healthy individuals with nicotinamide and measured IL-13 synthesis in plasma. Indeed, we found significant increased levels of IL-13 in plasma of treated individuals (p=0,003), suggesting activation of IL-13 by nicotinamide. Moreover, IL-13 downstream molecules mentioned above were regulated in CD33+ cells from healthy individuals treated with nicotinamide *in vitro*. The involvement of IL-13 in the GM-CSF/NAMPT triggered granulopoiesis is novel and interesting but is not fully understood yet.

Summary and Conclusions: Taken together, GM-CSF failed to induce granulopoiesis in CN patients due to its inability to activate NAMPT/C/EBPß-dependent "emergency granulopoiesis" and to regulate NAMPT/IL-13R signaling.

S740

COMPARISON OF TWO DOSE-REGIMENS OF PROTHROMBIN COMPLEX CONCENTRATES IN URGENT ANTICOAGULATION REVERSAL. A PROSPECTIVE, RANDOMISED STUDY.

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Background: Prothrombin Complex Concentrates (PCC) are haemostatic blood preparations indicated for urgent anticoagulation reversal, though the optimal dose for effective reversal is still under debate. The latest generations of PCC's include four coagulation factors, the socalled 4-factor PCC.

Aims: The aim of this study was to compare the efficacy and safety of two doses, 25 and 40 IU/kg, of 4-factor PCC in vitamin K antagonist (VKA) associated intracranial haemorrhage.

Methods: We performed a prospective, randomised study including patients with objectively diagnosed VKA-associated intracranial haemorrhage between February 2008 and April 2012. Patients were randomised to receive 25 or 40 IU/kg of 4-factor PCC. The primary endpoint was the International Normalised Ratio (INR) 10 minutes after the end of 4-factor PCC infusion. Secondary endpoints were changes in coagulation factors, global clinical outcomes and incidence of adverse events (AEs).

Results: A total of 58 patients were randomised: 28 in the 25 IU/kg and 30 in the 40 IU/kg group. Baseline demographics and clinical characteristics were comparable between the groups. The mean INR was significantly reduced to 1.2 and 1.5 in all patients of both groups -10 min after 4-factor PCC infusion. The INR in the 40 IU/kg group was significantly lower than in the 25 IU/kg group 10 min (p=0.001), 1 hour (p=0.001) and 3 hours (p=0.02) after infusion. The 40 IU/kg dose was also effective in replacing coagulation factors such as PT (p=0.038), FII (p=0.001), FX (p<0.001), protein C (p=0.002) and protein S (0.043), 10 min after infusion. However, no differences were found in haematoma volume or global clinical outcomes between the groups. Incidence of death and thrombotic events was similar between the groups.

Summary and Conclusions: Rapid infusion of both doses of 4-factor PCC achieved an INR of 1.5 or less in all patients with a lower INR observed in the 40 IU/kg group. No safety concerns were raised by the 40 IU/kg dose. Further trials are needed to evaluate the impact of the high dose of 4factor PCC on functional outcomes and mortality.

S741

TREATMENT OF REFRACTORY HYPERHEMOLYTIC TRANSFUSION REACTION IN HEMOGLOBINOPATHIES WITH CYCLOSPORIN

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Background: Hyperhemolytic transfusion reaction (HHTR) is a rare but potentially fatal complication of blood transfusion the distinguishing characteristic of which is severe hemolysis with a fall in hemoglobin (Hb) to below pre-transfusion level due to destruction of both transfused and recipient red cells and/or reticulocytopenia. First and most frequently reported in sickle cell disease HHTR has also been described in thalassemia disorders and patients without hemoglobinopathies. Proposed mechanisms include bystander haemolysis, suppression of erythropoiesis and destruction of red cells by activated macrophages. The mainstay of management in HHTR is avoidance of transfusion which exacerbates hemolysis with in severe cases steroids and high dose intravenous immunoglobulin (IVIG) +/- erythropoietin (EPO). If despite this further transfusion proves necessary IVIG and methylprednisolone (MP) may limit the risk of hemolysis. Cases of HHTR refractory to such measures pose a considerable challenge. Various treatment modalities including cyclophosphamide, rituximab, splenectomy, plasma exchange, and allogeneic hematopoietic stem cell transplantation have been advocated in individual cases. We describe the successful use of cyclosporin (CsA) for treatment of life-threatening refractory HHTR in sickle cell and thalassemia disorders.

Aims: To assess the efficacy of cyclosporin for treatment of refractory HHTR. Primary endpoints were Hb and transfusion requirement.

Methods: Patients with refractory HHTR treated in our tertiary haemoglobinopathy centre between 2007 and 2013 were identified retrospectively. HHTR was defined as post-transfusion hemolysis with a fall in Hb to below pre-transfusion value in the absence of other cause and refractory HHTR as the persistence or recurrence of severe anemia following transfusion despite treatment with steroids and IVIG +/- rituximab. Case notes, pathology results and transfusion laboratory records were reviewed.

Results: Three patients fulfilled the inclusion criteria (Table 1). Mean age was 30 years. HHTR developed between 5-21 days after initial transfusion of 2-4 red cell units with a fall in Hb to 28-34 g/L, on average 18±4% below pre-transfusion level. In all cases the direct anti-globulin test (DAT) was positive and multiple red cell alloantibodies were detected. In cases 1 and 3 the antibody profile reflected previous alloimmunisation. In case 2 there was no known history of alloimmunisation. Despite concurrent administration of IVIG+MP with transfusion of phenotype and cross-match compatible red cell units, EPO, prednisolone (cases 1 and 3), and rituximab (case 1) profound anemia developed with a Hb of 21-29 g/L, on average 20±6% below the initial nadir. Bone marrow examination undertaken in cases 1 and 3 revealed erythroid hyperplasia. CsA was commenced with a target level of 150-200 µg/L 2 weeks-8 months after the onset of HHTR and continued for 3-12 months. A gradual increment was observed in Hb which stabilised after 3 months at 55-69 g/L within or slightly below the patient's previous steady state range. Prior to commencing CsA therapy patients had received between 7 and 19 red cell units following the development of HHTR. CsA was associated a significant reduction in transfusion requirement. Two cases received no further transfusion after initiation of CsA therapy. Case 2 received one further transfusion within 2 weeks of commencing CsA and further transfusion was given for pregnancy related complication but after the post-partum period no further transfusion was required while receiving CsA. Following cessation of CsA therapy a reduction in Hb was observed after 6, 2.5 and 36 weeks. In two patients (cases 2 and 3) the Hb level was restored following reintroduction of CsA which in case 2 was discontinued after 6 months with no recurrence of hyperhemolysis during follow up over 5 years. One patient (case 1) died from sepsis and multiorgan failure.

Table 1.

Summary and Conclusions: This is the first case series reporting the use of the calcineurin inhibitor cyclosporin A in the treatment of refractory HHTR. This novel approach was associated with recovery in Hb and abrogation of transfusion requirements in patients with both sickle cell and thalassemia disorders with this potentially life-threatening complication. The optimal duration of CsA therapy in HHTR remains to be defined.

Gene & cell therapy

S742

OUTCOMES OF GENE THERAPY FOR BETA-THALASSEMIA MAJOR VIA TRANSPLANTATION OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS TRANSDUCED *EX VIVO* WITH A LENTIVIRAL BETA GLOBIN VECTOR.

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Background: In patients with β-thalassemia major, haematopoietic stem cell (HSC) gene therapy has the potential to induce production of β-globin, γ-globin or modified β-globin in the RBC lineage and reduce or stop the need for transfusions. A prior clinical trial (LG001) demonstrated benefit of autologous CD34+ cells transduced with a replication-defective, self-inactivating lentiviral vector (HPV569) containing an engineered β-globin gene ($\beta^{A\text{-}T87Q}$). A further modified $\beta^{A\text{-}T87Q}$, vector (LentiGlobin BB305) has achieved greater transduction efficiency and a similar pre-clinical safety profile. LentiGlobin BB305 is now being evaluated in the HGB-205 clinical trial.
Aims: To provide (i) long-term follow-up data on two subjects treated in LG001 and (ii) initial results from the HGB-205 study.
Methods: After the provision of informed consent, subjects with β-thalassemia major underwent HSC collection via peripheral blood apheresis and CD34+ cells were selected. Estimation of the mean *ex vivo* vector copy number (VCN) was obtained by qPCR performed on pooled *in vitro* colony-forming progenitors. Subjects underwent myeloablation with intravenous busulfan, followed by infusion of transduced CD34+ cells. Subjects were monitored for haematological engraftment, $\beta^{A\text{-}T87Q}$, expression (by HPLC) and transfusion requirements. Integration site analysis (by LAM-PCR and high-throughput sequencing on nucleated cells) and replication-competent lentivirus assays were performed.
Results: In LG001, two subjects (#1003 and #1004) with β^{E}/β^{0}, thalassemia major successfully engrafted following gene therapy with autologous HSCs transduced with HPV569. Neither subject experienced a cell infusion related adverse event. As reported previously (*Nature* 2010), #1003 became transfusion-independent one year post–transplant and remains so 5 years later, with the production of 2.5 - 3.5 g/dL $\beta^{A\text{-}T87Q}$-globin (~30% of total haemoglobin). The most recent VCN in #1003's peripheral neutrophils is 0.23. Subject #1003 also demonstrated partial dominance of a clone with vector integration within the HMGA2 gene that peaked at 4 years post-treatment (22% of the nucleated cells) and has now fallen to 6.8% while maintaining transfusion independence. For #1004, the current VCN (2 years post-treatment) in neutrophils is 0.016 and $\beta^{A\text{-}T87Q}$–globin accounts for ~5% of total haemoglobin. This subject remains transfusion dependent. Two subjects with β^{E}/β^{0}, thalassemia major (#1201 and #1202) have enrolled in the current HGB-205 trial and one has undergone transplantation. Transduction efficiency of the new BB305 vector compared to HPV569 is shown in Table 1. Data on the transplant outcomes and up to 6 months of follow-up in subjects treated in the HGB-205 trial will be presented at the meeting.

Table1. Comparison of gene transfer efficiencies and transplantation outcomes with HPV569 *vs.* BB305 $\beta^{A\text{-}T87Q}$-LentiGlobin vectors

Summary and Conclusions: Long-term transfusion independence is achievable with gene therapy for β thalassemia major. Use of the LentiGlobin BB305 vector has resulted in substantially higher VCN in CD34+ cells. It remains to be seen how the clinical outcomes will reflect this improvement.

S743

PHASE I TRIAL OF AUTOLOGOUS CD19-TARGETED CAR-MODIFIED T CELLS AS CONSOLIDATION AFTER PURINE ANALOG-BASED FIRST-LINE THERAPY IN PATIENTS WITH PREVIOUSLY UNTREATED CLL

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Background: Patient T cells may be genetically modified to express chimeric antigen receptors (CARs) targeted to antigens expressed on tumor cells. We have previously reported initial results from a phase I clinical trial treating patients with chemotherapy refractory and relapsed CLL with autologous T cells modified to express the 19-28z CAR targeted to the CD19 antigen expressed on most B cell malignancies (Brentjens RJ *et al.*, Blood 2011). While the anti-tumor activity of the CD19-targeted CAR-modified (CAR^+) T cells has been modest in the setting of rapidly progressive and chemotherapy refractory disease, we have observed significantly increased rates of durable responses in CLL patients with reduced disease burden and chemotherapy-sensitive disease (Park JP *et al.*, ASH Abstract 2011).
Aims: In order to address the previously recognized limitation of autologous T cells modified to express CAR targeting CD19 in patients with chemotherapy refractory and relapsed CLL, we designed a phase I trial wherein CLL pts with residual disease following the first-line chemotherapy will receive the CD19-targeted CAR^+ T cells as a consolidative therapy.
Methods: Patients with CLL who have achieved either partial (PR) or complete response (CR) with detectable minimal residual disease (MRD) to the first-line therapy consisting of 6 cycles of pentostatin, cyclophosphamide and rituximab (PCR) were enrolled. Autologous T cells were transduced with a retroviral vector encoding the anti-CD19 scFv linked to CD28 co-stimulatory and CD3ζ signaling domains (19-28z). Patients received cyclophosphamide conditioning therapy followed by 3 escalating doses of CAR^+ T cells. Response was assessed at 3 months according to the NCI-WG criteria. Serial bone marrow aspirate and blood samples were assessed for the modified T cell persistence (assessed by flow and RT-PCR) and cytokine profile analysis
Results: To date, 7 patients have received the CAR^+ T cells in 3 dose cohorts ($3x10^6$ - $3x10^7$ CAR^+ T cells/kg). 6 patients had unmutated IgHV and 2 patients had del11q. All 7 patients achieved PR following the PCR chemotherapy. 4 patients had palpable lymphadenopathy (1 pt with bulky lymph nodes) prior to the T cell infusion. Median follow-up was 11 months (range, 3 – 17 mos). No DLT was observed. Mild and self-limiting cytokine release syndrome (CRS) was observed in 3 patients, and there was a positive correlation between the development of CRS and the CAR^+ T cell persistence. 1 patient achieved CR; 2 patients achieved CR in the bone marrow (1 MRD negative CR) but had progressive disease in lymph node only; 3 patients achieved PR; and 1 patient had progressive disease but this pt had rapidly rising ALC at the time of T cell infusion.
Summary and Conclusions: Autologous CD19-targeted CAR^+ T cells appear to be safe and have promising anti-tumor efficacy in patients with high-risk CLL undergoing first-line chemoimmunotherapy. Our findings suggest that the CD19-targeted CAR^+ T cells are more effective in eradicating disease in the marrow *versus* lymph nodes, and further studies are being conducted to better understand the mechanism of resistance.

S744

LIVER-DIRECTED LENTIVIRAL GENE THERAPY PROVIDES STABLE BENEFIT IN HEMOPHILIA B DOGS WITHOUT EVIDENCE OF GENOTOXICITY IN SENSITIZED MOUSE MODELS

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Background: Lentiviral vectors (LVs) are attractive vehicles for liver-directed gene therapy by virtue of their ability to stably integrate in the genome of target cells and the lack of pre-existing humoral and cellular immunity against vector components in most humans. Over the past years, we have developed a LV platform that can achieve stable transgene expression in the liver, induce transgene-specific immune tolerance and establish correction of hemophilia in mouse models upon systemic administration. This LV is designed to stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation.
Aims: Although encouraging, these results were obtained in small animal models and it is crucial to assess the feasibility and safety of scaling-up liver-directed LV gene therapy in large animal models before clinical translation. In addition, whereas liver gene transfer by L*Vs* appeared to be safe in the treated mice, concerns remain regarding the risks associated with vector insertional mutagenesis and studies in more stringent models are warranted. Here we address these issues exploiting the large-scale manufacturing process of L*Vs* for clinical testing and tumor-prone mouse models to enhance the sensitivity of testing vector genotoxicity.
Methods: We produced 3 large-scale batches of L*Vs* qualified for *in vivo* administration according to methods and specifications previously set for use in clinical trials. Each LV batch was infused entirely in one adult hemophilia B dog by portal vein administration. We challenged the safety of lentiviral integration in liver cells, in different settings of escalating stringency, by analysis of LV integration site (IS) distribution in the genome of hemophilia B mice at different times and neonatal administration in 2 sensitive mouse models of genotoxicity.
Results: We observed long-term stable reconstitution of coagulation factor IX (FIX) activity up to 1% of normal and significant amelioration of the clinical phenotype in 3 treated dogs (>6 years cumulative follow up). LV infusion was associated with transient signs of inflammation and mild hepatotoxicity which could be abrogated by pretreatment with anti-inflammatory drugs. There was no detectable long-term toxicity or development of FIX inhibitors. Concerning integration safety, the analysis of genomic LV IS at different times after administration showed that any deviation from random represented LV-intrinsic integration biases and not the outcome of *in vivo* selection. We then exploited 2 sensitive mouse models of genotoxicity and an aptly designed genotoxic LV as a positive control for insertional mutagenesis. While this LV induced hepatocellular carcinoma (HCC) at high frequency, the therapeutic LV did not increase the spontaneous occurrence of HCCs in these mice. We retrieved >9,500 unique IS and did not detect any evidence of selection of cells transduced by the therapeutic LV. These studies did not uncover any biological or molecular evidence of genotoxicity by the therapeutic LV even after administration to newborn tumor-prone or tumor-promoted mice.
Summary and Conclusions: Overall, our study positions LV-mediated liver gene therapy for further pre-clinical development and future clinical translation. L*Vs* may thus complement other available vectors to address some of the outstanding challenges posed by liver gene therapy of hemophilia and conceivably other diseases.

S745

ACTIVITY OF RAPIDLY-GENERATED BROAD-SPECTRUM T CELLS AS TREATMENT FOR ADENOVIRUS, EBV, CMV, BK VIRUS AND HHV6 INFECTIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Viral infections remain a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). We have previously demonstrated that the adoptive transfer of small numbers of *ex vivo*-expanded trivirus-specific T-cells (tVSTs) targeting EBV, CMV, and Adv is safe, effective and protective *in vivo*. However, broader implementation is limited by a manufacturing process that is prolonged (8-12wks), complex and requires infectious virus (EBV) and vector (Adv). Moreover, antigenic competition limits extension to additional viruses.
Aims: To generate T-cell lines with activity against 5 common post-transplant viruses (EBV, CMV, Adv, BK, HHV6), using a simplified 10-day procedure that excludes viral components, and show that a single line can be clinically effective against multiple viruses.
Methods: With NHLBI-PACT support 48 clinical-grade donor-derived multivirus (m)VSTs have been generated by exposing $3x10^7$ PBMCs to overlapping peptide libraries spanning Adv (Hexon, Penton), CMV (pp65, IE1), EBV (LMP2, EBNA1, BZLF1), BKV (Large T, VP1) and HHV6 (U11, U14, U90) antigens.
Results: We expanded a median of $35.7x10^7$ cells (range: $9.9\text{-}82.5x10^7$) over 9-11 days. The lines were polyclonal, comprising both CD4+ (57±2%) and CD8+ (35±2%) cells, that expressed central CD45RO+CD62L+ (62±3%) and effector memory markers CD45RO+/CD62L- (10±1%). mVST specificity was dependent on the donor's prior viral exposure; 45/48 lines had Adv activity (Hexon: 470±71; Penton: 366±86 SFC/$2x10^5$), 26/48 against CMV (IE1: 356±157; pp65: 1048±446), 37/48 against EBV (LMP2: 137±76; EBNA1: 123±52; BZLF1: 99±75), 28/48 against BKV (Large T: 123±61; VP1: 208±89) and 29/48 against HHV6 (U90: 109±78; U11: 37±17; U14: 84±26). None of the lines reacted against recipient PHA blasts. Eleven allogeneic HSCT recipients have received mVSTs in a dose escalation study with informed consent; 4 on DL1 ($5x10^6/m^2$), 4 on DL2 ($1x10^7/m^2$) and 3 on DL3 ($2x10^7/m^2$). There were no immediate infusion-related toxicities. One patient developed *de novo* Grade II GvHD of the skin 4 weeks after mVSTs, which improved with the administration of topical steroids. Three patients received the cells prophylactically and remained infection free up to 3 months post-pVSTs. The other 8 patients were treated for one or more active infections. Based on viral load measurements a single infusion successfully controlled active infections associated with all our targeted viruses: CMV (2 CR, 1 PR); EBV (5 CR, including a frank PTLD case); Adv (1 CR); HHV6 (2 CR) and BKV (5 CR, 1 PR, 1 NR). Of note, all 3 patients with BKV hemorrhagic cystitis had marked improvement/disappearance of hematuria post-pVSTs. One subject had transient but severe bladder pain with inflammation, seen on cytoscopy, and a 5-log fall in urine BK viral load with detection of BKV-specific T-cells in his bladder. Our only partially non-responding patient had 3 viral infections (EBV, HHV6, BKV) and cleared EBV and HHV6 but not BKV following the infusion of a line that lacked specificity for this virus, likely reflecting the seronegative status of the donor.
Summary and Conclusions: Thus, infusion of mVSTs has been safe and clinically effective (94% overall response) against up to 4 simultaneous/sequential infections in a single HSCT recipient. We are currently extending this platform to include other clinically relevant viruses and are planning to assess the activity of "off the shelf" 3rd party mVSTs for broader implementation.

S746

SLEEPING BEAUTY-MEDIATED ENGINEERING OF CYTOKINE INDUCED KILLER (CIK) CELLS WITH CHIMERIC ANTIGEN RECEPTORS (CARS) FOR IMMUNOTHERAPY OF ACUTE LEUKEMIAS

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Background: T cell engineering with CARs has been recently proved to be effective in redirecting effector activity towards leukemic blasts.
Aims: Since the profile of efficacy, safety and feasibility of cell manufacturing and gene therapy by viral vectors still remain major concerns, we explored here the use of Sleeping Beauty (SB) Transposon-mediated gene transfer in CIK cells for targeting Acute Leukemias.
Methods: With an optimized clinical-grade stimulation protocol, we genetically modified CIK cells to express two distinct 3rd generation CARs specific for myelogenous leukemia (AML) CD123+ or acute lymphoblastic leukemia (ALL) CD19+ blasts.
Results: The nucleofection minimally affected the phenotype of CIK cells, and the optimized protocol was effective in inducing T-cell expansion, with a fold increase sufficient to be translated into adoptive cell therapy clinical protocols (Figure 1, panel A, proliferation of CIK cells followed overtime by cell count). Modified CIK cells displayed stable expression of CD123.CAR or CD19.CAR with a frequency of 51.4%±2.9 (n=13) and 48.8%±6.8 (n=7), respectively (Figure 1, panel B, CAR expression determined at day 21 of differentiation), and exerted efficient lysis of leukemic cell lines and primary blasts (Figure 1, panel C, apoptosis detection, 5:1 Effector:Target ratio). Interestingly, CAR triggering by the antigen expressed by leukemic cells promoted specific cytokine secretion and proliferation that was restricted to the modified fraction of CIK cells, suggesting activation and selection of modified CIK cells upon encounter with cancer cells in the patients. The loss of the expression of transposase during the differentiation was assessed to assure the genome stability of the cellular product treated by SB system by absolute quantification through RT-PCR. Finally, preliminary insertion-site analysis by LAM-PCR confirmed that the integrations in the genome of SB system do not correlate with the genes-enriched regions.

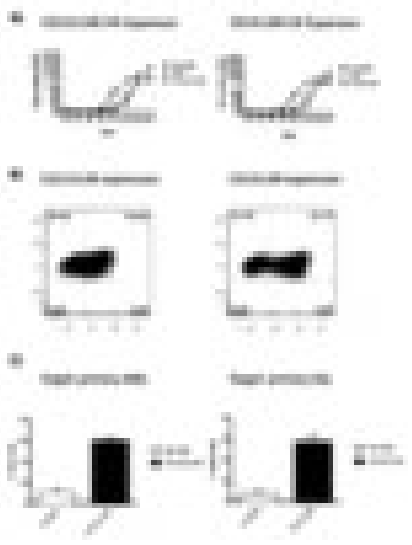

Figure 1.

Summary and Conclusions: In conclusion, SB system together with an optimized method of differentiation efficiently expand modified CD123.CAR+ and CD19.CAR+ CIK cells, redirect their activity towards AML and ALL cells, respectively, and retain a safe pattern of integrations in the genome. An easy clinical-grade adoptive cell therapy platform based on an innovative non viral method of gene transfer will be fundamental to improve the range of applications of immunotherapy to control relapse in leukemic patients. the genome of SB system do not correlate with the genes-enriched regions.

POSTER SESSION II

Acute lymphoblastic leukemia - Biology 2

P747

TARGETING THE DOPAMINE RECEPTOR SIGNALING REDUCES THE POLYCOMB PROTEIN BMI1 AND LIMITS THE SELF-RENEWAL OF BCR-ABL1 POSITIVE LEUKEMIA CELLS

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Background: Hematopoietic stem cells and leukemic stem cells share common features, including self-renewal, the capacity to differentiate, resistance to apoptosis, and limitless proliferative potential. Using a discovery platform that reveals differences between neoplastic and normal human pluripotent stem cells, the norvel dopamine receptor antagonist, thioridazine, was identified from libraries of known compounds that induce differentiation to overcome neoplastic self-renewal (Cell 149, 1284, 2012).
Aims: We investigated the molecular mechanisms by which dompamine receptor antagonist, thioridizine, regulates the self-renewal of primary BCR-ABL1 positive leukemia cells *in vivo*.
Methods: To identify the leukemia-propagating cell fraction of BCR-ABL1-positive leukemia, we serially transplanted human leukemia cells from patients with chronic myeloid leukemia blast crisis (n=1; T315I BCR-ABL1) or ponatinib-resistant Ph-positive acute lymphoblastic leukemia (n=2, Y253H/E255K/T315I BCR-ABL1 and T315I BCR-ABL1) into NOD/SCID/IL-2γc-/- mice. The cell fractions with CD34+ CD19+ could self-renew and transfer the leukemia in NOD/SCID mice. To investigate the effects of the domamine receptor antagonist on self-renewal and the relevance as a therapeutic target in ABL-tyrosine kinase-resistant BCR-ABL1 positive leukemia, we examined the activity of thioridazine against CD34+CD38-CD19+, CD34+CD38+CD19+ fractions transferred NOD/SCID mice *in vivo*.
Results: NOD/SCID mice were injected intravenously with BCR-ABL1 positive cells then treated with thioridazine (20 mg/kg; p.o.) for 28 days. All mice demonstrated the engraftment of leukemia by flow cytometry. However, the treatment with thioridazine reduced the population of CD34+CD38- positive cells. We isolated human CD45+ cells from the spleen of mice from each treatment group and injected equivalent numbers of leukemia cells into secondary recipients. Following 30 days, all mice received BCR-ABL1 cells from vehicle treated mice engrafted with leukemia. In contrast, leukemia engraftment was not detected in recipient mice (n=6) from thioridazine-treated donors. These results demonstrate the persistent effects of domapine receptor signaling inhibition on long term self-renewing BCR-ABL1-positive leukemia cells. We further examined the effects of hedgehog pathway modulation on *in vitro* clonogenic growth. CD34+CD38-CD19+ cells from T315I BCR-ABL1 (n=2) and WT-BCR-ABL1 (n=1) cells were treated with 5 μM of thiorizaxine for 72 hrs, washed free of drugs, and plated in quadruplicate in methylcellulose. At 14 days, colonies were counted as initial plating. The representative plate was then washed and cells were re-suspended and re-plated. After an additional 14 days, colonies were counted as secondary re-plating. Clonogenic recovery of untreated cells was normalized to 100% and plating results from all treatment groups were expressed as % control. Dopamine receptor pathway inhibition by thioridazine had only minimum effects on colony formation after initial plating over control cells. However, upon serial re-plating, secondary colony formations were significantly inhibited by thioridazine ($p<0.001$). To identify the mechanisms that limit the self-renewal of BCR-ABL1-positive cells by thioridazine, NOD/SCID mice engrafted with T315I -BCR-ABL1- positive CD34+ CD19+ fractions were treated with thoridazine (20 mg/kg; p.o.) for 14 days. Thioridazine induced the expressions of p21Cip1 and reduced the expression of polycomb protein BMI-1.
Summary and Conclusion: Our preclinical results indicate that thioridazine have potential as an important option for controlling the drug-resistant leukemia initiating cells in BCR-ABL1 positive leukemia. It is expected that the domapine receptor antagonist may become extremely useful therapeutic interventions in a number of hematological neoplasms, including BCR-ABL1 positive leukemia, where the persistence of cancer stem cells.

P748

COMPLEX GENETIC HETEROGENEITY INFLUENCES PROGNOSIS IN ADULT B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA NEGATIVE FOR RECURRENT FUSION GENES

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Background: Although recent high-resolution genome-wide profiling analysis of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) samples has contributed to the identification of many novel somatic genetic alterations, a deep molecular characterization of adult ALL is still challenging, especially for those cases lacking recurrent fusion genes.
Aims: In order to better molecularly dissect this ALL subgroup, we performed an integrative molecular approach including gene-candidate high-resolution screening and genome-wide profiling analyses.
Methods: We retrospectively analyzed 28 newly diagnosed *BCR-ABL1*-negative BCP-ALL subjects (19 males/9 females; median age 41.5 years; negative for known fusion genes) and 28 *BCR-ABL1*-positive BCP-ALL subjects as a comparison group. In *BCR-ABL1*-negative ALL karyotype was normal in 10/28 (36%). Copy number alterations (CNA) of know leukemia-related genes, such as *IKZF1, CDKN2A/B, PAX5, EBF1, ETV6, BTG1, RB1*, and genes within *PAR1*: *CRLF2, CSF2RA, IL3RA* have been assessed by the SALSA MLPA kit P335 IKZF1 (MRC Holland). In addition, sequence mutations have been investigated in *TP53, CRLF2, JAK2, LEF1, PAX5* and *IL7R* by next-generation deep-sequencing (NGS) (Roche Applied Science; IRON-II study oligonucleotide primer plates). Positivity for newly described *BCR-JAK2, PAX5-JAK2, ETV6-ABL1, EBF1-PDGFRB, NUP-ABL1* gene fusions occurring in *BCR-ABL1*-like ALL (Roberts KG *et al.*, Cancer Cell. 2012) was assessed by PCR amplification and sequencing. Finally, SNP arrays (SNP 6.0, Affymetrix), gene expression profile analyses (GeneChip® Human Transcriptome Array 2.0) and whole exome sequencing (Illumina) were performed to more fully assess genomic complexity.
Results: Overall, 76% of *BCR-ABL1*-negative subjects showed an abnormality of at least one of the analyzed known leukemia genes: 7 (25%) had one, 4 (14%) had two, 6 (21%) had three, and 6 (21%) had four or more alterations. In subjects showing no abnormalities, SNP arrays analysis revealed amplifications of chromosome 1q in 2/6 cases (33%). Deletions of *CDKN2A/B* were the most frequent (39%) and in 73%, they occurred together with other abnormalities, suggesting that multiple events are needed to induce the full leukemia phenotype. Other common CNA included: deletions of *IKZF1* (25%), *ETV6* (25%), *PAX5* (14%), *EBF1* (11%), *PAR1* region (11%) and *RB1* (7%). NGS showed mutations of *JAK2* and *CRLF2* in 7% (R683S/G) and 4% (F232C), respectively. No positivity for newly described fusion genes activating tyrosine kinase was confirmed. Importantly, subjects with no abnormalities showed better survival rates compared to those with one or more molecular alterations ($p<0.01$). The *BCR-ABL1*-positive subgroup shared the same CNA of *BCR-ABL1*-negative cases, such as deletions of *IKZF1* (71%), *CDKN2A/B* (21%), *PAX5* (14%), *BTG1* (11%), *EBF1* (11%), and *ETV6* (4%), but they did not show mutations in the analyzed genes.
Summary and Conclusion: BCP-ALL lacking recurrent fusion genes is a highly heterogeneous and complex disease. Current diagnostic procedures need to be revised to improve risk assessment and to guide therapeutic decisions.
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MODELING RESISTANCE TO TYROSINE KINASE INHIBITORS IN TEL/ABL+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: TEL/ABL+ acute lymphoblastic leukemia (ALL) represents a rare leukemia subtype with unfavorable prognosis. The activity of fusion kinase can be inhibited by tyrosine kinase inhibitors (TKI), which might be beneficial in the therapy of this ALL.
Aims: We aimed to study potential mechanism of TKI resistance in TEL/ABL+ ALL.
Results: Via long-term exposure of parental sensitive TEL/ABL+ cell line to slowly increasing doses of imatinib mesylate (IM) we have established panTKI-resistant daughter cell line. To control for the effect of long-term culturing, daughter sensitive cell line was established in parallel to resistant one and always used together with parental sensitive cell line in analyses comparing cells with resistant and sensitive phenotype. Using deep sequencing, FISH, qPCR and Western blot we excluded the most common mechanisms of IM

resistance described in BCR/ABL+ ALL (kinase domain mutations, genomic amplification or enhanced fusion gene expression) as the cause of resistance in our model. To study genomic background of resistance, we performed genomic profiling and whole exome sequencing. First round of profiling on high density SNP arrays did not reveal any changes acquired by resistant cells compared to sensitive cells. Second round of profiling on SNP array with different coverage revealed that the resistant cells acquired a 60 kb intragenic deletion in KDM6A gene encoding lysine-specific histone demethylase. This deletion is predicted to result in either the expression of aberrant KDM6A or in loss of function and we are currently studying its impact on transcript and protein level. Whole exome sequencing identified 25 non-synonymous nucleotide variants (SNVs) within gene coding regions that were gained by resistant cells and not detected in sensitive cells. Twelve SNVs affect genes expressed in the studied cell line and annotated to various biological processes including intracellular signaling (*e.g.* G protein GNB1 or JAK/STAT signaling component STAM2). These genes have not been associated with TKI resistance before and their potential contribution to resistance needs to be further clarified. To study gene expression changes associated with resistance we have performed gene expression profiling on microarrays and identified a set of genes differentially expressed between resistant and sensitive cells. The expression data for the top up/down-regulated genes were confirmed by qPCR on a set of multiple independently harvested and processed samples. The expression of several candidates was further assessed with regard to the presence of IM in growth media and 2 different expression patterns were observed. First group of genes, represented by SORBS2, was differentially expressed in resistant cells compared to sensitive cells irrespective of IM presence. In contrary, the change in expression of second subgroup of genes (involving BCL6 and SOCS2) was induced in resistant but not sensitive cells by high but still completely tolerated IM levels. Of interest, upregulation of BCL6 was recently described as a cause of IM resistance in BCR/ABL+ cells and its inhibition was shown to restore IM response (Duy, Nature, 2011).

Summary and Conclusion: To conclude, we have established a new model of TKI-resistance that is not driven by the most common resistance mechanisms described so far. Using genomic and expression profiling we have identified several genetic alterations and gene expression changes associated with resistant phenotype that may contribute to resistance mechanism and will be further studied.

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COMPARISON BETWEEN QUANTITATIVE CLONAL IG TARGETS AND FUSION TRANSCRIPTS IN THE DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Minimal residual disease (MRD) is a powerful prognostic indicator in acute lymphoblastic leukemia (ALL). Quantification of clonal antigen-receptor gene rearrangements by real time polymerase chain reaction (QPCR) is considered to be the standard methodology for MRD monitoring in ALL. An alternative less labor-intensive and less expensive approach is the quantification of fusion transcripts in those carrying recurrent chromosomal translocations by real time reverse transcriptase PCR (RQ-PCR) assay. Data of correlation between simultaneous measurements of clonal immunoglobulin (*Ig*) gene and common fusion transcripts have been very rare.

Aims: We aimed to compare the sensitivity between the two PCR-based assays for *Ig* targets and RQ-PCR for the 3 major fusion transcripts in MRD monitoring in ALL patients with t(12;21)/*ETV6-RUNX1*, t(9;22)/*BCR-ABL1* and t(1;19)/*TCF3-PBX1*.

Methods: Bone marrow mononuclear cells were collected at diagnosis and at different time points after therapy in 28 pediatric *ETV6-RUNX1* ALL patients with 44 follow-up samples; 10 children and 13 adults carrying *BCR-ABL1* (17 P190 and 6 P210) with 60 follow-up samples; and 12 children and 3 adults harboring *TCF3-PBX1* with 39 follow-up samples. Totally, 143 follow-up samples were examined simultaneously by using two PCR-based assays for each sample. RQ-PCR with TaqMan® assay was performed with *ABL1* gene as control for the measurement of 3 fusion transcripts according to the Europe Against Cancer Program. QPCR assay for clonal *Ig* targets was carried out following the Guidelines of the European Study Group on MRD detection in ALL. The detection sensitivity of *Ig*-QPCR assay was 10^{-5} in 62.1% of patients; the remaining patients had a sensitivity of 10^{-4}. The detection sensitivity of RQ-PCR for all three fusion transcripts was 10^{-5}. MRD-negative was defined as <0.01%.

Results: The overall correlation between *Ig*-PCR and RQ-PCR for fusion transcripts was good (correlation coefficient R^2=0.754, P<0.0001). A comparison between *Ig*-QPCR and *ETV6-RUNX1* RQ-PCR assays showed that MRD was positive in 15 samples (R^2=0.688, P=0.0001) and negative in 22 samples for both assays, whereas *ETV6-RUNX1* MRD (+)/*Ig*-MRD (-) was found in 6 samples and *ETV6-RUNX1* MRD (-)/*Ig*-MRD (+) was detected in 1 sample (Kappa 0.678; concordance 84.1%). For patients with *TCF3-PBX1,* MRD was both positive in 10 samples (R^2=0.877, P<0.0001) and both negative in 29 samples examined by *Ig*-QPCR and *TCF3-PBX1* RQ-PCR assays (Kappa 1; concordance 100%). Of the 60 samples with *BCR-ABL1* transcripts, both assays showed positive MRD in 35 samples (R^2=0.627, P<0.0001) and both negative in 11 samples (Kappa 0.478; concordance 76.7%). Fourteen samples had *BCR-ABL1*-MRD (+) and *Ig*-MRD (-), and none had *BCR-ABL1*-MRD (-) and *Ig*-MRD (+). There was no difference in the sensitivity between P190 and P210 subtypes of *BCR-ABL1* (Kappa 0.457 *vs* 0.353 with concordance 73.7% *vs* 77.3%, P=0.292). Taking together, MRD was positive when assayed with RQ-PCR in 20 of 104 ALL samples with *ETV6-RUNX1* or *BCR-ABL1* but negative with *Ig*-PCR assay.

Summary and Conclusion: The present study showed that *TCF3-PBX1* ALL exhibited an excellent concordance (100%) between QPCR assay for *Ig* targets and *TCF3-PBX1* RQ-PCR assay. In 19% of samples, RQ-PCR assay for *ETV6-RUNX1* or *BCR-ABL1* transcripts was more sensitive that *Ig*-QPCR assay for MRD monitoring.

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MEK1 INHIBITOR SELUMETINIB SENSITIZES B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) CELLS TO DEXAMETHASONE BY MODULATING AUTOPHAGY

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Background: Glucocorticoids (GC) have been extensively used as important therapeutic agents in the treatment of B-cell acute lymphoblastic leukemia (B-ALL). GCs in B-ALL induce cell death that involves autophagy and apoptosis. Although resistance to GCs has been recognized as a major adverse prognostic factor, the molecular mechanisms leading to GC resistance are not fully understood.

Aims: To elucidate the molecular mechanisms responsible for GC resistance in B-ALL and characterize the consequences of targeted intervention in these mechanisms for sensitivity of leukemic cells to GCs.

Methods: Bioinformatics analyses of GC-sensitive and -resistant B-ALL blasts were carried out using publicly available gene expression datasets and GenePattern program. GC-resistant B-ALL SEMK2 cells and GC-sensitive RS4;11 cells were incubated with dexamethasone (DEX, 0,05 or 2 μg/mL) in the presence or absence of MEK1 inhibitor, selumetinib (SEL, 200nM). Cell apoptosis was assessed using annexinV/PI staining and FACS analysis. The activities of ERK, mTORC1 and 4EBP-1 were determined with Western blotting and phospho-specific antibodies. Autophagy was measured by the presence of LC3II form using Western blot and by monodansylcadaverine (MDC) staining. Beclin-1 (BCN1) knockdown was achieved by retroviral infection of SEMK2 cells with BCN1-specific shRNA.

Results: To elucidate the molecular mechanisms of GC resistance in B-ALL blasts, we performed gene set enrichment analysis of gene expression profiles obtained from GC-resistant and -sensitive primary B-ALL blasts. These analyses revealed that GC-resistant cells exhibit significantly higher expression of MAPK/ERK pathway components, compared to sensitive cells (p<.001, FDR=0.17). To confirm these findings, we assessed DEX sensitivity in cells with high (SEMK2) or undetectable (RS4;11) activity of MAPK/ERK pathway. SEMK2 cells were resistant to DEX, whereas RS4;11 were highly sensitive to this drug. In SEMK2 cells, SEL completely abrogated ERK1/2 phosphorylation and increased cellular sensitivity to glucocorticoids by 1.8-2.6-fold (p<0.01). Since DEX toxicity in B-ALL cells involves induction of autophagy, we assessed LC3 processing in SEMK2 cells incubated with either DEX, SEL or combination of SEL/DEX. Either drug alone did not change the level of LC3II, but their combination significantly increased the LC3II abundance. Similar results were obtained with MDC staining, a dye that marks autophagolysosomes. Since mTORC1 is the critical regulator of autophagy, we assessed the levels of p-mTORC1 (Ser2448), which reflects the activity of this kinase. In RS4;11 cells, DEX decreased the level of p-mTORC1. In contrast, DEX or SEL alone had no effect on mTORC1 activity in SEMK2 cells, but incubation of SEMK2 cells with DEX/SEL combination decreased activity of mTORC1 and mTORC1 substrate, 4EBP-1. Finally, to assess whether the induction of autophagy is required for the observed synergy between SEL and DEX, we silenced BCN1, a gene required for autophagosome formation, in SEMK2 cells. In cells transduced with non-targeting shRNA vector, SEL sensitized cells to DEX, but in BCN1-deficient cells, the synergy of DEX and SEL was markedly decreased.

Summary and Conclusion: Taken together, we show that MEK1 inhibitor selumetinib sensitizes DEX resistant B-ALL cells to glucocorticoid treatment. The underlying mechanism of this synergy involves inhibition of mTORC1 signaling pathway and induction of autophagy that leads to cell death.

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DISEASE EVOLUTION CORRELATED WITH EX VIVO DRUG SENSITIVITY AND RESISTANCE IN TCF3-PBX1 DRIVEN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The t(1;19)(q23;p13) translocation occurs in 3% of adult patients with acute lymphoblastic leukemia (ALL) and results in rearrangement of the *TCF3*(*E2A*) transcription factor and *PBX1* homeobox genes. The TCF3-PBX1 fusion protein is an activating transcription factor that drives oncogenesis by deregulating gene expression. The prognosis of adult t(1;19) ALL patients is comparable to other forms of adult ALL. However, relapse and chemotherapy related toxicity remain a problem.
Aims: We aimed to determine the *ex vivo* drug sensitivity profile of a t(1;19) positive pre-B-ALL of a 25 year old male with relapsed disease. In addition, we aimed to identify molecular markers linked to drug sensitivity and resistance in the tumor. Furthermore, we sought to understand evolution of the tumor during disease progression by deep molecular profiling of serial samples.
Methods: Bone marrow (BM) aspirates were obtained after informed consent and the mononuclear cell fraction was used for analyses. Two relapse samples were profiled by *ex vivo* drug sensitivity testing, exome and RNA sequencing. In addition, we sequenced the exomes of a diagnostic sample, and a skin biopsy sample, which was the germline control for somatic mutation calling. *Ex vivo* drug sensitivity testing was done using a library of 306 of investigational and approved antineoplastic drugs in 5 concentrations to generate dose response curves. The leukemia specific drug responses were compared to those obtained for healthy BM samples.
Results: Exome sequencing detected a novel somatic in-frame codon insertion mutation in *MTOR* (p.M1724MW), a loss of function mutation in *PHF6* and a heterozygous deletion of *CDKN2A,* which were clonal at diagnosis and relapse. We also identified two relapse specific mutations in *TP53* as well as a subclonal deletion of 17p encompassing *TP53*. RNA-sequencing revealed extremely high expression of *CXCR4* in both relapse samples. To determine if high expression of *CXCR4* is common in TCF3-PBX1 driven ALLs we assessed CXCR4 gene expression levels in 19 TCF3-PBX1 driven adult ALLs by qRT-PCR. Results indicated that only the index patient's relapse samples had aberrantly high *CXCR4* expression, suggesting that TCF3-PBX1 alone is not sufficient to deregulate expression of this gene. *Ex vivo* drug sensitivity screening showed that the cells were markedly sensitive to several investigational mTORC1 inhibitors, including GSK2126458, AZD8055, PF-04691502, MLN0128, as well as the mTOR inhibitor everolimus, approved for other indications. In addition, the cells were selectively sensitive to glucocorticoids, as well as BCL-2 inhibitors ABT-199 and navitoclax, which corresponded to *BCL2* and *BCL2L1* gene expression.
Summary and Conclusion: The presence of the *MTOR* mutation in the tumor suggests a link to the *ex vivo* drug sensitivity phenotype. *CXCR4* mediated binding of leukemic cells to the bone marrow stroma can increase resistance to chemotherapy, thus the aberrantly high *CXCR4* expression levels observed in the index patient's tumor may have contributed to disease relapse. Furthermore, loss of p53 is associated with drug resistance in cell line models through impairment of apoptotic signaling. The emergence of several relapse specific *TP53* mutations in the index patient's tumor suggests that relapse and therapy resistance were also caused by progressive loss of *TP53* during tumor evolution. Although the TCF3-PBX1 is an important driving event in disease development, additional acquired mutations and changes in gene expression in t(1;19) pre-B-ALL may contribute to overall treatment response and outcome.

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THE DIFFERENCE OF RCSD1-ABL1-LEUKEMOGENICITY BETWEEN EXON3 OF RCSD1/EXON 4 OF ABL1 AND EXON 2 OF RCSD1/EXON 4 OF ABL1

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Background: The RCSD1 gene, located at 1q23 involved in t(1;9)(q23;q34) translocation in acute lymphoblastic leukemia (ALL) is known as a novel gene fusion partner of ABL1 gene. We have previously reported that there are two types of fusion genes in a case of RCSD1-ABL1-positive ALL. One is the fusion of exon 2 of RCSD1/exon 4 of ABL1 (R2A4; 2952bp) and another one is the fusion of exon 3 of RCSD1/exon 4 of ABL1 (R3A4; 3042bp).
Aims: The aim of this study is to examine the leukemogenicity of two kinds of fusion genes of RCSD1-ABL1.
Methods: We generated echotrophic retroviral vector expressing R2A4 or R3A4 and transduced with Ba/F3 murine interleukin-3 (IL-3) dependent cell line, and examined the multiplication activity and the IL-3 dependency of each transduced Ba/F3 cells. To analyze the leukemogenicity, $2x10^8$ of each Ba/F3 cells were intraperitonealy injected into SCID mice and examined the overall survivals (OS) and histopathology of each mice. Moreover, we examined phosphorylation receptor tyrosine kinase activity of each Ba/F3 without IL3 by Phosphorylation Antibody Array.
Results: Only R3A4 transduced Ba/F3 acquired significant independency of IL3. (Control *vs* R3A4; P=0.001 and Control *vs* R2A4; P=0.90). *In vivo* analysis, R3A4 transduced Ba/F3 injected mice showed significantly shorter survivals in comparison with those of control or R2A4 transduced Ba/F3 injected mice (42days *vs* >100days; P=0.0009). Leukemic change and splenomegaly were observed in only R3A4 transduced Ba/F3 injected mice but not R2A4 transduced Ba/F3 injected mice. Phosphorylation Antibody Array showed that ABL1 were activated only in R3A4 transduced Ba/F3.
Summary and Conclusion: Since R3A4 has leukemogenicity but not R2A4, the 90bp of difference may be the key of leukemogenicity and the leukemogenicity seems to be related to the phosphorylation of ABL1. This 90bp may be a promising target to treat RCSD1-ABL1-positive ALL.

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CD20 EXPRESSION IN ADULTS WITH PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) FROM UKALL14, ITS REGULATION BY DEXAMETHASONE AND RITUXIMAB INDUCED CYTOTOXICITY

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Background: UKALL14 is an on-going randomized trial for newly diagnosed adult ALL. All patients with B-ALL, regardless of CD20 expression, are randomized, after a dexamethasone pre-phase, to standard treatment alone or standard treatment plus rituximab (off-label use) during induction. CD20 expression will be quantified and related to MRD and clinical outcome. CD20 has been shown to be up-regulated by prednisolone *in vitro* and *in vivo* in paediatric B-ALL (Dworzak et al 2008). This correlated with increased rituximab-induced complement-mediated cytotoxicity.
Aims: To quantify CD20 expression at diagnosis in adult patients with B-ALL enrolled on UKALL14. To assess whether dexamethasone (compared to prednisolone) induces CD20 up-regulation in-vitro in both cell lines and primary diagnostic cells cultured on a stromal cell layer. To assess whether up-regulation of CD20 correlates with enhanced rituximab-induced complement-mediated cytotoxicity.
Methods: UKALL14 trail patients gave informed consent. CD20 was quantified using a PE-conjugated antibody after careful gating on viable B-ALL cells. The percentage of blasts expressing CD20 and mean fluorescence intensity (MFI) were determined. MFI was normalized to antibodies bound per cell (ABC) using a standard curve created by BD Quantibrite fluorescence beads. Eight adult primary B-ALL and 5 B-ALL cell lines (NALM-6, SEM, 697, REH and NALM-16) were cultured with or without different concentration of dexamethasone for 3 days, followed by CD20 quantification. The Burkitt's cell line, Raji, was used as a positive control. Primary cells were cultured on hTERT expressing human bone marrow mesenchymal stromal cells. Complement-mediated cytotoxicity was assessed by Promega CytoTox-Fluor Cytotoxicity Assay with and without rituximab by adding fresh donor serum to the media.
Results: Fifty nine patients, 33 males and 26 females, median age of 44 years (range 23-64) were included. Twenty seven (45.8%) patients were defined as "CD20 positive" by commonly accepted criteria defined as a leukemic population with greater than 20% of cells expressing CD20. Median CD20 expression was 17.8% (range: 0.4-99.9%). The median quantity of CD20 was 455 antibodies/cell (range: 92-30552). Of eight specimens which could be cultured *in vitro*, four showed significant up-regulation of both percentage of cells expressing CD20 and quantity of expression in response to dexamethasone, as shown in Figure 1. Of the cells that up-regulated CD20 expression, baseline CD20 expression was 25-30% prior to exposure to dexamethasone. The findings from the B cell lines were similar. Raji cells had a 100% baseline level of CD20 expression, but nonetheless showed marked increase in ABC upon dexamethasone exposure. The other cell lines showed no significant change in CD20 expression upon dexamethasone exposure.
Complement-mediated cytotoxicity data showed a trend toward additional rituximab-induced cytotoxicity after CD20 up-regulation by steroid. Further samples are currently being analysed.

Summary and Conclusion: We have shown that all of the 59 samples of B-ALL tested expressed some level CD20 albeit at lower levels than are considered 'positive' by conventional criteria. Dexamethasone can up-regulate both percentage of cells expressing CD20 and quantity of antigen expression but does not do so in all specimens. A relationship between basal expression and up-regulation remains to be confirmed. On-going work will determine the relationship with rituximab-induced complement-mediated cytotoxicity. The relationship between CD20 expression at diagnosis and anti-leukaemia response to rituximab in adults is unlikely to be linear and may be influenced by prior exposure to steroid.

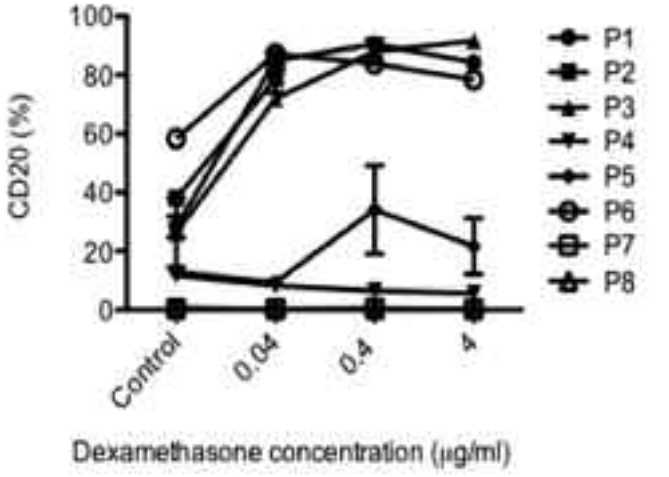

Figure 1. 8 diagnostic samples (P1-P8) showing percentage of blasts expressing CD20 following 3 days of incubation. Mean and standard error of duplicates shown.

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INHIBITION OF CLASS I PHOSPHATIDYLINOSITOL 3-KINASES (PI3KS) ISOFORMS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL): WHICH IS THE BEST THERAPEUTIC STRATEGY?

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Background: Class I phosphatidylinositol 3-kinases (PI3Ks) are heterodimeric lipid kinases consisting of a regulatory subunit and one of four catalytic subunits (p110α, p110β, p110γ or p110δ). p110γ/p110δ PI3Ks are highly enriched in leukocytes. In general, PI3Ks regulate a variety of cellular processes including cell proliferation, survival and metabolism, by generating the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). Their activity is tightly regulated by the phosphatase and tensin homolog (PTEN) lipid phosphatase. PI3Ks are widely implicated in human cancers, and in particular are upregulated in T-cell acute lymphoblastic leukemia (T-ALL), mainly due to loss of PTEN function. At present different compounds which target single or multiple PI3K isoforms have entered clinical trials.
Aims: To explore the role of the different PI3K isoforms in T-ALL, in order to identify the most effective targeted therapy strategy.
Methods: Both PTEN wt (ALL-SIL and DND-41) and PTEN deleted (Jurkat and Loucy) T-ALL cell lines were treated with class I pan-PI3K inhibitors (BKM120 and ZSTK454) or p110α (A-66), p110β (TGX-221), p110γ (AS-605240), p110δ (CAL-101) and p110γ/δ (IPI-145) selective inhibitors (all purchased from Selleck Chemicals, Houston TX, USA), and their effects were evaluated.
Results: In the tested cell lines, flow cytometry analysis evidenced a slight reduction in PIP3 levels after treatment with all the single-isoform inhibitors, whereas pan-PI3K and p110γ/δ inhibition significantly decreased PIP3 level. However, only pan-PI3K inhibition significantly affected cell viability in a dose and time-dependent manner after 48h treatment, with IC_{50} values ranging between 1 and 4 µM, for both BKM120 and ZSTK454, whereas for all the other drugs IC_{50} values were not attained. This finding was confirmed by a time-course analysis of viable cells, which revealed a rapid and drastic antiproliferative effect of the pan-PI3K inhibitors. Only after prolonged drug-exposure (64h) the combined p110γ/δ inhibition exerted a significant antiproliferative effect, in agreement with PIP3 decrease. Consistent with these results, Annexin V/PI staining analysis showed a significant increase of apoptosis after 48h treatment in both PTEN wt and deleted cell lines only after treatment with pan-PI3K inhibitors, whereas cell cycle was not affected either following pan- or single-isoform inhibition. PI3K signaling pathway analysis revealed the inactivation of the main downstream targets already after 1h treatment with pan-PI3K inhibitors, as evidenced by decrease of p-Akt, both at Thr308 and Ser473, p-P70S6K and p-S6RP. Of note, p110γ and p110δ inhibition slightly affected these PI3K targets, but only in some cell lines. Overall, no difference emerged in relationship with PTEN status.
Summary and Conclusion: Due to the roles played by PI3Ks in cancer, many efforts have tried to address the identification of compounds which specifically target PI3K isoforms for a better effectiveness and a lower toxicity. Here we demonstrated that, irrespective of PTEN status, only pan-class I PI3K inhibition is cytotoxic in T-ALL cells, implying that any isoform could sustain leukemic cell proliferation, and suggesting a redundant role played by each isoform. Therefore, our findings strongly support clinical application of pan-class I PI3K rather than single-isoform inhibitors in T-ALL treatment.

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CHARACTERIZATION OF CDKN1B-DELETED CHILDHOOD T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10-15% of pediatric leukemia. According to genetic alterations and gene expression signatures, T-ALL is subdivided into major subtypes comprising the *TAL/LMO*, *TLX1*, *TLX3*, and *HOXA* clusters. Furthermore, a multitude of genetic aberrations, which either occur across all subtypes or are specifically associated with one or the other, collaborate in the pathogenesis of the disease. Recent studies have revealed heterozygous deletions of *CDKN1B*-$p27^{KIP1}$ as one of the most frequent copy number alterations occurring in about 12% of pediatric T-ALL. However, it has not been determined yet whether *CDKN1B* deletions are associated with any of the major subtypes and/or specific mutation patterns.
Aims: The aim of our study was to genetically characterize pediatric T-ALL harboring deletions of the *CDKN1B* gene and to determine whether other recurrent aberrations found in T-ALL may also have an impact on its regulation.
Results: Array comparative genomic hybridization (aCGH) analysis of 102 pediatric T-ALL patients registered in the Austrian ALL-BFM 90, 95, and 2000 trials substantiated the high incidence of *CDKN1B* deletions in this disease. The data obtained by aCGH were confirmed by quantitative genomic PCR, which in one of the cases even revealed a homozygous deletion. In terms of other copy number alterations, the incidence of *CDKN2A/B* deletions in *CDKN1B*-deleted cases was significantly lower as compared to wild-type cases. Furthermore, fluorescence *in situ* hybridization (FISH) and RT-PCR were used to classify patient samples into the major molecular-genetic subtypes (*TLX1*, *TLX3*, *HOXA,* and *TAL/LMO*) based on chromosomal rearrangements. Out of 14 *CDKN1B*-deleted samples, three (21%) belonged to the *HOXA* and only one (7%) to the *TLX3* subtype, which usually account for 10% and 20-25% of cases, respectively. One case each harbored a *TRAD@-MYC* rearrangement and a cryptic *LMO2*-activating deletion. Intriguingly, 45% (5/11) of the *CDKN1B*-deleted patients showed increased *MEF2C* expression levels and were, therefore, assigned to the immature T-ALL subtype, which accounts for roughly 10% of T-ALL. Whereas in one of these cases a *BCL11B* rearrangement and in another case a potential *MEF2C* activating deletion were observed, in the other three patients the underlying genetic alterations resulting in up-regulation of *MEF2C* remain to be determined. To date, in all remaining cases none of the most frequent T-ALL-specific alterations has been detected. In order to assess the *CDKN1B* expression levels, the samples were subjected to quantitative RT-PCR and in several of them *CDKN1B* showed lower transcript levels than expected in cases of heterozygous deletions. Since several genes, such as *NOTCH1, FBXW7*, and *PTEN*, which are frequently affected in T-ALL, may be implicated in *CDKN1B* regulation, a mutation screening was conducted; however, no correlation between mutation patterns and *CDKN1B* expression levels was found.
Summary and Conclusion: Together our data show that *CDKN1B* deletions are a frequent event in T-ALL, that they commonly arise in the *HOXA*/immature subtype, and that *CDKN1B*-deleted cases have a significantly lower incidence of *CDKN2A/B* deletions. This work is supported by the Anniversary Fund of the Austrian National Bank; OeNB Project No. 14444.

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"GENOMIC INVERSE PCR FOR EXPLORATION OF LIGATED BREAKPOINTS" (GIPFEL) - A NEW METHOD TO DETECT THE MOST COMMON TRANSLOCATIONS IN CHILDHOOD LEUKEMIA

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Background: Detection of pre-leukemic cells is only possible in children who subsequently developed full-blown leukemia *e.g.* by backtracking leukemic fusion genes in Guthrie cards or cord blood samples. It remains enigmatic, however, if and at what frequency children with pre-leukemic cells remain healthy and leukemia-free in later life.
Aims: To enable the a priori detection of chromosomal breakpoints without any knowledge of its exact molecular make-up, we developed a PCR based method that allows the prospective detection of the five most common gene fusions associated with childhood leukemia (MLL-AF4, MLL-AF9, MLL-ENL, ETV6-RUNX1, and TCF3-PBX1).
Methods: The "Genomic Inverse PCR for Exploration of Ligated Breakpoints" (GIPFEL) method exploits the unique presence of a genomic translocation joining two different chromosomes. These fragments can be digested and re-circularized by ligation creating a junction across the restriction site whose sequence can be predicted from published genome data. Importantly, the ligation site is independent of the exact translocation position within the individual DNA circle. Published breakpoint regions of all genes involved in the respective translocations were analyzed *in silico* for restriction sites that allow digestion of all possible translocation events to yield fragments smaller than 50 kb. This condition was met for MLL breakpoints (digestion with BamHI), for ETV6-RUNX1 (SacI) and for TCF3-PBX1 (MfeI). Primer pairs were designed amplifying the complete set of theoretically predicted circularized fragments, requiring between 8 and 37 primers depending on the translocation. Genomic DNA was prepared from cell lines and diagnostic specimens by column purification. The equivalent of approximately 4x10e5 cells (2.5 µg DNA) was subjected to the appropriate restriction digest, ligated and removal of remaining linear DNA by exonuclease III. After ethanol precipitation the reaction products were subjected to a partially multiplexed, semi-nested PCR to quantify all possible ligation/junction products specific for the respective translocation. An internal genomic ligation product served as quality control and allowed relative quantification of the specific translocation product.
Results: Cell dilution and mixing studies with cell lines revealed that approximately 40 translocation positive cells in the entire input are sufficient to produce a reliable positive output signal. The method was then tested in a blinded study with 23, 20 and 22 samples obtained from MLL translocation patients and 60, and 30 samples for ETV6-RUNX1 and TCF3-PBX1 cases. There was no false positive result. Detection coverage was 83.3% for MLL-AF4, 66.6% for MLL-AF9, 60% for ETV6-RUNX1, 40% for TCF3-PBX1. MLL-ENL success rates were lower at 23.5 %. This phenomenon was accompanied by the serendipitous detection of more complex genomic rearrangements in two MLL-ENL cases where additional material from other chromosomes was fused to MLL. Taking into consideration the overall frequency of the individual translocations in childhood leukemia, GIPFEL is able to detect an estimated 40% of B-precursor acute lymphoblastic leukemia (ALL) and 30% of all childhood cases.
Summary and Conclusion: GIPFEL is able to uncover and to quantify the presence of common translocations in blood cells without prior knowledge of the exact breakpoint positions. About 4x10e5 nucleated cells are required per translocation to detect a leukemic clone at a frequency of 10e-4. In a defined clinical setting such as the prospective surveillance of patients at risk for leukemia (*e.g.* after previous treatment with topoisomerase inhibitors or following exposure to ionizing radiation) this method represents a valuable addition to the available diagnostic repertoire.

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BAFF-RECEPTOR EXPRESSION IN CHILDHOOD B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B cell-activating factor (BAFF) and its close relative proliferation-inducing ligand (APRIL) belong to the tumor necrosis factor (TNF) family. Physiologically, BAFF mediates the behavior of most B cells through interactions with their family receptors. Among them, only the BAFF receptor (BAFF-R) interacts specifically with BAFF, being the main responsible for primary B cell survival, selection and differentiation, in physiology and in disease conditions.
Aims: We aimed to investigate the potential role of BAFF/BAFF-R axis in Acute Lymphoblastic Leukemia involving B-Cell Precursor (BCP-ALL). With this purpose, we analyzed the pathway in hematological tumor cell lines as well as in primary BM and PB samples from children affected by BCP-ALL.
Methods: RT-PCR assays have been developed to determine BAFF and BAFF-R expression in cell lines. We implemented flow cytometry analysis to assess BAFF-R expression and to analyze the phenotype and the percentage of blast cells in BM and PB diagnosis and follow-up samples of BCP-ALL patients. ELISA analysis has been used to valuate BAFF concentration in plasma samples.
Results: Preliminary results demonstrated that BAFF-R is highly expressed in the B-lymphoid leukemic cell lines, such as REH, TOM1 and NALM-6 (RT-PCR analyses). Its expression is also detectable, although at lower levels, in mixed lymphoid/myeloid phenotype cell lines (such as THP1 and RS4;11), in myeloid K562 cells as well as in U937 histiocytic lymphoma cell lines. Supported by this data, we further collected BM and/or PB of 26 consecutive diagnostic samples of Pediatric BCP-ALL. We analyzed BAFF-R expression by flow cytometry, by indirect staining using a biotinylated antibody anti-BAFF-R and which was revealed by PE conjugated streptavidin antibody. In the same sample we assessed the CD19, CD10 and CD45 direct staining to recognize leukemic blast cells among the residual of normal cells. For each patient, in addition to the diagnostic sample, we analyzed at least one follow up sample (*i.e.* at day+8 or +15). We detected high levels of BAFF-R on CD19+CD10+CD45dim leukemic cells, which persisted during the follow up treatment. Moreover, we separated plasma by centrifugation of n=16 patients to analyze BAFF cytokine levels by ELISA technique, both in BM and PB samples. More interestingly, the ratio of BAFF level (ng/ml) over the number CD19+ blast cells (n°/mmc) revealed that the cytokine is consumed by blast cells at diagnosis and its level reaches physiological threshold after leukemic cell clearance, in follow up samples.
Summary and Conclusion: We assessed the expression of BAFF-R on leukemic blast cells of BCP-ALL patients. Of note, its expression is maintained on residual tumor cells during the early drug treatment, suggesting the potential targeting of this molecule in future advanced treatment approaches. All together, BAFF/BAFF-R axis could have a role in BCP-ALL, although additional studies are required to comprehend its role in the pathogenesis of leukemia.

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SIGNIFICANCE OF ADDITIONAL STRUCTURAL ABERRATIONS IN BONE MARROW CELLS OF 115 CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND HIGH HYPERDIPLOIDY

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Background: High hyperdiploidy (HeH), defined as the presence of 51 to 67 chromosomes in karyotype, is the most common cytogenetic finding in bone marrow cells of childhood B-cell precursor acute lymphoblastic leukemia (B-ALL), occurring in 25-30% of cases. HeH is characterized by nonrandom gain of specific chromosomes and clinically by favorable prognosis. Nevertheless, around a quarter of these children will suffer an adverse event within 5 years after diagnosis, which could be caused by increased genomic instability of leukemic cells leading to creation of additional cryptic structural aberration.
Aims: The aim of this study was to determine the frequency and the recurrence of structural chromosomal aberrations in hyperdiploid cells and to evaluate the impact of these aberrations for prognosis of children with ALL and HeH.
Methods: Karyotypes of all patients were analyzed at the time of diagnosis by conventional cytogenetic analysis and interphase fluorescence *in situ* hybridization (I-FISH) with a panel of Vysis DNA probes (Abbott Molecular) in order to detect heteroploid cells. In patients with structural or suspected cryptic aberrations multicolor FISH and multicolor banding (24*X*Cyte/*X*Cyte Probe Kit; MetaSystems) and array CGH (Cytochip Cancer 4x180K; BlueGnome) were performed. For overall and event free survival Kaplan-Maier analysis and Mantel-Cox test were done.
Results: During the years 1997-2013 we examined 115 children with B-ALL and high hyperdiploidy. This group included 64 boys and 51 girls with median of age 4 years (range 1 - 17) and median of the follow up to 82 months (range 1 - 210). Cryptic structural chromosomal rearrangements were found in 27 (23,5%) of them. Patients with ALL-specific chromosomal aberrations were excluded from the cohort. The majority of structural aberrations were unbalanced and chromosomes the most frequently affected were found to be Nos.: 1, 13, 6, 7 and 21. The most common recurrent abnormality was the duplication of the long arm of chromosome 1 (9x). The minimal duplicated region in all patients was 1q31 to 1q32.3 (22.5 Mb). In addition, the deletion of the long arm of chromosome 13 (5x), rearrangements of the long arm of chromosome 21 (3x) and the deletion of the long arm of chromosome 6 (2x) were proved. Event (relapse and/or exitus) occurred in six patients with high hyperdiploidy and five patients with high hyperdiploidy and structural aberrations, respectively. There was no difference in overall survival between these two cohorts. However, patients with cryptic structural changes showed a tendency to shorter event free survival (p=0,082).
Summary and Conclusion: Non-random structural abnormalities were found in approximately a quarter of children with B-ALL and high hyperdiploidy. Although high hyperdiploidy is well known as a powerful favorable prognostic marker in childhood ALL, our study demonstrates that prognosis may be

influenced by the presence of these structural aberrations. Therefore, a detailed cytogenetic analysis of every patient with high hyperdiploid cell clones by all available methods is necessary to identify those at an increased risk of relapse.
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IMMUNE STATUS IN RELATION TO THE TREATMENT OF CHILDHOOD BCP-ALL ACCORDING TO THE NOPHO ALL2008 PROTOCOL

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Background: Acute lymphoblastic leukaemia (ALL) is the most common type of cancer in children. All children with leukaemia in the Nordic countries are treated according to the common NOPHO ALL2008 protocol. Patients are allocated into risk/treatment groups based on karyotype and levels of minimal residual leukemic (MRD) cells in bone marrow after 1 and 3 months of chemotherapy. Even though the general outcome of the treatment in children with ALL is considered good (5-years EFS: 85%), severe side effects are frequently seen, not least potentially life-threatening infections due to the immune suppression.
Aims: To monitor the immune suppression in childhood B-cell precursor (BCP) ALL to investigate if immune status can predict severe/life-threatening infections.
Methods: A total of 299 samples from 49 Danish ALL patients treated according to NOPHO ALL 2008 treatment protocol were analysed. Consecutive blood samples were collected at diagnosis and at days 0, 15, 29, 79, 92 and during high-risk ALL block therapy (LHR). Lymphocyte subsets including B, T, NK, and CD4 RTE (Recent Thymic Emigrants) cells were quantified by single platform flow cytometry (BD TruCount beads). Data was evaluated in relation to initial treatment stratification of the patients in standard–risk/intermediate-risk (SR/IR), High-Risk (HR) and late HR-treatment (LHR).
Results: Preliminary results show a nadir in all lymphocyte subset counts at treatment-day 15, 29 and LHR, the most severe suppression found in the LHR group. The median CD4 T-cell count at day 15 in the SR/IR group is 200 cells per µL increasing through d29, d79 and d92 to 430, 720, and 930 cells per µL respectively. In the LHR group the CD4 T cell count is severely reduced with a median of 130 cells per µL. The CD4RTE cell count is moderately reduced in the SR/IR group on d15 and approaches normal levels at d29, and reach normal level at d79. In the LHR group, the median CD4RTE cell count is severely reduced throughout the intensive block therapy. For all time points the median B cell count (CD19posCD20pos) is reduced to below normal range (400 cells per µL) compared to age-matched controls without signs of reconstitution. In the LHR group the B cell counts (CD19 pos, CD20 pos) are severely reduced with a median of 5 and 4 cells per µL, respectively.
Summary and Conclusion: The CD4 T-cell count is reduced to critical levels during early induction therapy (d15) and during LHR block treatment. The CD4RTE cell count indicates close-to-normal thymus function in the SR/IR group from the end of induction therapy, while patients during LHR block treatment exhibit suppressed thymus function. B cell counts are reduced at all time-points during treatment and do not normalise throughout treatment. The relation to severe infectious event is currently under investigation, and may indicate when monitoring of CD4 T-cell count can be valuable.

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A COMPREHENSIVE NEW DNA TEST FOR DETECTION OF FUSION GENES IN LEUKEMIA

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Background: Leukemia patients carry a wide range of chromosomal abnormalities, which affect their prognosis and treatment options. Thus, a full and rapid diagnosis of these abnormalities is essential. Testing for this wide range of abnormalities is currently performed by a combination of techniques, including karyotyping, array, FISH, and RT-PCR. At the moment, the tests used are challenging to perform and at times inadequate. Given the sensitivity, specificity, costs and speed of current testing, there is an urgent need for improvement. The development of new technologies based on next generation sequencing (NGS) promises comprehensive analysis of all mutations and chromosomal abnormalities.
Aims: Recently a new technology, Targeted Locus Amplification (TLA), is developed uniquely enabling the targeted complete amplification and NGS of genes of interest including any Single Nucleotide Variants and/or structural variants that might occur. A such, the TLA technology enables the targeted comprehensive NGS of gene-fusions and will detect gene fusions irrespective of the position of the breakpoint and the identity of the fusion partner.
Methods: In a proof-of–principle, a TLA assay was developed for the *MLL* gene, having more than 50 translocation partners and involved in gene fusions in AML patients. We used four cell-lines, positive for a *MLL* gene fusion, namely a *MLL-AF4*, a *MLL-ELN*, a *MLL-AF6*, and a *MLL-AF9* gene fusion. TLA primers were designed on 5 positions across the *MLL* gene. DNA of the cells from the cell lines was cross-linked, digested, religated and TLA amplifications were performed with all 5 primers. The TLA amplicons were then sequenced on an Illumina MiSeq machine. Generated sequences were processed and mapped.
Results: The fusion partner of the MLL gene was correctly identified in all the samples. TLA was also performed in different mixtures of gene-fusion positive and healthy cells to determine the sensitivity. With the current protocol and existing data-analysis tools a sensitivity of 5% was already established. Results from bone marrow samples positive and negative for a MLL rearrangement will be presented.
Summary and Conclusion: Our first results demonstrate the capability of TLA in detection of structural abnormalities. Based on this results diagnostic assays can be developed which will simultaneously detect all types of structural genetic abnormalities and other aberrations relevant to almost all types of leukemia.

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AUTOPHAGY INDUCED BY DEXAMETHASONE MEDIATES GLUCOCORTICOID RESISTANCE IN LYMPHOID MALIGNANCY CELLS

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Background: Glucocorticoid (GC) resistance remains a major obstacle to the successful treatment of lymphoid malignancies, but the precise mechanism of this resistance has not yet been elucidated.
Aims: To explore the role of autophagy in dexamethasone (Dex) induced resistance of human lymphoid malignancy cells.
Methods: Flow cytometer (FCM) analysis was carried out to determine the GC-sensitive cells and GC-resistant cells. The effect of Dex on cell cycle was measured by FCM and western blot (WB) analysis. Autophagy of lymphoid malignancy cell lines CCRF-CEM, Raji and U-937 were detected by transmission electron microscopy (TEM), WB and MDC staining. WB was also carried out to evaluate the effects of Dex on phosphorylation of Akt, P70S6K and 4E-BP1 in Raji and U-937 cells. The effects of Dex combined with autophagic inhibitor chloroquine (CQ) or 3-methyladenine (3-MA) on cell viability and apoptosis were measured by CCK-8 assay and WB.
Results: Dex could significantly induced apoptosis in GC-sensitive CCRF-CEM cells but not in GC-resistant Raji and U-937 cells. Dex could significantly inhibited CCRF-CEM and Raji cell cycle at the G0/G1 phase, but not in U-937 cells. Dex triggered autophagy in Raji and U-937 cells: autophagosome formation was detected by TEM, LC3-I to LC3-II conversion and down-regulation of P62 were measured by WB, and the formation of acidic autophagic vacuoles was determined by MDC staining. Dex didn't induce autophagy in CCRF-CEM cells. WB showed a dramatic reduction of p-Akt, p-P70S6K and p-4E-BP1 expression in Dex-treated Raji and U-937 cells. CCK-8 assay and WB showed no significant effect on cell viability and apoptosis of Raji and U-937 cells treated with Dex, CQ or 3-MA separately, while combination Dex and autophagy inhibitors enhanced the effects of Dex on cell viability and apoptosis.
Summary and Conclusion: Dex induced autophagy in GC-resistant Raji and U-937 cells while failing to trigger autophagy in GC-sensitive CCRF-CEM cell. PI3K-AKT-mTOR pathway was involved in the autophagy induced by Dex. Pharmacological inhibition of autophagy enhanced the effects of Dex on viability and apoptosis in GC-resistant Raji and U-937 cells. Manipulation of autophagy has the potential to improve GC resistance therapeutics, might be offer a new strategy to overcome GC resistance.

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Abstract withdrawn

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EXTREMELY HIGH RATE OF COMPLETE HAEMATOLOGICAL RESPONSE OF ELDERLY PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS BY INNOVATIVE SEQUENTIAL USE OF NILOTINIB AND IMATINIB

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Background: We have explored if the administration of two TKIs, Nilotinib (NIL) and Imatinib (IM) can improve the results without increasing the toxicity in the elderly Ph+ Acute Lymphoblastic Leukemia (ALL) patients.
Aims: We investigate the type and number of BCR-ABL kinase domain mutations developing during and after the study.
Methods: We have designed a study (ClinicalTrials.gov. NCT01025505) in which patients more than 60 years old or unfit for intensive chemotherapy and SCT where treated with two TKIs, NIL 400 mg twice daily, and IM 300 mg twice daily, alternating for 6 weeks for a minimum of 24 weeks (study core) and indefinitely in case of response. The 6-weeks rotation schedule was respected, irrespectively of temporary discontinuations. The primary end-point was the rate of Disease Free Survival (DFS) at 24 weeks (4 courses of treatment); the secondary end points included the evaluation of CHR, CCgR and CMR rates.
Results: 39 patients have been enrolled in 15 Italian hematologic Centers (median age 66 years, range 28-84). Among these, 8 patients were unfit for standard chemotherapy or SCT (median age 50 years, range 28-59). 27 patients were p190, 5 were p210 and 7 were p190/p210. After 6 weeks of treatment, 36 patients were evaluable for response: 34 were in CHR (94%) and 2 in PHR (6%). 23 patients have already completed the study core (24 weeks), 87% were in CHR and 17 are currently continuing therapy in the protocol extension phase. Thus, the OS at 1 year is 79%, and 64% at 2 years. Overall, 1 patient was primarily resistant and 13 patients have relapsed, with a median time to relapse of 7.6 months (range 0.8-16.1 months), for a DFS of 51.3% at 12 months.
Summary and Conclusion: In this small cohort of Ph+ ALL elderly/unfit patients, the rates of relapse and progression were not likely to be different from the rates observed with Imatinib alone.
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GENETIC VARIATION IN FOLATE RELATED GENES AND THEIR ASSOCIATION WITH OUTCOME OF METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Methotrexate (MTX) is an important component of maintenance therapy in childhood acute lymphoblastic leukemia (ALL). However, some patients can develop resistance or adverse drug effects, which may hamper the efficacy of treatment or require drug discontinuation
Aims: Following the hypothesis that gene variants of the MTX action pathway can affect outcome of ALL, we analyzed the influence of thymidylate synthase (TS) enhancer 28-bp tandem repeat and reduced folate carrier gene (RFC80G>A) polymorphism on risk of relapse and toxicity in pediatric ALL.
Methods: The present study was conducted on 99 pediatric ALL patients (67 patients on maintenance therapy for two years and 32 relapsed patients within two years of diagnosis. Detection of TS tandem repeat polymorphism was done by PCR and RFC80G>A polymorphism by PCR – RFLP analysis.
Results: There was no significant association between the genotype frequency of both TS tandem repeats and RFC 80G>A with different grades of hematological and hepatic toxicity. Regarding risk of relapse in childhood ALL, a significant Odds ratio was found for the homozygous triple repeat 3R3R and heterozygous 3R2R compared with homozygous double repeat 2R2R and also, a significant Odds ratio was found for the homozygous AA genotypes compared with GG genotype.
Summary and Conclusion: carriers of TS 3R allele and RFC A allele have an increased risk of relapse. However, these alleles are not associated with toxicity of MTX therapy in childhood ALL. These findings open the possibility of drug dose adjustment and the patients who have lower frequency of chemotherapy toxicity might benefit from an increase in drug dose.

P766

6-MERCAPTOPURINE DOSAGE REDUCTION AND TPMT POLYMORPHISMS IN THE TREATMENT OF CHILDREN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: 6-Mercaptopurine (6MP) is used in the treatment of children with Acute Lymphoblastic Leukemia (ALL) for a long period of time (approximately 2 years). Thiopurine S-methyltransferase (TPMT) catalyzes methylation, deactivating this way the 6MP action. That enzyme is affected by functional polymorphisms which produce a defective enzyme altering 6MP intracellular metabolism and resulting in higher levels of thioguanine nucleotides and therefore in higher levels of toxicity.
Aims: To investigate the association between TPMT-deficient polymorphisms and the needed 6MP dose adjustments, as well as the prevalence of known side-effects such as hematotoxicity, infections, and mucositis.
Methods: We retrospectively studied a group of 47 children receiving 6MP as part of the DFCI 05-01 protocol between 2006 and 2013; 46 children were diagnosed with ALL and 1 with Lymphoblastic Lymphoma B; 22 patients were classified as standard risk, 19 as high risk and 6 as very high risk. This study focused on the continuation phase with a planned duration of 72 weeks, consisting of 24 three-week chemotherapy cycles. 6-MP dose adjustments were made to maintain white blood cell counts between 2 - 3 $x10^3$/mL or whenever related side effects were observed. We analyzed the following parameters: 6MP dose (mg/m^2/day), 6MP dose adjustments, hemoglobin level, leukocyte and platelet counts, infectious episodes, and mucositis. The infectious episodes were graded from 1 to 4: 1 if minor infection requiring no treatment; grade 2 if moderate infection requiring treatment; grade 3 if major infection requiring hospitalization and grade 4 if there was hypotension. *TPMT* genotype (*1, *2, *3A, *3B, *3C) was performed by direct sequencing (ABI3130, AB) in DNA extracted from whole blood.
Results: We studied 47 children, 28 boys and 19 girls, mean age 6.7 ± 3.7 (range 1-17 years). By the time of this first analysis, 40 (85%) had finished treatment, with a median of 24 continuation cycles (range 9-27). The average 6MP dosage was 45.57± 17.47 mg/m^2/day. Of the 47 patients, 44 (93.6%) were *TPMT* wild-type homozygotes and 3 (6.4%) were variant allele carriers: 2 *TPMT*1/*2* and 1 *TPMT*1/*3A*. In a univariate analysis, there was a positive association between 6MP dosage and leukocyte (r=0.6, P<0,0001), neutrophil (r=0.56, P<0,0001) and platelet counts (r=0.58, P<0,0001). Patients with *TPMT* variant alleles had a higher percentage of cycles with average dosage reduction of >50% (P=0.018). The group of patients with *TPMT* variant alleles presented higher percentage of cycles with infectious episodes, particularly grade 2 or more, although no statistically significant differences were found. Regarding the incidence of mucositis no differences were observed between groups.
Summary and Conclusion: Our data show that higher leukocyte and platelet counts are correlated with higher 6MP dosage, suggesting that even though dosage adjustments were made they may not have been enough to maintain white blood cell counts in the target range. Similar results have been reported in previous published studies. In our group, 6.4% of the patients were *TMPT* variant allele carriers, as it has been shown in other series. These individuals needed a significantly higher reduction in the 6MP dosage and showed a tendency to more infectious episodes. Despite these results, we are aware of the limitations of a small study group and therefore we plan to include more patients and analyze other variables in the forthcoming research.

P767

FREQUENCY AND CLINICAL SIGNIFICANCE OF OCCULT CENTRAL NERVOUS SYSTEM INVOLVEMENT AT DIAGNOSIS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS

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Background: 4-5% of adult ALL patients have central nervous system (CNS) involvement at diagnosis, assessed by cytologic analysis of cerebrospinal fluid (CSF). The frequency and clinical significance of occult CNS involvement as assessed by multiparameter flow cytometry –MCF- in adult ALL patients with a negative cytologic study of CSF, remains unknown.
Aims: To analyze the frequency and clinical significance of occult CNS leukemia at diagnosis in adult ALL patients.
Methods: CSF samples obtained at diagnosis from 61 adult patients with ALL were studied. Conventional cytologic (CC) analysis was performed in each participating center and the CC was considered positive if leukemic blast cells were observed after discard of traumatic punctures. MFC analysis was performed centrally. CSF cells were stained using fluorochrome-conjugated antibodies against various B-cell or T-cell antigens depending upon the immunophenotype of the leukemia at diagnosis. From 61 patients, 16 cases showing CC+ and MFC+ were excluded from the study. From the remaining 45 cases, patients with either CC-/MFC+ (occult CNS involvement) or CC-/MFC- (no CNS involvement) were compared.
Results: Median (range) patient age was of 43 (15,78) years and 26 (58%) cases were males. Eight patients (18%) had occult CNS involvement. No differences were found between CC-/MFC+ and CC-/MFC- groups in the clinical, hematologic, phenotypic and cytogenetic characteristics at diagnosis. In addition, no significant differences were observed in terms of complete remission (CR) rates (100% for CC-/MFC+ *vs*. 92% for CC-/MFC- patients, p=0.99), minimal residual disease (MRD) level<0.1% at the end of induction (71% *vs*. 67%, p=0.99) or<0.01% at the end of induction (57% *vs*. 37%, p=0.410), disease-free survival (DFS) at 3-yrs. (58% *vs*. 44%, p=0.522) or overall survival (OS) at 3-yrs (71% *vs*. 49%, p=0.354).
Summary and Conclusion: Although in this small series of adult patients with ALL occult CNS involvement at diagnosis was frequent, it had no impact on CR attainment, MRD level after induction, OS or DFS. Supported in part by Grant RD12/0036/0029 and RD12/0036/0048 from RTICC, Instituto de Salud Carlos III, Spain.

P768

TREATMENT OF RELAPSED ACUTE LEUKEMIA AFTER ALLO-HSCT BY SALVAGE CHEMOTHERAPY WITH LOW-DOSE CYTARABINE AND ACLARUBICIN COMBINED WITH G-CSF PRIMING: MORE EFFECTIVE AND LESS TOXIC?

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Background: Recurrence is a major cause of treatment failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute leukemia, and subsequential treatment options are very limited.
Aims: We evaluate the efficacy and toxicity of cytarabine and aclarubicin combined with granulocyte colony-stimulating factor priming (CAG regimen), consisting of concurrent use of granulocyte colony-stimulating factor (G-CSF) with low-dose cytarabine and aclarubicin, as a salvage therapy for acute leukemia patients who relapsed after allo-HSCT.
Methods: Fifty-nine patients (32 male and 27 female) with acute leukemia, with a median age of 27 years, relapsed post allo-HSCT and received salvage chemotherapy. Twenty-seven patients received CAG regimen while 32 patients received non-CAG regimen such as intensive chemotherapy.
Results: The overall response rate (ORR) of CAG was significantly superior to that of non-CAG groups (55.6% *vs* 28.1%, p=0.033). With regard to the disease type, comparing with non-CAG group, ORR of AML patients in CAG group was significantly higher (64.3% *vs* 20%, p=0.025).(When AML and acute lymphocytic leukemia (ALL) cases were analyzed separately, ORR in CAG group was significantly higher than that in non-CAG group among AML cases(64.3% *vs* 20%, p=0.025)). However, ORR of ALL in CAG group was similar with that in non-CAG group (50% and 35.2% respectively; P=0.471). Median overall survival (OS) from the starting of CAG chemotherapy and 2-year OS of CAG group were 9 (1-27) months and 16.1%. Meanwhile, median OS and 2-year survival of non-CAG group were 4 (1-49) months and 8.8%. Moreover, the median duration of neutropenia and thrombocytopenia of CAG group were significantly shorter than that of non CAG group, 6 (1-12) *vs* 11 (5-28) days (P=0.000) and 8(1-14) *vs* 14 (7-35) days (P=0.000). For the patients who received donor lymphocyte infusion (DLI) as a subsequential therapy, two-year OS of CAG and non-CAG group were 17.2% and 12.5% respectively (P=0.577). Treatment related mortality (TRM) was found in 2 cases in CAG group compared with 10 cases in non-CAG group. For CAG group, impact on ORR was significantly associated with the leukocyte level, and medullar blast percentage at relapse (p=0.005 and p=0.000 respectively).(For CAG group, characteristic associated with a higher likelihood of ORR on univariate analysis was lower tumor burden manifested by leukocyte level (p=0.005) and medullar blast percentage (p=0.000) at post-transplant relapse. Furthermore, the response to chemotherapy shown by multivariate analysis was the only factor that correlated with better survival (p=0.016, HR 0.426, 95%CI (0.213, 0.853))
Summary and Conclusion: CAG regimen as a salvage chemotherapy for relapsed acute leukemia post allo-HSCT could effectively reduce tumor burden with mild toxicity, especially for hypoplastic acute leukemia patients. For certain type of relapsed acute leukemia patients post allo-HSCT, CAG regimen may be an optimal choice as the bridge therapy followed by DLI or second allo-HSCT.

P769

QUANTITATIVE ULTRASOUND OF PROXIMAL PHALANXES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA SURVIVORS

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Background: The long term treatment related morbidities among the growing population of survivors from childhood acute lymphoblastic leukemia (ALL) are a matter of significant concern. Bone mineral density (BMD) impairment is a well-known complication in childhood ALL survivors. The optimal method to assess bone condition is still debated. Quantitative Ultrasound (QUS) of proximal phalanxes is a validated technique to assess BMD in children.
Aims: The aim of our study is to use QUS to assess BMD in ALL survivors and to evaluate the role of cytotoxic dosage in the development of long term bone damage.
Methods: From November 2012 to December 2013, 72 ALL survivors (40 female/32 male) were enrolled in the study in a single Pediatric Hemato-Oncology center. Mean age at diagnosis was 61 ± 45 months (95% CI, 51;41) while median age at QUS was 10.5 ± 4.3 years (95% CI, 9.49; 11.45); the mean period of follow-up from therapy completion was 41.2 ± 37.8 months (95% CI, 32.45; 55.89). Amplitude-dependent speed of sound (AD-SoS) was measured at the phalanxes of the non-dominant hand and expressed as a z-score. Linear correlation with cumulative dosages of cytotoxic agents, age at ALL treatment, Body mass index (BMI) and duration of follow-up were sought.
Results: Median AD-SoS z-score was -1,22 ± 1,19 (95% CI, -1,5; -0,94). Ten subjects (13.8%) presented z-score below -2 S.D. Vitamin D implementation was required in 9/72 (12.5%) survivors. A negative correlation was found between AD-SoS z-score and BMI (P=0.0001), as reported in literature, and age at diagnosis (P=0.01). Positive correlation was observed with duration of follow-up (P=0.01) (Figure 1). No significant correlation was found between z-score and cytotoxic dosage, corrected by sex, age and BMI.

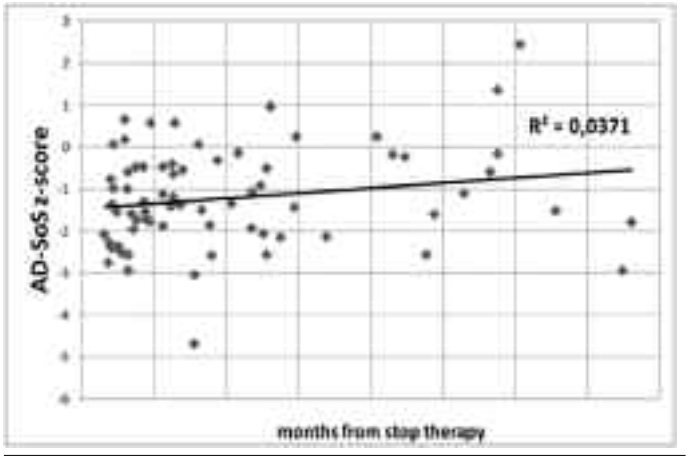

Figure 1.

Summary and Conclusion: Our report represents the largest cohort of childhood ALL survivors submitted to QUS after the stopping of therapy. As reported in Literature, QUS permits an early detection of bone impairment during childhood ALL treatment. In the present study, using QUS, we found that 13.8% of ALL survivors shows a BMD significantly lower than age-sex matched reference population. This effect decreases with duration of disease-free follow-up. No correlation was found with cytotoxic cumulative dosage, while age at

the onset of disease significantly affects BMD. Our data suggest that the major determinants of bone impairment are the disease itself and the phase of bone growth on which disease occurs.

P770

RELATIONSHIP BETWEEN MTHFR POLYMORPHISM AND SIDE EFFECTS OF HIGH-DOSE METHOTREXATE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common type of cancer in children. Administration of with high-dose methotrexate (HDMTX) followed by leucovorin rescue is an important component in the treatment of childhood ALL. methotrexate (MTX) toxicity includes mucositis, myelosuppression, and hepatic toxicity. MTX toxicity can vary with genetic variability in folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR).
Aims: To study the relationship between (MTHFR) gene polymorphism, and the occurrence of complications associated with (HDMTX) in pediatric acute lymphoblastic leukemia (ALL).
Methods: A total of 26 ALL children were studied. Clinical assessments, complete blood count liver and renal functions before and after HDMTX, MTX level measurement after HDMTX, and PCR (restriction fragment length polymorphism) for the MTHFR (C677T and A1298C) polymorphism were carried out. Informed consent was obtained from the legal guardians of the included children.
Results: Plasma MTX level had a relation to HDMTX toxicity specially mucositis. Regarding the C677T MTHFR allele, 14 patients (53.8%) had CT (heterozygous) polymorphism while 7 patients (26.9%) had TT (homozygous - mutant) polymorphism and 5 patients (19.2%) had CC (homozygous -wild) polymorphism. For A1298C allele, 18 patients (69.2%) had AC (heterozygous) polymorphism while 5 patients (19.2%) had AA(homozygous -wild) polymorphism and 3 patients (11.5%) had CC (homozygous -mutant) polymorphism. For C677TMTHFR allele, neutrophils recovery days were higher in TT followed by CT then CC polymorphism, platelet recovery days were higher in TT followed by CT then CC patients and days required for plasma MTX level to decrease to less than 0.1 µmol/l were higher in TT followed by CT then CC polymorphism. For A1298C allele, post-infusion platelet counts were lower in AA than AC and CC polymorphism, platelet recovery days was lower in AA than CC and AC patients. Neutrophils recovery days and days required for plasma MTX level to decrease to less than 0.1 µmol/l were higher in AA followed by AC then CC patients. For A1298C polymorphism, AC patients had the significant higher overall survival in relation to CC and AA patients (Log Rank=6.268, p value<0.05).
Summary and Conclusion: The increase in plasma MTX level after HDMTX might be associated with an increase in the risk for complications. Genotyping of folate polymorphisms might be useful in ALL children to optimize MTX therapy, reducing the associated toxicity with possible effects on survival.

P771

A PHASE I DOSE-ESCALATION STUDY OF MPEG-R-CRISANTASPASE ADMINISTERED BY INTRAVENOUS INFUSION IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY HEMATOLOGICAL MALIGNANCIES

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Background: Asparaginase (ASNase) is an important component of multiagent chemotherapy for treatment of acute lymphoblastic leukemia and lymphoma in children and young adults. However, hypersensitivity reactions still occur in 10% to 30% of patients receiving *Escherichia coli*–derived ASNases, often resulting in treatment discontinuation. To maintain clinical outcomes, patients may be switched to ASNase *Erwinia chrysanthemi,* an immunologically distinct ASNase. Due to the short half-life of ASNase *Erwinia chrysanthemi*, each two-week course of therapy requires 3 administrations a week. To improve pharmacokinetic properties and reduce immunogenicity, recombinant PEGylation technology was used to create a new *Erwinia chrysanthemi*–ASNase, mPEG-r-crisantaspase.
Aims: This is an open-label, multicenter, dose-escalation study to determine the maximum tolerated dose, safety, and pharmacokinetics of mPEG-r-crisantaspase in patients with relapsed or refractory hematological malignancies.
Methods: Patients were eligible for the study if they were aged 18 to 50 years with relapsed or refractory hematological malignancy for which standard curative or life-prolonging treatment was not an option. This study met institutional review board approval and all patients provided written consent. Exclusion criteria included active central nervous system disease or a previous allergic reaction (grade ≥2) to ASNase *Erwinia chrysanthemi*. All patients received 2 intravenous infusions over 60 minutes of mPEG-r-crisantaspase once every 2 to 4 weeks. Patients were allowed to continue dosing until disease progression if judged appropriate by the investigator. Based on preclinical data, 500 IU/m^2 was selected as the initial dose. Dose escalation was based on the number of patients experiencing a dose-limiting toxicity (DLT) within 14 days of the first infusion. If a given dose resulted in ASNase activity ≥0.1 IU/mL for ≥10 days, up to 6 patients could be enrolled at that dose.
Results: Ten patients (mean age 40.6 years) have been enrolled to date; planned enrollment is 36 patients. The study included 5 patients diagnosed with Hodgkin's lymphoma, 3 patients with diffuse large B-cell lymphoma, 1 patient with acute lymphoblastic leukemia, and 1 patient with acute myeloid leukemia. All patients have failed multiple lines of therapies. As the first 3 patients dosed at 500 IU/m^2 maintained ASNase activity >0.1 IU/mL at 14 days, an additional 3 patients were enrolled at this level. No DLT was observed and all 6 patients showed ASNase activity >0.1 IU/mL at 14 days (Figure 1). Fourteen days after the second dose, ASNase activity levels were generally higher compared to 14 days after the first dose, with several patients maintaining target activity levels ≥5 weeks. The Independent Dose Escalation Committee agreed to dose 3 patients at 750 IU/m^2 with a 21-day interval. These patients maintained enzymatic activity >0.1 IU/mL at 14 days, with 1 DLT observed (neutropenia lasting more than 7 days). The most common adverse events (>30%) were diarrhea, anemia, hypoalbuminemia, decreased antithrombin III, and nausea. Three deaths occurred following administration of 500 IU/m^2 mPEG-r-crisantaspase (1 due to cerebral hemorrhage and 2 due to disease progression), and 1 death occurred after infusion of 750 IU/m^2 (disease progression).
Summary and Conclusion: At a dose of 500 IU/m^2, mPEG-r-crisantaspase provides effective serum ASNase activity for 14 days following intravenous infusion. The safety data for 500 IU/m^2 and 750 IU/m^2 are consistent with the known safety profile of ASNase treatment and with comorbidities and disease progression in this patient population.

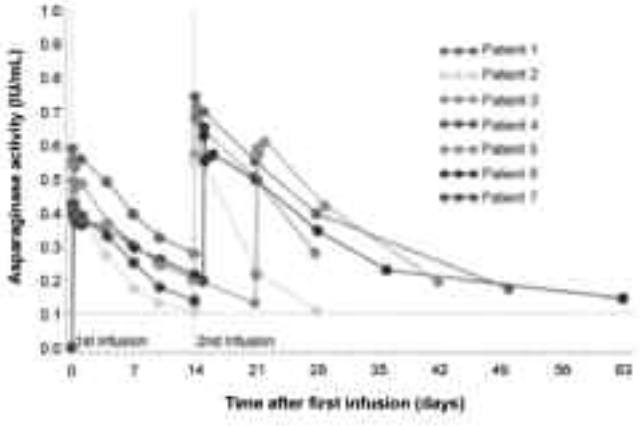

Figure 1. Asparaginase activity following 500 IU/m^2 mPEG-r-crisantaspase (One additional patient was recruited following the death of a patient due to disease progression after the first infusion).

Support: Study funded by Jazz Pharmaceuticals plc or its subsidiaries and conducted in 5 LYSA centers (registration N°NCT01551524).

P772

MINIMAL RESIDUAL DISEASE IN ADULTS WITH PHILADELPHIA NEGATIVE B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA - A SWEDISH POPULATION-BASED STUDY

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Background: Minimal Residual Disease (MRD) is an independent risk factor

for acute lymphoblastic leukemia (ALL). In Sweden, MRD is determined by flow cytometry in Philadelphia negative B-cell precursor ALL (Ph- BCP-ALL). Conversion to high risk protocol, *i.e.* allogeneic stem cell transplantation, is recommended when MRD levels are >1% after remission induction (MRD1) and/or do not reach<0.1% after consolidation therapy, even in the absence of conventional risk factors (WBC >30x10^9/L, t(4;11) or late remission).
Aims: To evaluate the introduction of MRD measurements for Ph- BCP-ALL in Sweden outside randomized controlled trials, in terms of feasibility, cut-off adequacy and correlation to relapse and overall survival in a population-based cohort >45 years old.
Methods: All patients diagnosed with ALL are reported to the Swedish ALL registry where clinical data is assembled prospectively. We included patients diagnosed with Ph- BCP-ALL from 2007 to 2011, aged >45 years and treated with ABCDV/VABA with curative intention. Statistical analysis and Kaplan Meier survival curves were calculated using StatView and SPSS, 95% confidence intervals (CI) were obtained. Overall survival (OS) was defined as time from diagnosis until last follow up or death. Continuous complete remission (CCR) was defined as time from morphologic complete remission to relapse, death in remission or date of last follow-up. The study was approved by the regional ethical committee in Uppsala.
Results: Thirty-five patients, 12 men and 23 women with a median age of 61(range 46-79) years, were included. Twenty-seven of them had a WHO-score 0-1 and eight patients scored 2 or higher. Four patients presented with t(4;11), nine had WBC >30x10^9/L and one had CNS leukemia. Thirty patients (86%) achieved morphologic complete remission (CR) after the first course and another two after the second. Among the responders to the first course, MRD1 was measured in 22 patients and evaluable in 20. Eight of these patients had no detectable MRD (reported as<0,1 or<0,01%) whereas twelve patients had a residual disease (five reported as 0,01-0,099%, two 0,1-0,09%, five >1%). Sixteen patients had an MRD measurement after the second course and twelve after the third. One patient was converted to the high-risk group solely due to MRD-levels. OS at 3 years was 53 (CI 36-79)% and at 5 years 45 (CI 27-62)%. Three patients died in CR because of therapy related complications. Eleven patients received only 1-2 courses and 24 patients followed the protocol. The OS was significantly lower in the former group (p=0,04). OS did not differ significantly between the patients with MRD1 >0.1% and<0,1%. Looking at CCR a good-risk group was identified with MRD1<0,1% and CCR at 3y was 83 (CI 62-100)%. Allogeneic stem cell transplantation was performed in ten patients. Five patients were transplanted in CR1 and five in CR2, no patient in the latter group survived.
Summary and Conclusion: MRD measured by flow cytometry is feasible but it has been proven difficult to obtain samples from all patients when outside a clinical study. Our results are compromised by this fact. The high intensity protocol was tolerable for most of the patients and incomplete treatment corresponded to adverse prognosis. We assume that the stipulated MRD cut-off levels are too high, since only one patient was converted to the high-risk group due to MRD. Patients with MRD<0,1% after remission induction seem to have a favorable prognosis, but validation is needed from a larger cohort.

P773

INVASIVE FUNGAL INFECTIONS IN PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA: RESULTS FROM AN UNIVERSITY HOSPITAL, 2005 TO 2013
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Background: Invasive fungal infections (IFIs) are important causes of morbidity and mortality in children with leukemia. Few reports available in the literature about IFI in pediatric patients with leukemia.
Aims: The aim of the study was to delineate the epidemiologic, clinical features, risk factors, and outcome of invasive fungal infections in this population.
Methods: The medical records of the patients with acute lymphoblastic (n=125) and acute myeloblastic leukemia (n=30) who were treated at Pediatric Hematology Department of Ege University School of Medicine, between 2005 and 2013 were retrospectively reviewed to determine the rate, causative agents, and the outcome of IFI in children.
Results: A total of 57 IFI episodes were recorded in 48 (male/ female=24/24) patients. The rate of IFI among the patients with acute leukemia was 57/155 (36.7%). Patients did not receive primary prophlaxis except four patients with relapsed AML. The median age of the patients was 8.2 (range 1.5-16) years. IFI was classified as proven in 18 (31,5%), probable in 14 (24,5%) and possible in 25 (43,8%) episodes. The causative fungus was microbiologically documented in 17 proven IFI episodes. In 13 of 18 proven IFI episodes *Candida spp. (3 albicans, 4 parapsilosis, 1 krusei, 1 guilliermondii, 3 tropicalis, 1 pellucilosa*) and in 3 proven IFI episodes *Geotrichum capitatum*, in 1 proven IFI episode *G.candidum* and in 1 proven IFI episode *T.asahii* was isolated from blood culture. In 1 episode, deep tissue infection with *Mucor* spp. was histopathologically documented. To be in the induction phase, prolonged and severe neutropenia were found to be major risk factors for IFI. Three month survival of these patients after the diagnosis of IFI was (87,7%). IFI was the main cause of death in 4 patients (7%).
Summary and Conclusion: Our results indicate that non-albicans *Candida* spp. was the most common fungi as the causative agent for IFI. The risk factors for IFI; severe and prolonged neutropenia and to be in the agressive phase of chemotherapy. Prevention, early detection, and advanced treatment strategies are needed to improve the outcome of IFI in children.

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ASPARAGINASE-ASSOCIATED PANCREATITIS (AAP) DURING TREATMENT FOR CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)
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Background: In spite of being a key agent in the treatment of ALL, Asparaginase has serious and possibly fatal adverse effects, such as AAP.
Aims: To look for adverse effects of Asparaginase treatment, particularly AAP, as well as possible factors related to its occurrence.
Methods: We assessed the clinical records of 80 children diagnosed with ALL in a single centre between 2002 and 2013, treated with DFCI protocol 91-01 and 05-01 who were able to complete Consolidation II – 30 weeks of Asparaginase (children under 1-year old, Philadelphia-positive ALL, refractory leukemia after induction, relapse before consolidation II and cases of early death were excluded of the analysis, as well as children with FAB L3). The median follow-up was 52 months [6;135].
Results: The median age at diagnosis was 5 years-old [1;17]; 53.8% were male. Patients presented with signs/symptoms related to cytopenias (86.1%), lymphadenopathy (47.4%), organomegaly (47.4%) and bone pain (19.5%); two (2.5%) had CNS involvement. Median haemoglobin was 8.0 g/dL and median platelet count was 46.5x10^6/L; 16 (20%) patients had >50x10^6/L leucocytes at diagnosis (median: 9.95x10^6/L). Immunophenotype was compatible with precursor B-ALL in 72 (90%) patients. Hyperdiploid karyotype (21.3%) and t(12;21) (18.4%) were the most frequent cytogenetic findings; 4 (5.3%) patients had MLL gene rearrangements. Twelve (16.9%) patients had minimal residual disease >0.01% at the end of remission induction. Patients were treated by standard (51.3%), high (32.6%) and very high risk (17.5%) regimens. Eighteen (22.5%) patients were treated with Native E.coli Asparaginase and 73 (91.3%) with PEG-Asparaginase; three patients had to switch to Erwinase due to allergic reaction. Twelve (15%) patients had a thrombotic event; 61.3% had hepatotoxicity. Eight (10%) patients had AAP; all were treated with the pegylated formulation. Median time to AAP was 21weeks [3;34] after beginning of consolidation treatment; 50% of patients had AAP after the 10th administration. Among the AAP group, 7(87.5%) patients had hypertriglyceridemia, compared to 29(40.3%) in the remaining group (p=.05). Seven (87.5%) had asymptomatic elevation of pancreatic enzymes previously to the AAP episode (p<.0001). Two (25%) patients of those with AAP had T-cell lineage, compared to only 8.3% in the remaining group (p=.035). Five (62.5%) were high/very high risk, compared to 47.2% in the not-AAP group. Two patients died, one (12.5%) due to septic shock (along with pancreatitis); and one other after relapse. The 5-year overall survival was inferior for patients with AAP (98.6% *vs.* 87.5%; p=.05).
Summary and Conclusion: In our group of patients, the incidence of AAP was 10%. Even if clinical or laboratorial parameters cannot predict AAP, close monitoring of pancreatic enzymes and triglyceridemia still play a very important role. The high/very high risk patients may have an increased likelihood of AAP, possibly due to the more aggressive chemotherapy regimens.

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MOLECULAR CYTOGENETIC ANALYSIS IN 108 CHILDHOOD ACUTE LEUKEMIA PATIENTS FROM SAUDI ARABIA: CORRELATION TO MOLECULAR AND OTHER BIOLOGIC FACTORS
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Background: Pediatric acute leukemias are generally characterized by recurrent molecular and cytogenetic abnormalities in both childhood acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The identification of those abnormalities is clinically important because they are considered significant risk-stratifying markers.
Aims: No sufficient data exist regarding cytogenetic profile in Saudi acute pediatric leukemia patients. 108 cases of acute childhood leukemia were examined to determine cytogenetic profile, *FLT3* mutations and frequency of the most chromosomal abnormalities associated with pediatric ALL and AML, and did correlations to other biologic factors and patient outcome.

Methods: *Patients:* from 2004 to 2013, we reviewed all cases with established diagnosis of childhood ALL and AML. Of the 108 patients, 75 were B-lineage ALL, 24 AML and 9 T-cell ALL. ALL patients were treated according to UKALL 2003 protocol while AML according to UKAML 12 protocol. Informed consent was obtained from parents. *Cytogenetic Analysis:* Chromosome banding analysis and fluorescence *in situ* hybridization (FISH) were used to detect genetic aberrations. *Analysis of FLT3 mutations:* Bone marrow or blood samples were screened for *FLT3* mutations (internal tandem duplications, ITDs and point mutations, D835) using polymerase chain reaction methods (PCR).
Results: Table 1 summarizes patient's characteristics and patient's outcome. Cytogenetic analysis showed chromosomal anomalies in 59 out of 92 cases (68 ALL and 24 AML) with overall incidence 64.1% (43/68, 63.2% in ALL and 16/24, 66.7% in AML). The most frequent chromosomal anomalies in ALL were trisomy 21, t(9;22), and t(12;21) while t(8;21), t(15;17), trisomy 8, and MLL in AML. Our data are in accordance with those published showed that *FLT3*-ITD mutation have a strong bad prognostic factor in pediatric AML patients and associated disease progression while t(9;22) and MLL gene rearrangements were signs of a bad prognosis in childhood ALL, with high rate of relapse, shorter overall survival; (P=0.031) and event-free survival (EFS) was also worse (P=0.040) compared to standard risk group.
Summary and Conclusion: These data confirm that the frequencies of cytogenetic abnormalities and its prognostic significance were similar to those reported in the literatures. FLT3 mutations occur in a significant percentage of Saudi Pediatric AML and had bad prognostic relevance while not common in pediatric ALL and did not affect clinical outcome.

Table 1. Patient's characteristics and cytogenetic profile.

Clinical features and outcome			FLT3 Mutations and Cytogenetic alterations		
Parameter	**ALL**	**AML**	**Parameter**	**ALL**	**AML**
Male: Female	51:33	11:13	FLT-ITD	1/46 (2.2%)	2/15 (13.3%)
Median age (years)	5	11	FLT-D835	1/46 (2.2%)	2/15 (13.3%)
Median WBCs Count ($x10^9$/L)	9.2	27	Karyotype	24 normal 6 abnormal	9 normal 6 abnormal
Median Hb (x gm/dl)	8	9	**Risk (Available)**	**n=68**	**n=19**
Median Platelets Count ($x10^9$/L)	37	30	t(9;22)	7 (10.8%)	-
Median BM Blasts	90%	70%	t(12;21)	7 (10.8%)	-
Risk Groups			t(1;19)	1 (1.5%)	-
High	20	4	t(8;21)	-	2 (10.5%)
Intermediate	24	16	t(15;17)	-	2 (10.5%)
Standard	40	4	Inv(16)(p13q22)	-	1 (5.3%)
Remission Status			MLL	3 (4.6%)	2 (10.5%)
CR1	66	18	MYC	1 (1.5%)	-
CR2	7	2	+21	8 (12.3%)	-
CR3	5	1	+9	3 (4.6%)	-
Refractory	6	3	+8	1 (1.5%)	2 (10.5%)
Outcome			Del 12p	4 (6.2%)	-
Alive	69	12	Hyperdiploid	3 (4.6%)	1 (5.3%)
Died	15	8	Pseudodiploidy	2 (3.1%)	0
Lost follow up	0	4	Tetraploidy	1 (1.5%)	1 (5.3%)
5-year survival rate	82%	60%	Others	2 (3.1%)	1 (5.3%)
			Normal FISH	22 (33.9%)	7 (36.8%)

Internal tandem duplications (ITD)
Fluorescence in situ Hybridization (FISH)

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LENALIDOMIDE FOR TREATMENT OF REFRACTORY/RELAPSED ADULT B-ALL PATIENTS: RESULTS OF A MULTICENTRIC PHASE 2 STUDY

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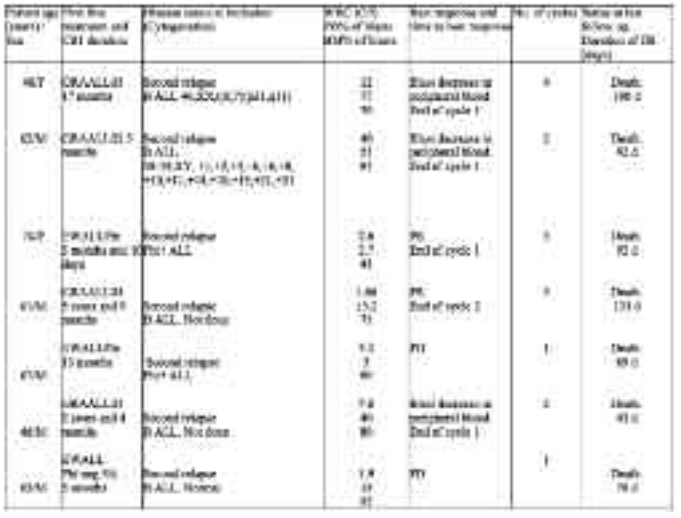
Background: The prognosis of refractory/relapsed adult patients with acute lymphoblastic leukemia (ALL) remains very poor. With conventional therapies, less than half of these patients will achieve a second remission and few will be eligible for allogeneic HSCT, which is regarded as the treatment of choice at this stage of the disease. Novel agents are needed to improve outcomes. Lenalidomide is an immunomodulatory agent with promising results in the treatment of patients with B-cell malignancies. In the past, polychemotherapy (VAD regimen) had demonstrated significant antitumor activity both in ALL and in multiple myeloma. Lenalidomide in combination with dexamethasone is indicated for the treatment of multiple myeloma in relapse.
Aims: Based on pre-clinical data in B-cell malignancies and clinical data in multiple myeloma, this study was conducted to evaluate the safety and efficacy (CR, CRp, PR) of lenalidomide in combination with dexamethasone for the treatment of adult patients with refractory or relapsed B-cell lineage ALL.
Methods: Eight patients with B-ALL (6 patients with precursor B-lymphoblastic ALL and 2 patients with Phi+ ALL with a T315I mutation) were enrolled between February 2010 and August 2013. Lenalidomide was given orally at a dose of 25 mg/day on days 1 through 21 of 28-day cycles, in combination with dexamethasone 40 mg per os once daily on days 1,8,15,22 until achievement of CR, disease progression or intolerable toxicity. One subject was not evaluable because of his death by hemorrhagic complication before the beginning of the treatment. Therefore, only 7 patients were included in the analysis.
Results: Patients had a median age of 63.5 years (range, 46 to 74 years), a median of 2 prior therapies (range, one to three therapies), and received a median of 2 treatment cycles (range, one to four cycles) of therapy. The overall response rate was 28.6% with 2 patients achieving partial response after one course. Three other patients who had shown sufficient treatment activity (with a blast decrease ≥ 50% from the baseline in the peripheral blood) carried on with a second cycle of treatment. Response duration was short and no patient could undergo bone marrow transplant procedure. The hematologic toxicity included grade 4 neutropenia and grade 4 thrombopenia in 4 and 5 patients, respectively. The median duration of ANC<0.5 G/l and platelets<20 G/l were 15 and 13 days, respectively. Infectious complications requiring hospitalization occurred in 5 patients (gram-negative bacteremia, *Listeria monocytogenes* bacteremia, pneumococcal bacteremia and 2 pneumonias not otherwise specified). No patient experienced tumor lysis syndrome or thrombotic complications. Median OS was 92 days and death was the result of progressive disease in all patients.
Summary and Conclusion: Although the lenalidomide-dexamethasone regimen failed to improve the outcome of this very high risk population, this study showed some responses to lenalidomide with a manageable toxicity, comparable to any other anti-leukemic treatment. Combination of lenalidomide with chemotherapy or monoclonal antibodies should be tested in the future.

Table 1.



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ENDOCRINE SIDE EFFECTS AFTER CHEMOTHERAPY IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Focus on long-term side-effects after cancer therapy in childhood has become of the crucial importance. The exposure of chemotherapy at young ages has increased vulnerability to long-term treatment induced sequelae. Also contribution of radiation may increase adverse sequelae.
Aims: We aimed to evaluate endocrine side effects in patients with acute lymphoblastic leukemia being followed-up 1-5 years after treatment at İzmir Dr. Behçet Uz Children's Hospital. Study included 41 patients (M/F=20/21) and 20 of them were applied prophylactic cranial RT and remaned weren't.
Methods: Patients were screened for endocrine side effects, growth, blood glucose metabolism, lipid metabolism abnormalities, sexual development, thyroid metabolism, bone mineral density and adrenal insufficiency. Height, weigth, biochemical parameters (Ca, P, ALP, preprandial blood glucose, serum lipid profile), hormones parameters (PTH, 25-OH D vitamine, FSH, LH, Testosteron, Estrogen, TSH, fT3, fT4, thyroid antibody, ACTH, Cortisol), IGF-1 and IGFB-3 were noted from patient records. Thyroid ultrasound, pelvic

ultrasound in girls, DEXA Z for bone mineral density (BMD) were analized. Body mass index, HOMA-IR, glucose/insuline were calculated with the data gathered from patient files. All of the patients were evaluated fort he effects of RT and chemotherapy (KT).
Results: Mean age of the patients were 10±3,1 years. In 35 of 41 (85,3%) patients at least one endocrine complication was detected. One patient's (2,4%) height was under 3 percentile, 12 (29,3%) patients were obese. All the patients had normal preprandial blood glucose but 14 (34,1%) patients had insuline resistance. Three (7,3%) patients had IGF levels under -2 SDS, 2 (4,9%) patients had high FSH-LH levels. There was no puberte precox or tarda. One (2,4%) patient had subclinical hypothyroidy, one (2,4%) patient had positive thyroid antibodies. Eight (19,5%) patients had thyroiditis, three (7,3%) patients had nodules, two (4,9%) patients had cysts at thyroid ultrasound. Twenty (48,9%) patients had low levels of cortisol. All patients had normal levels of DEXA Z scores. Six (14,6%) patients had subclinical D vitamine deficiency, two (4,9%) patients had subclinical hyperparathyroidy, three (7,3%) patients had isolated hypercalcemia. Only insulin resistance rate was higher in patient who were apllied RT than those who weren't apllied RT. Endocrine side effects were evaluated according to the time elapsed after treatment only D vitamine had significant difference. In our study, RT only posesed a risk for insulin resistance. This could be because of low RT dose and short time interval after RT treatment. In our study, lack of difference in obesity rate between patients who were and weren't apllied RT, and obesity prevalance being higher than general population suggest the contribution of CT, especially steroids with well known effect. Due to low numbers of patients with T-cell ALL and/or HRG, which are treated with high doses of chemotherapeutic agents, the relation between side effects and CT doses could not be evaluated. Although endocrine side effects were more common in the 4-5 years after treatment, significant increase was seen in the second year also these side effects are expected to be more frequent as the time after treatment increase.
Summary and Conclusion: Endocrine side effects were observed in ALL patients who survived after treatment. These patients should be followed-up closely for endocrinological side effects, and early diagnosis and treatment should be performed for endocrinological disases which may develop later in life.

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TRANSFUSION IRON INTAKE IN TRANSPLANT ELIGIBLE PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: RETROSPECTIVE ANALYSIS OF IRON LOAD AT THE END OF CHEMOTHERAPY PROGRAM AND BEFORE TRANSPLANTATION

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Background: Iron overload is an important adverse prognostic factor for patients undergoing hematopoietic stem cell transplantation (HSCT), increasing the risk of infections, veno-occlusive disease and hepatic dysfunction. Moreover, it seems from literature that elevated pretransplant ferritin levels are associated with higher non relapse mortality following HSCT and might influence the risk of acute and chronic GVHD. The majority of data derives from studies regarding patients with thalassemia and myelodysplastic syndromes, with established iron chelation strategies in these diseases. On the contrary, the extent of transfusion iron overload in transplant eligible acute lymphoblastic leukemia (ALL) and the need of iron chelation therapy (ICT) in this patients remains debated.
Aims: To evaluate transfusional iron intake in potentially transplant eligible patients with ALL treated with conventional chemotherapy regimens, focusing on patients who underwent HSCT or completed consolidation chemotherapy courses.
Methods: we retrospectively analysed 29 potentially transplant eligible ALL patients from January 2010 to February 2014 (15 males, 14 females, median age 46years): 24 were B-ALL, 8 of them Philadelphia (Ph) positive; 5 were T-ALL. All patients completed their treatment program: 16 of them were treated with induction and consolidation chemotherapy followed by HSCT, while 13 patients received only chemotherapy. All patients underwent induction therapy according to IVAP regimen (prednisone, cyclophosphamide, idarubicin, vincristine, L-asparaginase, dexamethasone), followed by sequential consolidation therapy: high dose MTX/cytarabine or MTX/L-asparaginase, alternated with low dose chemotherapy. Ph positive patients received TKI in association with standard chemotherapy, omitting L-asparaginase. Iron intake until HSCT or until the end of consolidation chemotherapy, expressed in mg of iron, was calculated as total amount of red blood cells (RBCs) transfused X 1.08. Ferritin levels at the end of the treatment were also evaluated.
Results: Mean transfusional iron intake was 0.45 mg/kg per day, among a mean reatment period of 7.62 months. Mean ferritin level at the end of the treatment or before transplant conditioning regimen was 1380 µg/L (median 1051 µg/L).
Summary and Conclusion: This retrospective evaluation confirms a relevant transfusion iron load in ALL in a short time interval; of note, patients with β-thalassemia major or other refractory anemias receiving 2-4 units of blood per month have a transfusion iron intake of 0.3-0.6 mg/kg per day. It is known that serum ferritin is not specific for iron overload and is a poor predictor of body iron burden, and maybe it is even less specific in ALL as a reliable marker in monitoring iron loading. Moreover, the optimal ICT in ALL patients is still to be defined: subcutaneous deferoxamine infusion is inconvenient and troublesome due to thrombocytopenia and neutropenia, while oral deferasirox administred in proven iron overloaded patients may not be sufficiently rapid in his action if we consider the short time available before transplantation and concomitant toxicity of chemotherapy administred, particularly on hepatic function. Efficacy of early low dose deferasirox administration at the beginning of chemotherapy in ALL patients could be an interesting field of investigation; special attention to the possible interactions with chemotherapy suggests appropriate clinical studies with monitoring of safety.

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DECREASE EXPRESSION OF MIR-20A PROMOTES CANCER CELL PROLIFERATION AND PREDICTS POOR SURVIVAL OF ACUTE MYELOID LEUKEMIA

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Background: MicroRNAs (miRNAs) are a family of 19- to 25-nucleotides non-coding small RNAs that primarily function as gene regulators. Growing evidences indicate miRNAs play important roles in cancer development, progression, metastasis and may constitute robust biomarkers for cancer prognosis. To date, although certain miRNAs have been established a clear oncogenic role in hematological malignancies, other individual miRNAs potentially involved in human leukemogenesis still remain elusive.
Aims: This study aimed to determine the clinical characteristics and prognostic significance of microRNA-20a (miR-20a) in adult de novo acute myeloid leukemia (AML) patients.
Methods: The expression levels of miR-20a in bone marrow mononuclear cells were measured in 98 newly diagnosed AML patients and 20 cases of normal healthy donors by real-time quantitative polymerase chain reaction. Kaplan-Meier and Cox proportional regression analyses were utilized to determine the association of miR-20a with survival of patients. The potential functions of miR-20a on proliferation were evaluated by proliferation and flow cytometry analysis. The direct target gene of miR-20a was also identified by luciferase reporter assays.
Results: miR-20a was expressed at significantly lower levels in the bone marrow of AML patients compared with normal controls. Patients with lower miR-20a expression had significantly poorer complete remission (CR) rates (Log rank p<0.001), relapse-free survival (RFS, Log rank p<0.001) and overall survival (OS, Log rank p<0.001). Low miR-20a expressers had lower CR rates and OS within the Southwest Oncology Group classification. Multivariate analysis revealed that lower miR-20a was an independent predictor of poor prognosis. MiR-20a restoration could result in low levels of cAMP and weak activity of PKA, thus relieving the inhibitory effect of PKA on mononuclear-leukemic cell proliferation. Subsequent investigations revealed that miR-20a directly reduced the endogenous protein level of myeloid cell leukemia sequence 1 (Mcl-1) in mononuclear-leukemic cell.
Summary and Conclusion: MiR-20a is decreased in AML and correlates with AML prognosis. Down-regulation of miR-20a increases the proliferation abilities of AML cells. Our findings suggest miR-20a may represent a novel potential therapeutic target and biomarker for survival of AML patients.

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COPY NUMBER GAINS OF CHROMOSOME 1P36 LEAD TO PRDM16 OVEREXPRESSION IN AML PATIENTS

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Background: PRDM16 gene (1p36) is rearranged in AML/MDS with t(1;3)(p36;q21), t(1;21)(p36;q22) and t(1;12)(p36;p13). These translocation resulted in PRDM16 overexpression through juxtaposition to the enhancer of RPN1 at 3q21 or through fusion transcript formation with RUNX1at 21q22 or ETV6 at 12p13, respectively. AML/MDS with t(1;3)(p36;q21) showed similar clinical and prognostic characteristics with AML/MDS with inv(3)/t(3;3) and EVI1 rearrangements. PRDM16 overexpression has been reported in AML in absence of 1p36 rearrangements, but the mechanisms are still unknown and the studies not conclusive.
Aims: To characterize PRDM16 in cases with 1p36 abnormalities and to assess PRDM16 expression in a cohort of AML without 1p36 involvement.
Methods: The study group was composed of 14 AML and MDS cases with 1p36 abnormalities and a cohort of 80 AML without 1p36 involvement by conventional cytogenetic (CC) and were analysed by relative RQ-PCR and FISH using 3 BAC probes covering PRDM16 and its flanking regions (BlueGnome Ltd. Cambridge, UK).
Results: We identified 14 cases with 1p36 abnormalities: FISH analysis of 13 available samples identified 4 cases with PRDM16 rearrangement. Two cases showed a t(1;3)(p36;q21), one a t(1;21)(p36;q22) and the last one an add(1)(p36) in CC. In 3 cases the breakpoint was at 5' of PRDM16, whereas in t(1;21) it was at 3' of PRDM16, which is a rare event. We were not able to identify the chromosome partner involved in the case with add(1)(p36). Other 3 cases showed copy number gains of PRDM16 identified as the presence from 3 to 5 signals with all the three probes used in interphase FISH. To exclude the amplification of the whole p arm we used a control probe on 1p32 that did not show amplification. Metaphases FISH localized the site of amplification on the der(1)(p36) in two cases, whereas it was localized on unidentified chromosome in the last one. Rearrangements and amplification of PRDM16 were associated with overexpression by RQ-PCR. High levels of PRDM16 expression (greater that three standard deviations above the mean 2-ΔΔCt of 10 normal bone marrow controls) were observed in a significant subset of AML with normal karyotype (AML-NK) (12/25;48%) and with adverse cytogenetic prognostic group (3/11;27.3%) but they were also associated with isolated rare translocations (4/10;40%). In 3 cases with a complex karyotype FISH analysis detected an extra copy of PRDM16. Cases with gene amplification were associated with older age, complex karyotype involving chromosome 5 and 7 abnormalities, higher WBC and AML compared to cases with PRDM16 translocations.
Summary and Conclusion: PRDM16 gene is a frequent target of 1p36 abnormalities in AML. Copy number gain of PRDM16 is a recurrent genetic abnormality in AML with 1p36 abnormalities and, although less common, in AML with complex karyotype but undetectable 1p36 abnormalities. Copy number gain of chromosome 1p36 has not been previously associated with PRDM16 overexpression in patients with myeloid malignancies. We also demonstrated overexpression of PRDM16 in different subgroups of AML without PRDM16 rearrangements or amplification, especially in AML-NK subset. Since the poor prognosis associated to PRDM16, the role and prevalence of PRDM16 expression should be addressed further in a larger cohort of patient. Although the limited case series, amplification seems to be associated with distinct cytogenetic and clinical features and could reflect a different pathway or role of PRDM16 in the pathogenesis of these AML patients. *Supported by University of Bologna RFO, BolognAil and Coop Reno.*

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CHARACTERIZATION OF THE RARE TRANSLOCATION T(3;10)(Q26;Q21) IN AN ACUTE MYELOID LEUKEMIA PATIENT

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Background: Recently, we introduced a flexible strategy for mapping cytogenetically identified unique abnormalities down to the single nucleotide level. This strategy has enabled us to design clone-specific assays for sensitive minimal residual disease (MRD) monitoring in acute leukemia patients (Jancuskova *et al*. Leuk Res. 2013) as well as to elucidate regions/genes involved in congenital chromosomal aberrations (unpublished data). Here we characterize the rare chromosomal translocation t(3;10)(q26;q21), involving the *MECOM* gene (MDS and EVI1 complex locus located in band 3q26), identified in an acute myeloid leukemia (AML) patient.
Aims: Our aim was to use our strategy to identify the fusion partner on chromosome 10q21 and to characterize the precise nucleotide sequence of the chromosomal breakpoint.
Methods: The chromosomal translocation was revealed by standard cytogenetic techniques (G-banding, mFISH), and involvement of the *MECOM* gene was confirmed by FISH with the use of a commercially available probe set. The derivative chromosome 10 was isolated using fine-needle microdissection followed by whole genome amplification (WGA). Ten dissected fragments were sequenced on the GS-Junior next-generation sequencing platform. The reads obtained were aligned to reference sequences of chromosomes 3 and 10 using in-house developed software. The last mapped reads from both chromosomes were used as docking sites for primers for long-range PCR to amplify the putative breakpoint. The long-range PCR products were directly sequenced using Sanger sequencing to reveal the precise nucleotide sequence of the breakpoint.
Results: Using a combination of cytogenetic and molecular approaches, we mapped the t(3;10)(q26;q21) to the single nucleotide level, revealing a fusion of the *MECOM* gene (3q26.2) and *C10orf107* (10q21.2).
Summary and Conclusion: In AML patients, the *MECOM* gene can be rearranged with a variety of other partner chromosomes and partner genes. According to the Mitelman database, only one case with a t(3;10)(q26;q21) translocation has been reported, but neither the fusion partner of the *MECOM* gene nor DNA sequence were identified. The approach described here opens up new possibilities in characterizing acquired as well as congenital chromosomal aberrations. In addition, DNA sequences of chromosomal breakpoints may be a useful tool for unique molecular MRD target identification in acute leukemia patients.

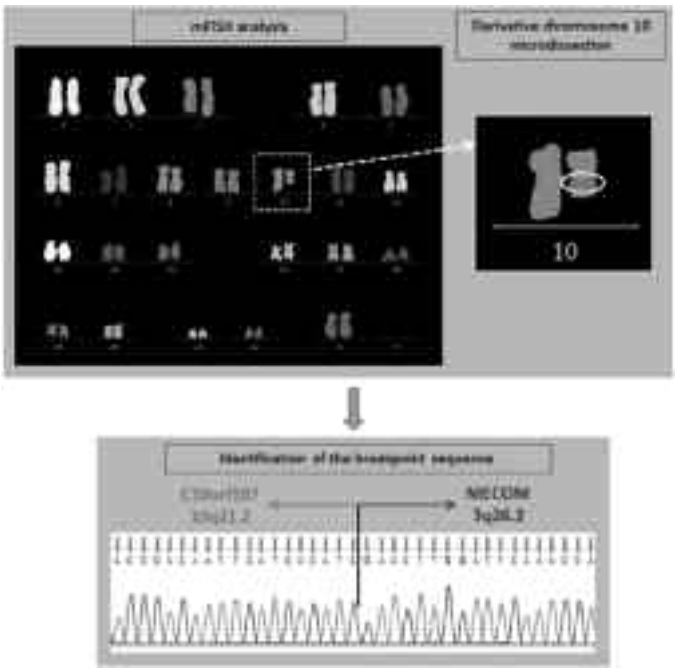

Figure 1.

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GSX2 DEREGULATION IN CD7+ ACUTE MYELOID LEUKEMIA BEARING 4Q12 TRANSLOCATIONS WITHOUT FUSION GENES

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Background: CD7 positivity (CD7+) in approximately 30% of acute myeloid leukemias (AML) is assumed to arise from an early precursor maintaining both myeloid and T-lymphoid potentialities[1]. Interestingly, several chromosome translocations in AML were associated with CD7 expression[2,3,4]. One is the rare t(4;12)(q12;p13), with an estimated incidence of under 1% of adult AML, which is characterized by immature phenotype and poor clinical outcome[2]. Molecular characterization of t(4;12)(q12;p13)[3] showed that 12p13 breakpoints fell within *ETV6* gene, while 4q12 breakpoints involved either *CHIC2* or no genes. Whatever genomic site recombined with 4q12, the pathogenetic effect appeared to be linked to ectopic *GSX2* gene expression[3]. This homeobox gene is normally expressed only in neural stem cells as a key regulator of neurogenesis[5].

Aims: To investigate molecular features of translocations with 4q12 breakpoint in CD7+ acute myeloid leukemias.

Methods: FISH and expression studies were performed on bone marrow cells from two patients with AML-M0 and a 4q12 chromosomal breakpoint. Both had small pseudo-lymphoid blasts[6] with agranular basophilic cytoplasm and CD7 expression. Karyotypes were: 37-46, XX, del(3) (p?), t(4;12) (q12;p13), del (5) (q13q31), -15, -16, del (17) (q21q22), del (22) (q?), +mar1, +mar2 [cp9]/46, XX in patient 1 and 46, XY, t(4;17) (q12;q22)[12]/46, XY[8] in patient 2. The 4q12 region was investigated with LSI 4q12 Tricolor Rearrangement Probe (Vysis, Abbott Molecular) and with homebrew BAC and fosmids (Figure 1). A break apart FISH assay (RP11-434C1 for the 5' and RP11-418C2+RP11-297N18 for the 3') investigated *ETV6* at 12p13 in patient 1; fosmids used for 17q22 in patient 2 are listed in Figure 1. qRT-PCR was performed using Light Cycler 480 (Roche) and TaqMan assay probe (Applied Biosystems) Hs00370195_m1for *GSX2* gene. *ABL1*(Hs00245445_m1) was the endogenous reference control. RNA from GL15 cell line was our positive control for *GSX2* expression (http://www.ncbi.nlm.nih.gov/UniGene/). In silico analysis was performed using ucsc database (http://genome.ucsc.edu/).

Results: Breakpoints at 4q12 fell about 15 kb centromeric to *GSX2* in patient 1, and 50kb telomeric to *GSX2* in patient 2. Translocation partners were, respectively, *ETV6* (12p13) and *MSI2* (17q22). *GSX2* was significantly over-expressed in both cases compared with healthy donors and a group of CD7+ AML without 4q12 involvement.

Summary and Conclusion: *GSX2* de-regulation derived from two different recombinations involving 4q12 and *ETV6* or *MSI2* respectively. A t(4;17)(q12;q22) with a *MSI2* rearrangement has never been reported so far, although in myeloid malignancies with a t(3;17) translocation over-expressed *EVI1* rearranged with MSI2[7]. Interestingly, haematological features of all the available cases suggest that *GSX2* ectopic expression delineates a genetic subgroup among CD7+ AML. Although the fine mechanisms of *GSX2* de-regulation remain to be understood, in our cases we hypothesize that they are related to inactivation of silencers located close to *GSX2,* at 5' in patient 1 and at 3' in patient 2.

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A

Clone	Region	Bp	Gene/Locus
RP11-3H20	4q12	54471086-54623941	
RP11-512P14	4q12	54760890-54951875	CHIC2
G248P800746D1	4q12	54918796-54958873	5' CHIC2
G248P87717F7	4q12	54946506-54984553	GSX2
G248P84085H6	4q12	54974879-55013975	
G248P86829F10	4q12	55025784-55068881	
RP11-601I15	4q12	54997333-55137334	5' ADGRA
RP11-231C18	4q12	55127326-55257486	3' ADGRA
RP11-28O10	4q12	55208225-55360614	

B

Clone	Region	Bp	Gene/Locus
RP11-169P13	17q22	55039702-55213145	SCRIB1, AKAP1
RP11-426D19	17q22	55094894-55458319	5' MSI2
G248P84443B9	17q22	55451728-55490010	MSI2
RP11-226M10	17q22	55458320-55566881	MSI2
G248P852H2	17q22	55482207-55536853	MSI2
G248P8569B4	17q22	55509086-55553237	MSI2
G248P82274C7	17q22	55554187-55594685	MSI2
G248P8510B5	17q22	55583294-55636954	MSI2
RP11-118E18	17q22	55566862-55712080	MSI2
RP11-34I18	17q22	55658517-55831437	MRPS23, CUEDC1
RP11-113K1	17q22	56729425-56892390	5' TEX14, RAD51C, 5' PPM1E

Figure 1.

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TELOMERE LENGTH IS SIGNIFICANTLY SHORTENED IN AML PATIENTS IN CYTOGENETIC REMISSION: POSSIBLE IMPLICATIONS ON THE ORIGIN OF AML

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Background: Telomere length (TL) reflects the replicative history of cells and represents an established marker for tissue aging. Critical short telomeres have been found to play an important role in the development of chromosomal instability and malignant transformation. Acute myeloid leukemia (AML) is a malignant, genetically heterogeneous disease primarily occurring in later adulthood. Despite of tremendous progress, the origin of AML and especially the relationship to aging is not well understood.

Aims: The question whether AML originates from prematurely aged hematopoietic stem cells (HSC) is still unclear. In the present study, we address this issue by sequentially analyzing TL in AML patients at diagnosis, in remission and during refractory disease or relapse.

Methods: TL of bone marrow (BM, n=88) and peripheral blood (PB, n=9) of 45 newly diagnosed AML patients was analyzed using monochrome multiplex quantitative-PCR. PB leukocytes of 87 healthy controls were used for age-adaption of TL. Mean age of the analyzed AML patients was 50.4 years (range 21-75). Follow-up included the following timepoints: at diagnosis (n=20), after two cycles of induction chemotherapy (IC, n=42), after three additional cycles of consolidation chemotherapy (CC, n=30) and one year after diagnosis (n=5).

Results: Newly diagnosed AML patients showed a significantly shortened age-adapted TL (mean±SE: -0.65±0.2 T/S ratio, p=0.001). After induction chemotherapy and compared to TL at diagnosis, TL increased in patients with complete remission (CR, -0.33±0.1 T/S ratio, n=34), but still remained moderately shortened compared to age-matched controls (p=0.001). In comparison, patients with persistent AML showed no change in TL (-0.61±0.2

T/S ratio, n=8). TL of patients in CR remained stable following three additional cycles of consolidation chemotherapy (-0.23±0.1 T/S ratio, n=26, p=0.03) as well as after one year (-0.25±0.1 T/S ratio, n=5, p=0.28). Patients with relapse after consolidation chemotherapy remained substantially shortened (-1.0±0.2 T/S ratio, n=4, p=0.04). To exclude treatment related effects as a predominant reason for TL shortening, we longitudinally followed selected individual patients. We found no evidence for accelerated TL shortening in CR after induction (-0.28±0.1 T/S ratio, n=24), after consolidation chemotherapy (-0.18±0.1 T/S ratio, n=24) and at one year follow-up (-0.25±0.1 T/S ratio, n=5) supporting the notion that chemotherapy does not influence TL during CR.
Summary and Conclusion: In this study we show that TL of AML at diagnosis is substantially short and increases - reaching cytogenetic remission - mostly due to a shift from leukemic cells (with shortened TL) to non-clonal cells. However, age-adapted TL of patients in cytogenetic remission is still significantly short in first CR and remains stably shortened after further treatment up until one year of follow-up. In summary, our data provides first evidence that AML possibly originates from HSC with prematurely shortened TL. The degree of TL shortening found in non-clonal cells during first CR can be translated into approx. 22.6 years of additional aging.

P784

COMPLEX CHROMOSOMAL REARRANGEMENTS LEADING TO EVI1 OVEREXPRESSION ARE RECURRENT MECHANISMS IN CASES WITH VARIOUS 3Q ABNORMALITIES

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Background: Chromosomal rearrangements involving 3q26 region are a recurrent finding in myeloid malignancies. These abnormalities lead to up-regulation of EVI1 gene that has been associated with a very poor prognosis. EVI1 is also overexpressed in different subgroups of AML without cytogenetic abnormalities of 3q26, such as AML with -7/7q- abnormalities, which are also the most frequent additional abnormalities to EVI1 rearrangements. In some cases, FISH analysis revealed cryptic EVI1 rearrangements.
Aims: To identify EVI1 rearrangements in cases with myeloid malignancies and 3q or -7/7q- abnormalities.
Methods: The cases were selected according to the presence of 3q abnormalities or -7/7q- by conventional cytogenetics (CC) and were analysed by FISH using EVI1 breakapart probes (Cytocell, UK), and by relative RQ-PCR.
Results: We analysed 97 AML, 28 SMD and 9 MPD cases with 3q (n=75) or -7/7q- (n=60) abnormalities. Among cases with 3q abnormalities, 24 showed inv(3)/t(3;3), 13 balanced 3q26 translocations, 11 balanced 3q21 translocations and 27 various 3q abnormalities involving different loci from 3q26. EVI1 rearrangements were detected in 32/37 (86.5%) cases with 3q26 abnormalities, whereas 5/37 (13.5%) had 3q26 abnormalities without EVI1 involvement suggesting the presence of other genes implicated in leukemogenesis. Unexpectedly, 4/11 (36.4%) cases with balanced t(3q21) displayed EVI1 rearrangements. To better characterize these latter cases, metaphase FISH analyses was performed and revealed that EVI1 rearrangements were the consequence of complex mechanisms involving multiple breakpoints on 3q arm that masked 3q26 region involvement. Multiple breakpoints were also identified in a t(3;8)(q26;q22) by interphase FISH suggesting that this mechanism can occur in apparent classical 3q26 translocation, too. EVI1 rearrangement was also detected in 2/27 (7.4%) cases with various 3q abnormalities: metaphase FISH revealed a t(3;6)(q26;q25) and a t(1;3;13)(p34;q26;q14) that were cryptic because of suboptimal quality of metaphases. All cases with EVI1 rearrangements detected by FISH showed overexpression by RQ-PCR compared to 10 normal bone marrow samples. Among cases with 7/7q- abnormalities, only one case demonstrated EVI1 involvement by FISH. A further examination of the karyotype allowed the identification of a sub-clone with 3q abnormality harbouring EVI1 gene amplification (>50 copies) not previously identified. Gene amplification is a mechanism leading to overexpression that has been rarely described for EVI1. Other 8 cases showed elevated EVI1 expression without EVI1 rearrangements. Thirty-nine cases showed EVI1 rearrangement. In 12 (30.8%) cases it was the sole cytogenetic abnormality; -7/7q- was found in 25 cases (64.1%); del(5q) was seen in 7 cases (17.9%) and complex karyotype in 10 cases (25.6%). The majority of patients were diagnosed as *de novo* AML or MDS (61.5%), median age was 51 years; 24 were male and 15 female. Only 5 of 24 treated patients (20.8%) achieved a complete cytogenetic remission after more than one cycle of chemotherapy. The median survival of EVI1 rearranged cases was 10.2 months, with an overall survival of 33% at 1 year, and 5% at 3 and 5 years.
Summary and Conclusion: Although 3q26 abnormalities are strictly associated with EVI1 rearrangements, FISH and RQ-PCR identified cases with 3q26 abnormalities involving EVI1 and cases with EVI1 rearrangements without involvement of 3q26 by CC. These events could be the consequence of a low quality of metaphases, but they were often the result of complex chromosomal rearrangements that occurred frequently in t(3q21) cases. Because of poor prognosis of EVI1 overexpression, a screening for EVI1 rearrangements should be performed in myeloid malignancies with chromosome 3q abnormalities. Furthermore, FISH should be performed in cases with 7q abnormalities when the quality of metaphases is suboptimal. *Supported by University of Bologna RFO, BolognAIL and Coop Reno.*

P785

DEVELOPMENT OF MOLECULAR BEACON BASED PCR ASSAY FOR EASY DETECTION AND QUANTIFICATION OF CHIMERIC TRANSCRIPTS OF AML-ETO [T(8;21)] IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: *Acute myeloid leukemia* (AML) is a malignant disease of blood arising as a result of cancer transformation and disorders in differentiation of hematopoietic cells on the level of myeloid cell precursor cells. Tumor cells of such patients often contain mutant forms of certain oncogenes, and therefore mutations in these genes are believed to be responsible for malignization of the hematopoietic system. In 20% of patients with AML, leukemic cells carry a translocation between chromosomes 21 and 8 resulting in formation of the chimeric oncogene *AML1-ETO,* producing a fused protein AML1-ETO, which has the activity of a transcription factor. Molecular rearrangements are constant and occur in exon 5 of the *AML1* gene and in exon 2 of the *ETO* gene. Protein AML1-ETO contains the N-terminal region of AML1 protein, which includes the DNA and Core Binding Factor β (CBFβ)-binding Runt Homology Domain (RHD), whereas the C-terminal part belongs to ETO protein including its four Nervy Homology Region (NHR) domains. AML1 activates transcription and thus promots differentiation of granulocytes due to transactivation of series-specific target genes while formation of the fused protein, AML1-ETO, results in the replacement of the AML1 activation domain by the ETO repressor domain. The fused protein AML1-ETO binds with DNA *via* the RHD domain of the AML1 moiety and effectively recruits corepressor complex *via* the NHR domain of ETO moiety, and thus inhibits the expression of *AML1* target genes instead of activating them. Patients having AML-ETO translocation have a relatively favourable prognosis and thus its detection helps the clinician to take better therapeutic decisions. Cytogenetic analysis by G-banding (karyotyping) is required at the time of diagnosis of all AML patients; however this method is often time-consuming as it requires culturing of leukemic cells and their capture at metaphase. FISH analysis for AML1-ETO fusion can also be performed using locus-specific probes. Dual color probes against AML1 and ETO resulting in fusion-signals are commercially available and commonly used but are not cost effective. qPCR is a quick and sensitive method that is recommended for detection of the fusion transcript but is highly expensive and requires infrastructure as well as expertise. Most of these methods are therefore not performed routinely in developing countries and therefore, it becomes imperative to develop a cost effective and highly sensitive method for the detection of AML-ETO translocation [t(8;21)] in Acute Myeloid Leukemia patients in developing countries.
Aims: To develop a PCR based easy to visualize quantitative assay for detection of AML-ETO translocation [t(8;21)] in Acute Myeloid Leukemia patients.
Methods: Peripheral blood samples were collected from untreated AML patients from Safdarjung and AIIMS hospital. Cost effective RNA isolation was standardized from these blood samples by comparing expression of four housekeeping genes in leukocytes. In-house primers and molecular beacons were designed for the detection of AML-ETO transcript and PCR assay was standardized for quantification of transcript using beacon strategy. The assay was evaluated against qPCR using published primers.
Results: Using the technique of molecular beacons, we successfully developed a cost effective and highly specific and sensitive reverse transcriptase-PCR assay for AML-ETO detection. Our assay could detect as low as 100fg of AML-ETO chimeric DNA and shows a linear increase in fluorescence with increase in template concentration (Figure 1). The specificity of our assay was confirmed using competition experiments. The clinical evaluation of the assay has been carried out using 100 leukemic samples.
Summary and Conclusion: We envisage that the current method is as sensitive and specific as commercially available method. Since the method is highly cost effective, and does not require expensive infrastructure and expertise, it can be used in resource poor settings.

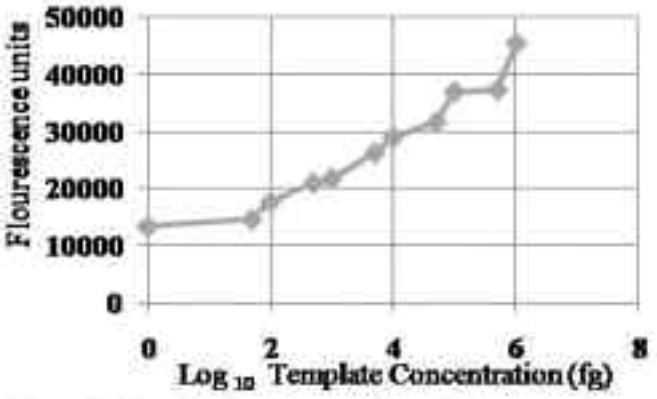

Figure 1. Graphical representation of fluorescent intensity of product obtained by carring in-house standardized AML-ETO specific PCR with different concentrations of positive close (TA vector containing AML-ETO insert), with 50nM beacon added to each, measured on Elisa reader (at 550,590nm).

P786

ANALYSIS OF NPM1 SPLICE VARIANTS REVEALS DIFFERENTIAL EXPRESSION PATTERNS OF PROGNOSTIC VALUE IN ACUTE MYELOID LEUKEMIA

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Background: The process of mRNA splicing has been reported to play an important role in human disease development and many cancer-related genes are regulated by alternative splicing. In addition, first analyses of alternatively splicing in bone marrow of AML samples identified novel splice variants specific for AML patients in comparison to normal cells.
Aims: Since splicing variants play an important role in cellular functioning the current study focuses on the characterization of *NPM1* splicing variants expression as well as its impact on the biology and prognosis of AML patients.
Methods: For the first cohort of patients (104 samples) qRT-PCR was performed and total expression (Rt) as well as levels of the three splice variants of *NPM1* were evaluated: R1 (exons 1- 9 and 11-12), R2 (exons 1- 10), R3 (exons 1-7, 9 and 11-12). We found prognostic significance of the expression level of R2, therefore we decided to validate it in independent cohort of AML patients. We consolidated 104 patients previously analyzed with 97 patients from the new cohort of total 201 cases and preformed the final analysis for R2. The existence of R2 at the protein level was evaluated with the use of Western Blot technique. To investigate whether R2 might disrupt localization of the NPM1 wild type protein, immunohistochemistry analysis for NPM1 in 23 AML cases was performed.
Results: Total expression as well as expression of R1 and R3 were significantly higher in 104 AML patients compared to healthy volunteers (HVs) with a median expression of 8.59 *vs* 0.93 (p=0.001), 1.73 *vs* 0.55 (p=0.014), and 2.54 *vs* 0.11 (p<0.001), respectively. We evaluated the existence of R2 at the protein level in AML patients samples and AML cell lines. We found that the expression of R2 was significantly higher in all AML patients compared to HVs with a median expression of 1.64 *vs* 0.33 (p=0.009). High R2 expression was associated with longer OS when CN-AML patients were analyzed (880 *vs* 438 days, p=0.028). Longer OS was observed in CN-AML patients with high R2 expression without concomitant *FLT3*-ITD mutations compared to the rest of groups. Most importantly, in CN-AML cases survival differences seen between the established ELN groups according to a *NPM1*/*FLT3*-ITD stratification were less impressive (p=0.03) than between groups stratified according to R2 expression/*FLT3*-ITD mutational status (p=0.003). Multivariate analysis revealed R2 expression (HR, 0.470; 95%>CI, 0.098 to 0.842; p=0.042), *FLT3*-ITD alteration (HR, 3.325; 95%>CI, 3.000 to 3.650; p<0.001), and age (HR, 1.035; 95%>CI, 1.023 to 1.047, p=0.004), but not WBC or *NPM1*mut as significant variables for OS. Finally, in cases with high R2 expression we were able to determine a cytoplasmic localization of NPM1 even in the absence of *NPM1* mutation. Therefore, we provide further evidence that the cytoplasmic localization of NPM1 might depend not only on its mutational status, but might also be influenced by the distribution of its splice variants.

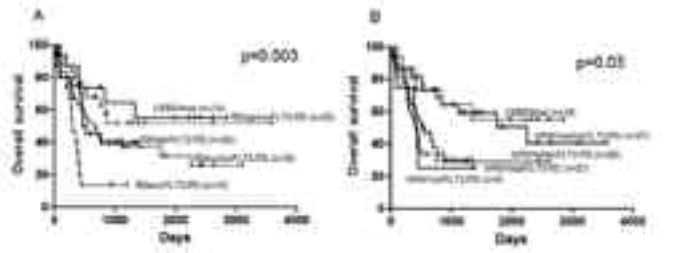

Figure 1. (A) OS in five groups of CN-AML patients: R2high/noFLT3-ITD, R2low/noFLT3-ITD, R2low/FLT3-ITD, R2high/FLT3-ITD and CEBPAmut. (B) OS in five groups of CN-AML patients: NPM1mut/noFLT3-ITD, NPM1wt/noFLT3-ITD, NPM1wt/FLT3-ITD, NPM1mut/FLT3-ITD and CEBPA-mut.

Summary and Conclusion: In our study we found that the expression level of R2 was elevated compared to HVs suggesting that not only *NPM1* mutation but also its splice variant expression might play some role in the process of the tumorigenesis. As the R2 represents a truncated form of *NPM1* gene, this isoform mostly localizes in the nucleoplasm, and thus might also have a biological impact in the malignant cells. In our cohort of cases survival differences seen between the established ELN groups according to a *NPM1*/*FLT3*-ITD stratification were less impressive than between groups stratified according to R2 expression/*FLT3*-ITD mutational status. In summary, the expression of R2 might be of biological importance for CN-AML patients. Moreover, R2 splice variant provides prognostic value for CN-AML patients and it might give information in addition to the *NPM1* mutational status.

P787

HIGH LEVELS OF HOTAIRM1, A LONG INTERGENIC NON-CODING RNA RELATED TO HOX GENES, IS ASSOCIATED WITH POOR PROGNOSIS AND A DISTINCTIVE MICRORNA SIGNATURE IN INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA

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Background: During the last years non-coding RNAs (ncRNAs) have emerged as key regulators of diverse cellular processes. They are classified according to their size in short (*i.e.* microRNAs) and long (*i.e.* lincRNAs) ncRNAs. LincRNAs are long ncRNAs located in intergenic regions with multiple regulatory functions including gene expression regulation. Interestingly, an active crosstalk between microRNAs and lincRNAs has been shown. LincRNAs are known to be deregulated in some cancers but their potential importance in acute myeloid leukemia (AML) is so far unknown. HOX genes play an important role in hematopoiesis and are deregulated in AML. Some lincRNAs, including *HOTAIRM1, HOTTIP and HOTAIR,* are located in the HOX genomic regions, but their expression level and prognosis role have not been studied in patients with AML. Since the intermediate risk cytogenetic (IR)-AML is a group with highly diverse prognosis; we analyzed the prognosis value of expression of these lincRNAs in this subgroup of patients.
Aims: To investigate whether the expression of HOX-related lincRNAs is associated with molecular characteristics, miRNA expression, and clinical outcome in IR-AML.
Methods: We have analyzed bone marrow samples from 77 IR-AML patients (median age, 52; 51% males) who received intensive chemotherapy following CETLAM trials in a single institution. Forty-two patients harbored NPM1 mutation (*NPM1*mut, 54%), 35 *FLT3*-ITD (45%) and 7 patients, a biallelic *CEBPA* mutation (9%). The expression of HOTAIRM1 (Hs03296533_g1), *HOTAIR* (Hs03296631_m1) and *HOTTIP* (Hs00955374_s1) was analyzed using TaqMan® Gene Expression Assay (Applied Biosystems). Statistical analysis was performed with BRB Array Tools, SPSS v15.0.1. MaxStat program from R software was used to determine the optimal cutoff point.
Results: *HOTTIP* and *HOTAIRM1*, but not *HOTAIR*, were expressed in most of our AML samples. *HOTAIRM1* expression was associated with *NPM1*mut (p=0.005). In the entire cohort, high *HOTAIRM1* expression was associated with shorter 5-year survival (OS) (p=0.003, 14±18% *vs*.44±14%), shorter 5-year disease-free survival (p=0.002, 52±12% *vs*.91±26%), and a higher risk of relapse at 5 year (p=0.012, 85±25% *vs*.35±14%). This effect was maintained both within the subgroup of *NPM1* mutated patients (OS, p=0.013, 20% ±24% *vs*. 52±18%) and within subgroup lacking favorable molecular features (*i.e.*, absence of *NPM1* mutation and *CEBPA* biallelic mutation and/or *FLT3*-ITD) (OS, p=0.017, 10±18% *vs*.40±15%). In the multivariate analysis including age, WBC, *NPM1*mut, *FLT3*-ITD as covariates, *HOTAIRM1* expression level showed independent prognostic significance (p=0.003; HR=3.052, 95% CI: 1.5-6.3)

both in the entire cohort and the unfavorable molecular subgroup (p=0.017; HR=2.8, 95% CI: 1.2-6.5). Supervised analysis by means of t-test based on multiplex permutations revealed a distinctive 33-miRNA signature which correlated with *HOTAIRM1* expression. Importantly, a positive correlation was observed between *HOTAIRM1* and miR-196b (p<0.001), a miRNA located in the same HOX genomic region.

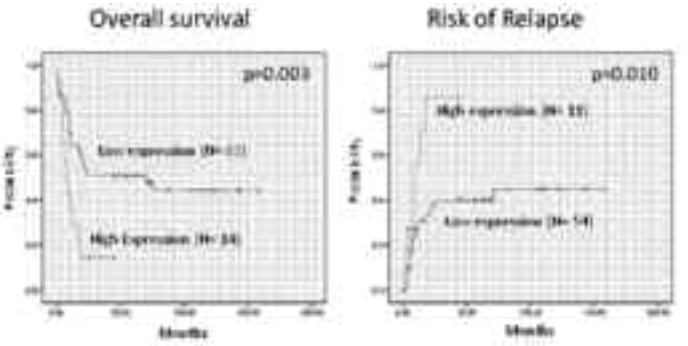

Figure 1. Prognostic value of HOTAIRM1 levels.

Summary and Conclusion: In this series of IR-AML patients, *HOTTIP* and *HOTAIRM1* were expressed in most patients. The expression level of the HOX-related lincRNA *HOTAIRM1* showed independent prognostic value. Interestingly, *HOTAIRM1* expression levels showed a strong positive correlation with its neighboring gene miR-196b. Nonetheless, confirmation of the prognostic impact of this lincRNA and search of potential underlying mechanisms accounting for this prognostic effect is warranted.
Acknowledgments: ISCIII RH13/00205, SEHH, FIS13/00999

P788

CD123 (IL-3Rα) IS CONSISTENTLY EXPRESSED ON ACUTE MYELOID LEUKEMIA (AML) CARRYING NUCLEOPHOSMIN (NPM1) GENE MUTATION

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Background: Antibody-based immunotherapy is a promising strategy to target and eliminate chemoresistant leukemic cells. Bispecific T cell engaging antibodies (BiTEs) are a novel class of antibodies which recruit *in vivo* the patient's own cytotoxic T cells and retarget them directly at specific surface antigens on leukemic cells. The interleukin-3 receptor a chain (CD123) has been identified as a potential immunotherapeutic target because it is overexpressed in AML compared to normal hematopoietic stem cells. Notably, CD123 is selectively overexpressed not only on AML blasts, but also on CD34+CD38- AML leukemic stem cells (LSCs) that are considered to be major players in chemotherapy drug resistance and leukemia relapse. CD123 as immunotherapeutic target in AML has been validated in preclinical studies with either CD123xCD3 bispecific antibodies or CD123 CAR-engineered T cells and clinical trials with these agents are expected in the next future. *Nucleophosmin (NPM1)* mutation is the most common genetic lesion in AML accounting for about 30% of cases. Immunohistochemical studies, have previously shown that in *NPM1*-mutated as well as in FLT3-ITD AMLs, CD123 is more frequently expressed. However, a comprehensive and quantitative evaluation of CD123 expression in AML has not been reported.
Aims: Here, we quantified CD123 expression by flow cytometry correlating expression intensity with cytogenetic and molecular disease characteristics in order to identify patient cohorts suitable for CD123-targeted therapy.
Methods: CD123 expression levels were evaluated by flow cytometry in fresh samples from 130 consecutive adult AML patients at diagnosis, using an antibody combination including CD34-FITC/CD123-PE/CD45-PerCP-Cy5.5/CD38-APC, acquiring at least 50000 events and reporting both percentage of CD123 positive cells (PPC) and CD123 median fluorescence intensity (MedFI) in both bulk and CD34+CD38- cell population. Blasts were gated using both FSC/SSC and CD45/SSC dot-plots. For all patients *NPM1* gene mutation status was assessed by immunohistochemistry, Western Blot and/or molecular analysis. FLT3 mutational status was assessed by RT-PCR. 60/130 (46%) carried *NPM1* gene mutation; 27/93 (25%) were *FLT3*-ITD mutated.
Results: According to karyotype (n=94), CD123 was more expressed in AML with normal karyotype than in AML with one or more karyotype abnormalities (mean values of MedFI 36.2 *vs* 19.5, p<0.01, Mann-Whitney). According to *NPM1* gene status (n=130), *NPM1*-mutated (NPMmut) AML showed brighter CD123 expression as compared to *NPM1* wild type (NPMwt) (mean values of MedFI 43.3 *vs* 22.1, p<0.01, Mann-Whitney). According to *FLT3* gene status (n=93), *FLT3*-ITD AML were associated with higher CD123 expression when compared to the *FLT3* wild-type (FLT3wt) counterparts (mean values of MedFI 49 *vs* 25.1, p<0.01, Mann-Whitney). As shown in Fig.1 the highest expression was reported in *NPM*mut/*FLT3*-ITD genotype AML in both bulk and CD34+CD38- leukemic cell populations (mean value of MedFI 55.1 and 93.6, respectively).

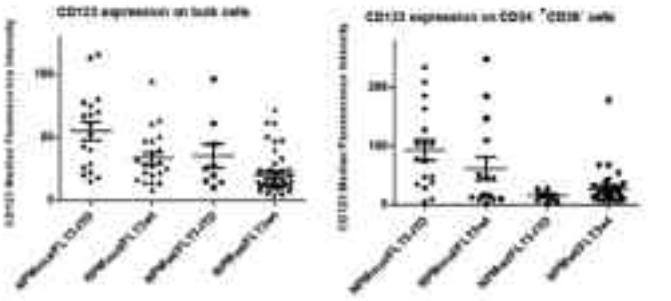

Figure 1.

Summary and Conclusion: Our results indicate that *NPM1* and *FLT3*-ITD mutations correlate with higher CD123 expression levels in AML. Although CD123 was globally positive in most cases of AML in our series, it was markedly expressed in AML carrying mutations of both *NPM1* and *FLT3* either on leukemic bulk cells as well as on CD34+CD38- putative LSCs, making this AML subtype a suitable candidate for CD123-targeted immunotherapy.

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THE PROGNOSTIC VALUE OF LEUKEMIC STEM CELLS IN PEDIATRIC AML

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Background: Pediatric acute myeloid leukemia (AML) patients exhibit a high risk of relapse (30-40%) and therapy related mortality (5-15%), resulting in a 5 year overall survival rate of only 65-70%. Because of the rarity of AML in children, little is known about the pathogenesis and few adequate prognostic parameters have been identified. The cancer stem cell model proposes that AML cells are hierarchically organized into compartments that contain leukemic stem cells (LSC) which have unlimited self-renewal capacity and are capable of propagating leukemia and relapse. Although in adult AML it has convincingly been demonstrated that the number of LSC (CD34+CD38-) at diagnosis is an important prognostic factor[1], this has not thoroughly been investigated in pediatric AML.
Aims: Despite complete remission rates of about 70%, the majority of pediatric patients with AML relapses within 3-5 years from diagnosis. Therefore, there is great need of identifying more sensitive prognostic factors that can predict relapse (originating from LSC outgrowth). We explored whether the number of LSC at diagnosis, detected with flow cytometry, is correlated with the risk of relapse and time to relapse.
Methods: Within the framework of the DB-AML01 treatment protocol (a collaboration between BSPHO and DCOG), serial samples from diagnosis till end of treatment were collected. Sixty-seven Dutch and 42 Belgium diagnostic samples were available for flow cytometric analysis. Our initial analyses included the Dutch samples. Stem cells were analyzed using the labeling CD45-CD34-CD117-CD38-CD2+56-CD123-CD7-HLA DR. LSC were defined as CD34+/CD38- cells (fixed cut-off values 10^3 and $3x10^3$, respectively) and expressed as a percentage of the total amount of CD34+ cells. For all analyses the Infinicyt software was used. Statistics were performed using SPSS (version 22.0).
Results: Mann Whitney U-Test showed that the percentage of LSC was significantly associated with the risk of relapse (p=0,034) and the risk of adverse event (relapse or death) (p=0,023). ROC curve analysis showed that at a cut-off of 23,5%, relapse can be predicted with a sensitivity of 69% and a specificity of 75%. Kaplan-Meier survival analysis showed that this percentage was significantly associated with relapse free survival (p=0,006). Neither the percentage of blasts, the percentage of CD34+ cells, the number of white blood cells (WBC) or the presence of genetic abnormalities (inv(16), FLT3, NPM1 and t(8;21) at diagnosis proved to be significantly associated with the risk of relapse or relapse free survival. ROC curve analysis showed that at a cut-off of 17,1% LSC, the occurrence of an adverse event can be predicted with a sensitivity of 76% and a specificity of 72%. Kaplan-Meier survival analysis showed that this percentage was significantly associated with event free survival

(p=0,002). Kaplan-Meier survival analysis also showed that the amount of WBC was significantly associated with event free survival (p=0,002).

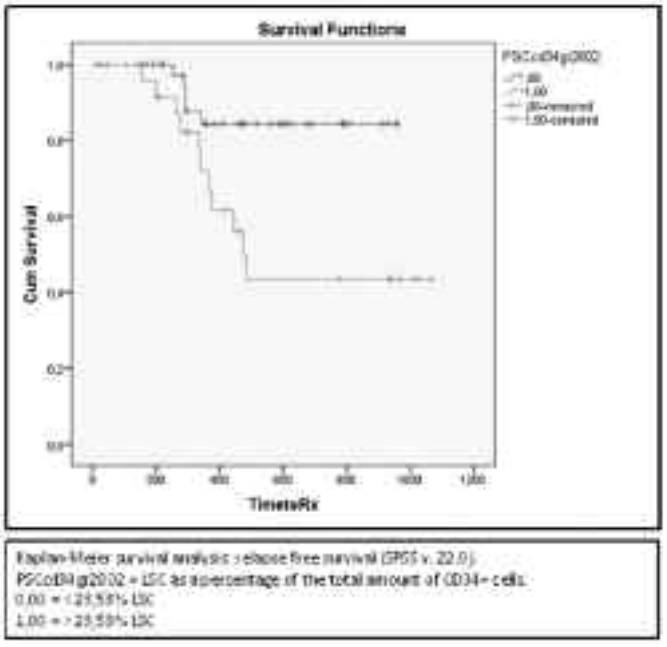

Figure 1.

Summary and Conclusion: The percentage of stem cells, detected at diagnosis with flow cytometry, is a prognostic factor for relapse in pediatric AML patients. Our current studies focus on refinement of the analysis strategy which will be applied to the 42 Belgian patients at diagnosis. In addition, the value of measuring LSC in the MRD setting will be evaluated. Furthermore, these studies will be extended in the recently started NOPHO-DBH AML 2012 trial. [1]van Rhenen A et al (2005). Clin Cancer Res 11: 6520-6527. This study was supported by KIKA, the Netherlands.

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BCL11B UP-REGULATION IN ACUTE MYELOID LEUKEMIA WITH CD2 T-ANTIGEN EXPRESSION AND FLT3 MUTATION

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Background: *BCL11B*/14q32 belongs to kruppel zinc finger family of transcription factors directly binding a GC-rich consensus sequence in target genes. It interacts with the Nucleosome Remodelling Deacetylase (NuRD) complex and is a subunit of mammalian SWI/SNF, the most frequently mutated chromatin-regulatory complex in human cancer. [1,2] In hematological diseases, *BCL11B* might behave as tumor suppressor gene or oncogene. It is downregulated by mono-allelic deletions or mutations in 10-15% of T-ALL[3,4] and in cases with inv(14)(q11q32)/t(14;14)(q11;q32).[5,6] *O*ver-expression occurred in 4 AML cases.[7]

Aims: To investigate AML with 14q32 reciprocal translocations

Methods: Karyotyping identified t(2;14)(q22;q32) and t(6;14)(q25;q32) respectively in 6 and 2 *FLT3* mutated AML, with CD2 positivity. Other expressed T-antigens were CD7 and/or cyCD3. Fluorescence *in situ* hybridization (FISH), RT-PCR, cloning and sequencing investigated partner gene breakpoints. qRT-PCR (Light Cycler 480, Roche) evaluated *BCL11B* expression. Affymetrix SNPa, (Cytogenetics Whole-Genome 2.7M Array Platform), Exome and RNA sequencing (Hiseq 2000, Illumina) were performed. FISH studied cryptic *BCL11B* rearrangements in 689 cases.

Results: 14q32 breakpoints fell at the *BCL11B* gene 5' end in all cases. 2q22.3 breakpoints involved the Zinc finger E-box Binding homeobox 2 (*ZEB2*) gene. *ZEB2* exon 2 was fused in-frame to *BCL11B* exon 2. Western blotting detected 2 *ZEB2-BCL11B* splicing isoforms with or without *BCL11B* exon 3. Breakpoints at 6q25 fell in a region where no genes have been mapped. *ZEB2-BCL11B* and *BCL11B* expression was high in t(2;14) and t(6;14) AML, respectively. SNP analysis confirmed all karyotypic changes and detected non-recurrent copy number variations. High Throughput Sequencing did not find any other common fusion or mutation. Genes like DNMT3A, TET2, WT1, EP300 were involved in 6/8 patients. Gene Expression Profiling identified a specific *BCL11B* translocation signature. Differential expression of 14/39 BCL11B targets indicated common downstream targets in t(2;14) and t(6;14). No cryptic *BCL11B* translocations were found.

Summary and Conclusion: Two recurrent translocations, (2;14)(q22.3;q32) and t(6;14)(q25;q32), deregulated *BCL11B* in a CD2+, FLT3+, AML and have a specific gene expression signature. In the t(2;14) the BCL11B involved in the ZEB2/BCL11B fusion was overexpressed. Notably the ZEB2-BCL11B protein and BCL11B share a RRKQXXP NuRD interaction motif at the N-terminal[1,8] and the same C-terminal containing BCL11B C2H2-zinc finger DNA binding domain. The mechanism of BCL11B up-regulation in the t(6;14) remains to be determined. A super enhancer mapped at 6q25, however, is an interesting candidate. In our study we showed that as a consequence of these translocations BCL11B and ZEB2-BCL11B may act as similar transcription factors.

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PSEUDO-EXHAUSTION OF T CELLS IN RELAPSED AML

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Background: The prognosis of acute myeloid leukemia (AML), particularly when associated with adverse chromosomal or molecular aberrations, is poor due to a high relapse rate after induction chemotherapy. Postremission therapy for elimination of minimal residual disease remains a major challenge. Immunotherapeutic strategies aim at the stimulation of AML-specific immunity, especially of $CD8^+$ T cells. However, the functionality of these cells in AML patients is not well described. T cell exhaustion has been suggested to contribute to immune evasion in various solid and hematological malignancies. Primarily demonstrated in chronic viral infections, exhausted T cells are characterized by an increased expression of several inhibitory molecules, reduced proliferation and an impaired capability of cytokine secretion and cytotoxicity.

Aims: To characterize T cell phenotype and function in AML patients at different stages of disease.

Methods: $CD8^+$ and $CD4^+$ T cells from AML patients at primary diagnosis, with refractory disease, at relapse and at relapse after allogeneic stem cell transplantation (alloSCT) (23, 4, 9 and 7 individuals, respectively) were analyzed by flow cytometry-based assays. Surface expression of CD244, CD160, PD-1, TIM-3 and LAG-3 was determined. T cell proliferation and production of the cytokines IFN-γ, TNF-α and IL-2 were measured in response to different stimuli. Results were compared to healthy controls (HC) (30 individuals), while untreated HIV-infected patients (10 individuals) served as positive controls for an exhausted T cell state.

Results: In HIV-infected patients, we observed a pronounced upregulation of the inhibitory molecules CD244, CD160 and PD-1 on $CD4^+$ and $CD8^+$ T cells as well as globally impaired cytokine production, clearly indicating T cell exhaustion. In contrast, T cells from AML patients at primary diagnosis showed an expression pattern of inhibitory surface molecules that was similar to T cells from age-matched HCs. Interestingly, AML patients with a relapse after alloSCT showed a 3- and 6-fold increased overall expression of PD-1 on $CD8^+$ ($p=0.0084$) and $CD4^+$ ($p<0.0001$) T cells, respectively. This PD-1 expression pattern correlated to an increased proportion of memory T cells, which have an inherently higher expression of PD-1. Both, relapsed patients after conventional chemotherapy and relapsed patients after alloSCT, displayed a shift from the naïve towards the memory T cell compartment, indicating reinforced T cell differentiation. Functionally, no defect in T cell proliferation in any of the AML patient cohorts was detected. Of note, however, we observed a 2-fold decrease ($p=0.0068$) in IFN-γ production by $CD4^+$ T cells exclusively in patients at primary diagnosis.

Summary and Conclusion: We show that T cells of newly diagnosed and relapsed AML patients are fully functional. Moreover, we demonstrate enhanced T cell differentiation in relapsed AML patients. We therefore hypothesize that bulk T cells in AML are in a status of activation, not exhaustion. Thus, immunotherapies that aim at eliciting tumor-specific immune responses, *e.g.* dendritic cell based vaccines, may be particularly suited for postremission therapy.

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PROSPECTIVE LONG-TERM MINIMAL RESIDUAL DISEASE MONITORING ON PERIPHERAL BLOOD IN RUNX1-RUNX1T1 ACUTE MYELOID LEUKEMIA: RESULTS OF THE FRENCH CBF-2006 TRIAL

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Background: Core-binding factor acute myeloid leukemias (AML) are associated with a favorable prognosis. However, around one third of patients will present hematological relapse. In AML with translocation t(8;21)(q22;q22), quantification of *RUNX1-RUNX1T1* fusion transcript with real-time quantitative polymerase chain reaction (RQ-PCR) allows to monitor minimal residual disease (MRD) during and after therapy. In the CBF2006 trial, we showed that early MRD response was a major prognostic factor (Jourdan E. et al, Blood 2013). Until now, the prognosis of MRD long-term follow-up has been rarely explored in prospective trials.
Aims: The main objective of the study was to assess the usefulness of prospective MRD monitoring on peripheral blood (PB) by RQ-PCR to predict relapse.
Methods: In the multicenter CBF-2006 study, 96 patients with t(8;21) AML in complete remission after intensive chemotherapy entered a prospective peripheral blood (every 3 months) and bone marrow (every year) MRD monitoring for 2 years. Transcript ratios were normalized to *ABL* as (*RUNX1-RUNX1T1/ABL*) x 100 and complete molecular response (CMR) was defined by a transcript ratio ≤0.001% on peripheral blood. Prognostic impact of CMR and loss of CMR on cumulative incidence of relapse (CIR) and overall survival (OS) were evaluated in a time-dependent manner (Mantel-Byar analyzes).
Results: During the 2-year follow up, 77 patients reached CMR. Median time between complete remission and CMR was 2.5 months [IQ: 1.4 – 4.6] up to 16 months. As expected, CMR achievement was associated with significantly reduced incidence of relapse (3-year CIR: 23% *vs* 51%, P=.001) and better overall survival (3-year OS: 90% *vs* 56%, P=.008). However, time to CMR was not predictive of CIR or OS. Among the 77 patients who achieved CMR, 23 patients presented a positive MRD (>0.001%) with a median time of 6.9 months [IQ, 3.9 – 13.3] up to 23 months after CMR achievement. Loss of CMR was confirmed on a second sample in 13/23 patients (57%). Cumulative incidence of relapse was significantly higher in patients with a confirmed loss of CMR when compared to patients with a non confirmed loss of CMR (3-years CIR, 83% *vs* 14%, p=.03) with no difference in overall survival. In patients with confirmed loss of CMR, time between first positive MRD and hematological relapse was 3.9 months [IQ: 3.3 – 6.9].
Summary and Conclusion: Contrarily to early MRD reduction on therapy, time to CMR achievement is not predictive of relapse in patients with *RUNX1-RUNX1T1* AML. Monitoring peripheral blood MRD every 3 months up to 2 years after CR allowed to predict and anticipate hematological relapse.

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FREQUENT MUTATIONS IN PML-RARA IN RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Two therapeutic agents, ATRA and arsenic trioxide (ATO), induce differentiation of promyelocytes *in vivo* and clinical remission of acute promyelocytic leukemia (APL) patients. However, mutations in the *RARA* and *PML* parts of the *PML-RARA* fusion gene have been reported to confer resistance to ATRA (Imaizumi *et al.*, Blood 1998) and ATO (Goto *et al.*, Blood 2011), respectively.
Aims: The evaluation of mutations within the *PML-RARA* fusion gene in relapsed APL.
Methods: We screened 12 cases with APL who showed hematologic or molecular relapse for mutations within the *PML-RARA* fusion transcript. Amplified *PML-RARA* fusion transcript was analyzed by direct Sanger sequencing. The detection limit of this analysis was 1:10 allowing the detection of 1 mutated allele in a background of 10 wildtype alleles. Eight patients were male and four patients were female with a median age of 47.15 years (range: 16.7-73.2 years).
Results: Overall, in 7/12 relapsed patients (Pt) (58.3%), mutations within the *PML-RARA* fusion transcript were detected. In these seven patients we performed mutation analysis also in samples taken at primary diagnosis. In none of the cases these mutations were detectable at initial diagnosis and thus all were acquired mutations. All mutations were missense mutations with mutation loads of 10-100%. Two of seven patients were treated with ATRA and had a mutation in the RARA region of *PML-RARA*. One of these mutations was previously described (p.Ser287Leu), whereas the other was novel (p.Val135Leu). Five of seven patients were treated with ATRA and ATO and harbored mutations in the *PML* region of *PML-RARA.* All five patients had at least two and up to four mutations in the *PML* region of *PML-RARA* (Pt1: p.Gly269Ser + p.Val301Met + p.Ala303Val; Pt2: p.Leu81Pro + p.Glu277Lys + p.Ala303Thr + p.Gln354Arg; Pt3: p.Ala263Ser + p.Glu296*; Pt4: p.Ala125Thr + p.Arg282His + p.Val287Ile; Pt5: p.Glu281X + p.Gly317Asp). All *PML* mutations were located in functional domains and were not described before. In three patients material from different relapse time points was available. In the first patient three relapses occurred and all three mutations in the *PML* region of *PML-RARA* were for the first time detectable at the time point of third relapse after treatment with ATRA and ATO. The second patient had two relapses. Four mutations in the *PML* region of *PML-RARA* were detected at first relapse after treatment with ATRA and were not detectable at the time point of second relapse after treatment with ATRA and ATO. The third patient also had two relapses (no treatment information available). Mutations in the PML region of *PML-RARA* were detected at first relapse and were not detectable at second relapse.
Summary and Conclusion: We demonstrate a high incidence of acquired mutations within the *PML-RARA* fusion transcript in relapsed APL patients. Although functional data is still missing, these mutations may confer resistance to ATRA and ATO. These data may have implications for further treatment strategies of such patients.

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NFATC1 MEDIATES SORAFENIB RESISTANCE IN FLT3-ITD POSITIVE ACUTE MYELOID LEUKEMIA

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Background: Internal tandem duplications (ITD) in the fms-related tyrosine kinase 3 (FLT3) are the most frequent mutations in normal karyotype acute myeloid leukemia (AML). FLT3-ITD is associated with a dismal prognosis. Although FLT3 inhibition with sorafenib is rational and clinically effective in chemotherapy-refractory FLT3-ITD+ AML patients, sorafenib resistance essentially always develops after rather short latency. Mechanisms of sorafenib resistance are not well defined. The transcription factor, nuclear factor of activated T-cells (NFATc1) is expressed in pancreatic cancer and certain lymphatic leukemias. It was recently shown that inhibiting NFATc1 with the immune suppressant and calcineurin inhibitor cyclosporine A (CsA) regulates imatinib resistance in BCR-ABL-positive leukemia cells.
Aims: Here we characterized NFATc1 expression in AML. We subsequently asked, whether NFATc1 has a role in the regulation of sorafenib response of FLT3-ITD positive AML.
Methods: NFATc1 mRNA expression level were screened using cDNA array data of 47 FLT3-ITD$^+$ and 222 FLT3-ITD$^-$ AML patients. NFATc1 mRNA and protein expression was analyzed in 14 AML cell lines and primary AML patient material. The role of NFATc1 in regulating sorafenib response was studied *in vitro* by conducting proliferation and apoptosis assays: sorafenib-sensitive and –resistant FLT3-ITD-positive MV4-11 cells and primary patient blasts were treated with sorafenib, NFATc1 inhibitors (VIVIT, CsA) or both. The role of NFATc1 in controlling sorafenib response was also studied *in vitro* and *in vivo* by genetic knock down of NFATc1 using NFATc1-specific shRNA vectors or by expression of a constitutively nuclear (active) NFATc1 variant.
Results: NFATc1 mRNA and the nuclear (active) NFATc1 protein were found to be overexpressed in AML cell lines and primary cells, especially if they were FLT3-ITD+. NFATc1 regulated sorafenib sensitivity of FLT3-ITD+ cells: pharmacological inhibition or genetic knockdown of NFATc1 using CsA/VIVIT or inducible shRNA constructs, respectively, synergized with sorafenib in mediating proliferation inhibition and apoptosis induction of sorafenib-sensitive FLT3-ITD+ MV4-11 cells. Moreover, in derived sorafenib-resistant MV4-11 clones, blocking NFATc1 with CsA overcame resistance. The magnitude of this effect correlated with the extent of NFATc1 overexpression. Vice versa, co-expression of a constitutively nuclear NFATc1-mutant with FLT3-ITD in myeloid progenitor cells (32D-FLT3/ITD-NFATc1), resulted in instant and FLT3-ITD-independent sorafenib resistance *in vitro* and in a syngenic mouse model *in vivo*. Finally, AML blasts derived from sorafenib-resistant FLT3-ITD-positive AML patients could be resensitized to sorafenib in presence of CsA *in vitro*.
Summary and Conclusion: CsA may improve sorafenib treatment outcome of FLT3-ITD-positive AML patients by overcoming or delaying sorafenib resistance development. Our results also imply a so far unrecognized anti-leukemic role of the routine clinical application of CsA as immune suppressant post allogenic stem cell transplantation. This may warrant reconsideration of the CsA tapering practice in FLT3-ITD positive AML.

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EXPRESSION OF FLT3-ITD DYSREGULATES THE DBC1-SIRT1-P53 SIGNALING AND PROMOTES THERAPY RESISTANCE

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Background: SIRT1 is a NAD+ dependent histone deacetylase, which has been shown to act as an important regulator of apoptosis, DNA-repair and genomic integrity under conditions of stress. Beside deacetylation of histones SIRT1 has several other substrates including KU70, FOXO1 or p53. SIRT1 deacetylates p53 at lysine 382 thereby reducing its transcriptional activity followed by loss of p53 dependent apoptosis in response to cell damage. TP53 mutations represent one of the most common genetic events in human cancers. Interestingly, TP53 alterations are rarely detected in acute myeloid leukemia (AML). Recent reports have shown high expression of SIRT1 in CML cells. In previous work we and others demonstrated increased SIRT1 protein expression in AML samples harboring activating mutations in signal transduction pathways such as FLT3-ITD, c-KIT or RAS-GTPases (EHA Abstract Book 2013, Blood 2013 vol. 122 no. 21 3789). Expression was regulated by FLT3 tyrosine kinase activity and regulated at protein but not RNA levels.
Aims: In this study we aim at understanding mechanisms of FLT3-ITD mediated SIRT1 regulation, the role of p53 as an effector protein and SIRT1 as a therapeutic target.
Methods: We established a doxycycline-inducible system enabling pharmacologic control of shRNA expression targeting SIRT1 in several AML-cell lines. In addition AML cells were treated with Tenovin-6 or EX527 with and without genotoxic agents. Cell death was analyzed by Annexin-V staining and cell cycle analysis. To investigate the functional role of p53 upon pharmacologic SIRT1-inhibition cells were transduced with lentivirus expressing p53-shRNA. Finally, to gain insight into *in vivo* effects upon SIRT1-inhibition, we performed xenotransplantation assays using SIRT1-shRNA-expressing MV4-11 cells. Transplanted animals were treated with doxycycline to induce SIRT1-knockdown and PKC412 alone or in combination.
Results: Targeting SIRT1 using the SIRT1/2 inhibitor Tenovin-6 (TV-6), EX527 or knockdown of SIRT1 resulted in a slight increase in apoptotic cell death in primary AML samples and cell lines. In contrast, inhibition of SIRT1 significantly sensitized leukemic blasts to FLT3 inhibitor therapy or genotoxic agents. In TP53-wildtype cells this effect was strongly dependent on p53, as knockdown of p53 cells abrogated the inhibitor effects. However, SIRT1 inhibition also sensitized TP53-mutant cells suggesting alternative downstream targets of SIRT1. In colony assays using murine leukemia models driven by MLL-AF9 and FLT3-ITD SIRT1 acts as a safeguard to counteract oncogenic stress and leukemic blasts become dependent on SIRT1 activity. Further, pharmacologic inhibition of SIRT1 attenuates the replating capacity without affecting cell death. *in vivo*, knockdown of SIRT1 in the AML cell line MV4-11 expressing a doxycycline-regulated SIRT1-shRNA resulted in prolonged survival in a xenotransplantation model and survival was further enhanced upon PKC412 combination therapy. Finally, we were interested to investigate the role of mutated FLT3 in SIRT1 regulation. Inhibition of FLT3-ITD kinase activity caused an increased phosphorylation of the physiological SIRT1-inhibitor DBC1 followed by binding of SIRT1 as revealed by co-immunoprecipitation experiments. We also noticed a slight increase in ATM/ATR phosphorylation and an increase in ATM-DBC1 binding upon FLT3-ITD inhibition indicating SIRT1 inhibition via the ATM-DBC1-SIRT1 axis. These effects were substantially enhanced upon treatment with genotoxic agents (cytarabine; irradiation) in combination with PKC412 compared to either agent alone.
Summary and Conclusion: Pharmacologic targeting of SIRT1 sensitizes AML cells to TKI therapy or genotoxic agents and partially restores the FLT3-ITD associated defective stress response pathway ATM-DBC1-SIRT1 resulting in activation of p53. In murine xenotransplantation models, inhibition of SIRT1 in combination with PKC412 treatment significantly prolonged survival of transplanted mice. These data suggest that SIRT1 represents a promising target in AML therapy.

P796

THE COMBINATION OF SELINEXOR (KPT-330), A SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE), & THE FLT3 INHIBITOR QUIZATINIB SHOWS ANTI-TUMOR ACTIVITY IN ACUTE MYELOID LEUKEMIA (AML) *IN VITRO* AND *IN VIVO*

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Background: AML cells overexpress the nuclear exporter Exportin 1 (XPO1/CRM1) and higher XPO1 levels correlate with poor outcomes in AML and other cancers. Selinexor (KPT-330), a novel SINE, antagonizes XPO1 and shows potent cytotoxicity for AML cells *in-vitro* and *in-vivo*, independent of genotype, while largely sparing normal hematopoietic cells. Mechanistic studies show that selinexor induces nuclear localization and activation of multiple tumor suppressor proteins (TSPs) and reduces levels of oncoproteins such as FLT3 and KIT, leading to rapid apoptosis of AML cells. We have recently reported preliminary Phase 1 results in which treatment with oral selinexor has led to durable disease stabilization and responses in patients with relapsed/refractory AML across multiple genotypes. Quizartinib is a selective inhibitor of FLT3, a receptor tyrosine kinase for which the FLT3-ITD mutant is frequently a driver for AML. Quizartinib has shown significant activity in AML and the drug is scheduled to enter Phase 3 in FLT3-ITD+ AML patients this year. We report here the results of *in-vitro* and *in-vivo* studies of Selinexor and quizartinib alone or in combination on proliferation and survival of MV4-11 AML cells.
Aims: To characterize the *in-vitro* and *in-vivo* effects of combining selinexor with quizartinib on AML cell survival relative to treatment with either drug alone.
Methods: FLT3-ITD+ MV4-11 AML cells were used for cell culture and xenograft studies. MTT was used to measure *in-vitro* cell proliferation. For xenograft studies, MV4-11 cells were grown as subcutaneous tumors in NOD-SCID mice.
Results: Selinexor and quizartinib each showed highly potent inhibition of MV4-11 proliferation *in-vitro*, and the combination had additive efficacy. In the MV4-11 AML xenograft model in NOD-SCID mice, a combination of selinexor with quizartinib had an apparently synergistic effect on tumor growth relative to the drugs administered as monotherapies. Suboptimal doses of selinexor

(5 or 10 mg/kg PO QODX3) had no effect or induced a 60% median tumor growth inhibition (TGI), respectively, by the end of the study. Quizartinib at 0.5 or 10 mg/kg QDX7 PO induced 60% TGI or 91% regression, respectively, over the same timeframe. The combination of the suboptimal doses of Selinexor (5 mg/kg) and quizartinib (0.5 mg/kg) induced 67% tumor regression. While 10 mg/kg quizartinib induced modest weight loss, selinexor, 0.5mg/kg quizartinib, and the combination were well-tolerated with expected weight gain and full survival.

Summary and Conclusion: Selinexor is a potent inhibitor of *in-vitro* and *in-vivo* AML cell survival and functions by nuclear localization / activation of TSPs and reduction in FLT3 and other oncogproteins. Direct FLT3 inhibition with quizartinib also results in killing of FLT3 abnormal AML cells. The combination of selinexor and quizartenib shows additive cytotoxicity on AML cells *in-vitro*. Moreover, the combination of suboptimal doses of selinexor with quizartinib *in-vivo* was well tolerated and was dramatically more effective than either drug alone, leading to robust xenograft regression. This synergistic effect provides rationale for the investigation of a selinexor/quizartinib combination in the clinic with the potential for enhanced efficacy in AML relative to treatment with these drugs as monotherapies.

P797

CABOZANTINIB IS SELECTIVELY CYTOTOXIC IN ACUTE MYELOID LEUKEMIA CELLS WITH INTERNAL TANDEM DUPLICATION OF FLT3 (FLT3-ITD)

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Background: Internal tandem duplication of *FLT3* (*FLT3*-ITD), one of the most common mutations in acute myeloid leukemia (AML), is found in approximately 30% of AML patients and is associated with poor prognosis. *FLT3*-ITD is a potential target for drug development. Cabozantinib (XL-184, *N*-(4-((6, 7-Dimethoxyquinolin-4-yl) oxy) phenyl)-*N*-(4-fluorophenyl) cyclopropane-1, 1-dicarboxamide) is an oral multikinase inhibitor that targets MET, VEGF2, RET, KIT, TIE2, and FLT3. Previous studies showed that cabozantinib inhibited multiple receptor tyrosine kinases and cell viability in various cancer cell lines, and exhibited effective anti-tumor activity in many cancer models. The US FDA approved cabozantinib for the treatment of medullary thyroid cancer in 2012. Clinical trials for treatment of prostate cancer, renal cell carcinoma, hepatocellular carcinoma, and non-small cell lung cancer are ongoing. However, there is very little information about the application of cabozantinib in leukemia.

Aims: We aim to elucidate the efficacy and mechanism of action of cabozantinib in acute myeloid leukemia.

Methods: Human leukemia MV-4-11, MOLM-13, OCI-AML3, THP-1 cell lines, and leukemia cells from patients with AML were used. MTS assay was performed to assess growth suppression of cabozantinib on AML cells. Western blotting, flow cytometry and quantitative real-time PCR were applied to elucidate potential molecular mechanism. Leukemic cell xenografts in zebrafish embryo were used for investigating efficacy of cabozantinib as *in vivo* model. The study was approved by the institutional review board of National Taiwan University Hospital and written informed consents were obtained from all participants in accordance with the Declaration of Helsinki.

Results: Cabozantinib was significantly cytotoxic in MV4-11 and MOLM-13 cells, both harboring *FLT3*-ITD, with IC_{50} of 2.4nM and 2.0nM, respectively. On the other hand, K562, OCI-AML3 and THP-1 were resistant to cabozantinib, with IC_{50} at the micromolar range. In addition, cabozantinib, up to 20μM, had no toxic to peripheral blood mononuclear cells from healthy controls, suggesting drug potency with biosecurity and cell line-specificity. Cabozantinib arrested MV4-11 cell growth at G_0/G_1 cell cycle within 24hrs, and the cell cycle phase changes are associated with decreased phosphorylation of FLT3, STAT5, AKT and ERK. In addition, annexin-V and PI staining analysis revealed that cabozantinib induced MV4-11 cell apoptosis in a dose-dependent manner; cleavage of caspase 3 and PARP-1 were also observed. Further investigations on apoptosis-related proteins revealed that cabozantinib-induced apoptosis was accompanied by down-regulation of anti-apoptotic proteins survivin and Mcl-1 and up-regulation of pro-apoptotic protein Bak. In zebrafish model, cabozantinib decrease the leukemic burden in embryos xenografted with MV4-11 or MOLM-13 leukemic cells, but did not demonstrate any toxicity or anti-angiogenesis effect on normal embryos with up to concentration of 100nM.

Summary and Conclusion: Cabozantinib is selectively cytotoxic to leukemia cell with *FLT3*-ITD, but not those without this mutation. Although the mechanism of action is unclear, the growth suppression is related to cell cycle phase arrest and apoptosis. Our results suggest that clinical trials for the efficacy of cabozantinib in AML with *FLT3*-ITD are indicated.

P798

PRE-CLINICAL ACTIVITY OF THE PI3K INHIBITOR BKM120 ON ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is frequently characterized by genetic and molecular alterations that induce constitutive activation of different signal transduction pathways such as PI3K/Akt/mTOR. Its inhibition through several small molecules, alone or in combination with chemotherapy, represents an attractive strategy to improve current treatment regimens. BKM120 is a highly selective pan-class I PI3K inhibitor which has been evaluated with encouraging results on various solid tumors and hematological malignancies, particularly T acute lymphoblastic leukemia (T-ALL).

Aims: In the present study we aimed at investigating pre-clinically the activity of BKM120 on AML cell lines and primary samples.

Methods: BKM120, kindly provided by Novartis, was tested on several AML cell lines and on 9 primary AML samples at concentrations ranging from 0.5 to 5μM. Cell cycle changes and apoptosis rate were analyzed by Acridine-Orange (AO) and Annexin V (AnnV)/PI staining. Western blot analysis was performed to evaluate intracellular signaling modulations induced by BKM120 up to 24h.

Results: Basal expression and phosphorylation levels of critical PI3K/Akt/mTOR pathway components were first assessed by western blot analysis. Despite some heterogeneity, AML cell lines (U937, NB4, OCI-AML2, HL-60/MX2, MOLM-13, OCI-AML3, HL-60 and KG-1) displayed a constitutive activation of the PI3K/Akt/mTOR axis, as documented by higher levels of p-Akt (Ser473), p-GSKα/β (Ser21/9), p-mTOR (Ser2448 and Ser2481), p-p70S6K (Ser371) and p-4EBP1 (Thr37/41) in comparison to normal peripheral blood lymphocytes (NPBLs). Three hours of BKM120 exposure induced a dose-dependent dephosphorylation of Akt (Ser473) in all cell lines, although with different sensitivity profiles. Akt dephosphorylation was associated with the downregulation of p-GSKα/β (Ser21/9) expression indicating a functional blockade of the PI3K pathway. BKM120 also affected both mTORC1 and mTORC2 activity inducing the dephosphorylation of p-mTOR (Ser2448), p-p70S6K (Ser371) and p-4EBP1 (Thr37/41) (mTORC1 downstream targets) and of p-mTOR (Ser2481) (mTORC2). Selective inhibition of the PI3K/Akt/mTOR pathway correlated with a marked reduction of cell growth (IC50s at 72h ranging from 0.7 for U937 to 1.2μM for KG-1) and with a significant ($p<0.005$) dose- and time-dependent induction of apoptosis in all cell lines. Analysis of cell cycle revealed, at 24h, a temporary accumulation of cells in G2/M phase which, at 72h, turns into apoptosis, as documented by the dramatic increase of the sub G0/G1 peak. Efficacy of BKM120 was then confirmed *ex-vivo* on 9 primary AML samples. At 144h, a mean increase of AnnV positive cells from 18.7% ± 0.1 (vehicle) to 26.4% ± 0.12, 31.6% ± 0.14, 40.4% ± 0.13 ($p<0.001$) and 45.4% ± 0.14 ($p<0.001$) was obtained at 0.5, 1, 2 and 5μM, respectively. Only 2 out 9 samples showed a limited increase of apoptosis induction (<15%). BKM120 was also tested on normal and activated PBMCs isolated from 5 healthy volunteers failing to show induction of apoptosis (8.0% and 4.4% apoptosis net increase at 5μM, respectively).

Summary and Conclusion: BKM120 treatment significantly impaired proliferation and induced apoptosis in all AML cell lines and in the majority of primary AML samples tested by selective inhibition of PI3K/Akt/mTOR signaling, suggesting the potential therapeutic activity of this molecule in AML.

P799

THE CXCR4 ANTAGONIST BL-8040 SYNERGIZES WITH THE FLT3 INHIBITOR AC220 TO INDUCE APOPTOSIS AND REDUCE MINIMAL RESIDUAL DISEASE OF AML CELLS *IN VIVO*

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous group of diseases characterized by the uncontrolled proliferation of hematopoietic stem cells and progenitors with a reduced capacity to differentiate into mature cells. While many patients with AML achieve a complete remission with traditional chemotherapy, the majority eventually relapse within a year. FLT3 is a class III receptor tyrosine kinase that is expressed on early hematopoietic stem cells/progenitors and is vital for development of normal levels of mature myeloid and lymphoid cells. FLT3 gene mutations represent one of the most common molecular perturbations in AML, accounting for ~30% of de novo cases. FLT3 internal tandem duplication (ITD) mutation in AML patients confers poor response to chemotherapy, high relapse rates and only transient response to FLT3 inhibitors. CXCL12 and its receptor CXCR4 are key players in mediating the interactions between the BM microenvironment and AML cells. CXCL12,

which is constitutively secreted from the BM stroma and AML cells, is critical for the survival and retention of AML cells within the BM. CXCR4 expression is associated with poor prognosis in AML patients with or without a mutated FLT3 gene, and inhibition of CXCR4 was shown to sensitize AML blasts toward chemotherapy. It was found that FLT3-ITD mutation activate CXCR4 signaling and is associated with increased CXCR4 expression in primary AML cells.
Aims: In this work we studied the effect of the high affinity CXCR4 antagonist BL-8040 on the survival of AML cells with FLT3-ITD mutation alone or in combination with the FLT3 inhibitor AC220 (Quizartinib).
Methods: In this study, human AML MV4-11 cells *(FLT3-ITD*) were used. Cells were incubated in-vitro for 48 hrs in the presence of BL-8040 (20μM), AC220 (50nM) or their combination. Cells viability and the percentage of apoptotic events were evaluated by FACS analysis. In the in-vivo study an AML model of NOD scid gamma (NSG) mice engrafted with MV4-11 cells was used. Three weeks after the engraftment mice were treated daily for seven consecutive days with SC injections of BL-8040 (400ug/mouse) or with oral administration of AC220 (10mg/Kg) or their combination. The survival and apoptosis of AML cells were examined in the blood, BM and spleen of the engrafted mice.
Results: In-vitro, treatment of AML cells with BL-8040 directly inhibited cell growth by 35% and increased cell death by 40%. AC220 was found to induce cell death in 60% of the cells and the combination of BL-8040 with AC220 further increased the apoptotic effect achieving 97% reduction in cell viability and inducing cell death by 93% of the AML cells. In-vivo, BL-8040 was found to reduce the AML blasts in the blood from 13.5% in the control to 1.7%. Treatment with AC220 with or without BL-8040 reduced this level to 0.1%. Interestingly, the level of total mouse WBC following AC220 was significantly reduced in 65% compared to the control. This deep reduction in normal WBC was prevented when AC220 was combined with BL-8040. BL-8040 was found to decrease the number of AML cells in the BM to 2.6% compared to 12.6% in the control mice while AC220 reduced this level to 0.05%. The combination of AC220 with BL-8040 was found to further decrease this level to as low as 0.006% of AML cell in the BM. In 3/5 mice in this group the combination treatment completely eliminated the AML cells from the BM. Similar effect was observed in the spleen when BL-8040 reduced the level of AML cells from 21% in the control to 0.4% and AC220 reduced this level to 0.09%. The combination of AC220 with BL-8040 was further decreasing this level to 0.02%. The reduction in the number of AML cells in the blood, BM and spleen was accompanied with the induction of AML cells apoptosis.
Summary and Conclusion: The CXCR4 antagonist BL-8040 rapidly and efficiently induces cell death of AML cells both in-vitro and in-vivo. The combination of BL-8040 and AC220 was found to reduce the minimal residual disease of AML cells in this mice model. These results suggest potential therapeutic advantages of BL-8040 in AML patients with the FLT3-ITD-mutations by targeting not only AML anchorage in the BM but also AML survival. Furthermore, it could provide a rational basis for BL-8040 therapy in combination with the FLT3 inhibitor AC220 in this patient population.

P800

PHARMACOLOGICAL PROFILES OF AML TREATMENTS IN PATIENT SAMPLES TO PERSONALIZE TREATMENT

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Background: To aid in the identification of effective treatments for individual patients, *ex vivo* assays for detecting cell death inducible by drugs for hematological malignancies have been in development for over 20 years. We have developed an automated flow cytometry-based platform (ExviTech) that can address previous artifacts thus providing clinically predictive pharmacological data measuring leukemic cell depletion analyzed with pharmacodynamic population models.
Aims: The purpose of this study is to derive the *ex vivo* pharmacological profiles across the AML patient population of single drugs and combination treatments as a tool for individualized treatment selection.
Methods: Bone-marrow samples from 180 patients diagnosed with AML were sent to Vivia from 24 hospitals across Spain within 24 hrs. The plates were incubated for 48-hours prior to analysis with ExviTech. The percentage of leukemic cell death was determined via labeling with monoclonal antibodies and AnnexinV-FITC. Dose-response curves of cytarabine, idarubicin, daunorubicin, mitoxantrone, etoposide, fludarabine, clofarabine, and 6-thioguanine were measured in these patient samples. The added benefit of combining these drugs into 12 combination treatments was assessed by measuring their synergy in each individual patient.
Results: There was a large range of interpatient variability in the response to a single drug and even larger in the synergism between drugs. Alternative treatments are found even among CYT-(IDA/DAU/MIT). The Population Pharmacological Profiles for an individual patient is shown on the figure below. The relative drug potency in terms of their percentile ranking within the population is shown in the left panel from 0 (weakest) to 100 (most potent). Green lines represent the individual patient potency relative to the population ranking, with confidence intervals. Third column lists when a drug leaves a significant % of leukemic cells alive, potential resistant clones. The panel on the right side shows the synergism of the drug combinations treatments shown as box-plots at 10-25-75-90% to highlight their distribution. The synergism value for an individual patient in each combination is shown in green, with confidence interval as parallel dotted green lines. This representation of the Pharmacological Profile of an individual patient sample quickly identifies extreme values, when a drug or combination is very sensitive (rightward shift green lines, green boxes) or very resistant (leftward shift green lines, red boxes). This patient showed average sensitivities for most drugs though highly resistant to Clofarabine (red box) that leaves 45% alive. However this patient showed lack of synergism in multiple treatments (right, red boxes). CYT and IDA show average potencies but lack of synergism, suggesting CYT-DAU might be a more efficient treatment. These representations lead to clear guidelines in >90% samples.

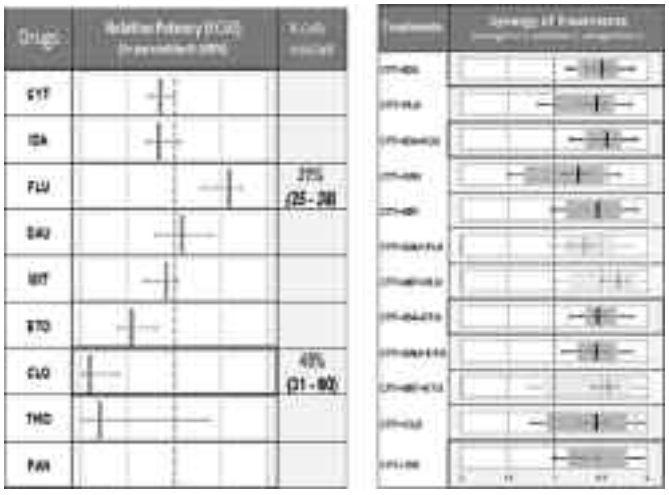

Figure 1.

Summary and Conclusion: We have developed an improved a methodology to measure the pharmacological activity of drugs and drug combinations in AML patient samples as well as modeling their pharmacological behavior. This information may be useful in selecting the optimal treatment for the individual patient, especially relapse/refractory patients in need of therapeutic alternatives. By testing the drugs used in the treatment protocols for AML directly on patient samples, a pharmacological based model has been developed to infer drug resistance or sensitivity, patient by patient.

P801

XPO1 INHIBITION USING SELINEXOR RESTORES TOPOISOMERASE IIA (TOPO IIA) LOCALIZATION TO THE NUCLEUS AND SENSITIZE PIMARY REFRACTORY AND RELAPSED ACUTE MYELOID LEUKEMIA (AML) BLASTS TO CHEMOTHERAPY

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Background: Inhibitors of topoisomerase IIα (topo IIα) are clinically effective in the management of AML. The efficacy of drugs targeting topo II is often limited by resistance and patients with primary refractory (PR) or relapsed (R) AML after induction therapy with cytarabine and Topo IIα inhibitors have a poor prognosis. Several *in vitro* studies have provided insights on potential mechanisms. Among them, a shift in Topo IIα localization from the nucleus to the cytoplasm could potentially lead to resistance to Topo IIα inhibitors. When Topo IIα is exported to the cytoplasm, it is not in contact with DNA, and Topo IIα inhibitors such as anthracyclines are unable to induce DNA-cleavage complexes and cell death. Topo IIα is exported from the nucleus by exportin-1 (XPO1), which is a nuclear protein export receptor that is over-expressed in AML. Selinexor, a selective inhibitor of XPO1 nuclear export, is currently being tested in a Phase 1 clinical trial in AML. Here, we hypothesize that increasing Topo IIα nuclear accumulation, by using Selinexor, may sensitize PR/R AML blasts to Topo IIα inhibitors.
Aims: To assess Topo IIα protein cell localization in PR/R AML blasts and to investigate the anti-leukemic activity of Selinexor in combination with Topo IIα inhibitors in AML cell lines and PR/R AML blasts.
Methods: Topo IIα cell localization was measured by Western Blotting (WB) and confocal microscopy. IC50 values for Selinexor and Topo IIα inhibitors were determined using MTS assays in AML cells lines and PR/R AML samples obtained from the OSU leukemia tissue bank. Synergy was calculated using the Chou-Talalay method. Idarubicin resistant MV4-11 cell line (R-MV4-11) was generated by exposing MV4-11 cells to low doses of idarubicin overtime (R: IC50 10 nM *vs*. no-R: 3 nM).
Results: To assess Topo IIα cell localization in PR/R AML, we examined 10 patient samples (5 PR, 5 R) by using WB and confocal microscopy. While Topo IIα expression was mostly nuclear we found variable expression of Topo IIα in the cytoplasm in 7 cases. We also found that R- MV4-11 cells exhibit a significantly higher cytoplasmic localization than MV4-11 cell lines (3 fold, $p<0.01$). While we did not found any correlation between CRM1 levels and Topo IIα expression in cell lines and patients, CRM1 inhibition using Selinexor restored nuclear localization of Topo IIα in all cell lines and PR/R AML samples. To evaluate whether the combination of Selinexor and idarubicin induce synergy, we treated the AML cell lines; MOLM-13, MV4-11, R-MV4-11 and 4 PR/R patient's blasts with Selinexor and idarubicin at two-fold dilutions of their individual IC50 values, and measured cell proliferation/cytotoxicity using the MTS assay. We found that concomitant treatment of Selinexor with idarubicin resulted in synergy (combination index (CI) values<1) in all cell lines and patient samples. These studies were repeated in MOLM-13 and MV4-11 AML cell lines using two other different Topo IIα inhibitors; etoposide and mitoxantrone. We observed synergy for all compounds (CI<1). For one patient, pretreatment and at relapse samples were available. While in the pretreatment sample, Topo IIα was exclusively nuclear, in the relapse sample Topo IIα was mostly localized in the cytoplasm. Interestingly, this patient initially received anthracycline based induction therapy and achieved complete remission. However, the patient's leukemia was refractory following a second induction using anthracyclines at relapse several months later. Treatment with Selinexor restored Topo IIα nuclear localization in this patient sample *in vitro* and strongly synergized with idarubicin (CI: 0.2).
Summary and Conclusion: Treatment with Selinexor restores nuclear localization of Topo IIα and sensitizes PR/R AML blasts to Topo IIα inhibitors.

P802

IMGN779: A CD33-TARGETED ANTIBODY-DRUG CONJUGATE (ADC) UTILIZING A NOVEL DNA ALKYLATOR, DGN462, IS HIGHLY ACTIVE IN VITRO AGAINST PRIMARY PATIENT AML CELLS AND IN VIVO AGAINST AML XENOGRAFTS IN MICE

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Background: Despite high initial response rates (about 80%) to chemotherapy, many acute myeloid leukemia (AML) patients experience a relapse of the disease, thought to be due to the outgrowth of persistent leukemic stem cells (LSC). The differential expression of CD33 on LSC compared to normal hematopoietic stem cells (HSC) makes CD33 an attractive target for treatment of AML with antibody-drug conjugates (ADCs). We have developed a new highly potent DNA alkylator, DGN462, which consists of an indolino-benzodiazepine dimer containing a mono-imine moiety. IMGN779 is an ADC comprising DGN462 conjugated to the anti-huCD33 antibody, Z4681A, via a cleavable disulfide linker.
Aims: To evaluate the *in vitro* and *in vivo* activity of the ADC IMGN779 as a potential therapeutic for CD33-positive AML.
Methods: CD33 levels and P-glycoprotein (Pgp) activity were measured by flow cytometry. Cytotoxic potencies of DGN462 and IMGN779 in AML cell lines were evaluated using continuous exposure up to 7 days, with WST-8 viability staining. Potency of IMGN779 against primary AML samples and normal bone marrow (NBM) was evaluated using colony formation assays after 24-hour exposure and after long term liquid culture to assess the potency in leukemic progenitors and LSC, respectively. The antitumor activity of IMGN779 was assessed in SCID mice bearing subcutaneous HL60/QC and EOL-1 xenografts. Pharmacokinetic parameters in CD-1 mice were determined from plasma concentrations of IMGN779 conjugate and its total Z4681A antibody component at various time points, measured by ELISA. The bioactivity of a subset of these plasma samples was confirmed by assay of cytotoxic potency against AML cells. The tolerability of IMGN779 was evaluated in CD-1 mice, with measurements of body weight, clinical observations and clinical chemistries.
Results: IMGN779 demonstrates highly potent and CD33-specific *in vitro* cytotoxicity against primary patient AML cells isolated from peripheral blood or bone marrow samples. IC_{50} values ranged from 10 to 1500 pM with the highest activity generally observed in samples with CD33 expression levels >5000 antigens per cell. In long term cultures, IMGN779 showed a dose dependent decrease of leukemic colony formation in patient AML samples. In contrast, colony formation increased in NBM, indicating that HSCs were spared. Pgp activity inversely correlated with CD33-expression levels and IMGN779 cytotoxicity. IMGN779 was highly active against AML cell lines, including Pgp-expressing cell lines, with IC_{50} values ranging from 2 to 3000 pM. IMGN779 was highly active against AML xenografts, with a minimal efficacious dose (MED) of 0.6 mg/kg (conjugate dose). Conjugate half-life was approximately 3-4 days in mice, with bioactivity maintained for at least 3 days, indicating that the conjugate remains intact and active during circulation. IMGN779 had favorable tolerability in mice (maximum tolerated dose of 40 mg/kg) without delayed toxicity or liver toxicity.
Summary and Conclusion: IMGN779 is a CD33-targeted ADC utilizing a novel DNA-alkylating agent, DGN462. Its favorable preclinical tolerability profile suggests that IMGN779 may confer a therapeutic advantage over existing clinical agents for AML that demonstrate activity, but with significant toxicity. The highly potent, CD33-targeted activity of IMGN779 against AML cell lines and primary patient AML cells *in vitro*, the anti-tumor activity observed against AML xenografts in mice and the favorable safety profile support its advancement as a potential treatment for AML.

P803

VALIDATION OF BI-SPECIFIC T-CELL ENGAGER (BITE®) ANTIBODY ACTIVITY IN VITRO AND IN VIVO AGAINST DIFFERENTIALLY EXPRESSED CELL SURFACE TARGETS IN ACUTE MYELOID LEUKEMIA

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Background: BiTE® antibodies bind both tumor-associated surface antigens and CD3 on cytotoxic T-cells, and induce specific lysis of target positive cells. BiTE® AMG 330, targeting CD33, is being developed to treat acute myeloid leukemia (AML). However, BiTE® antibodies to other AML targets are needed to mitigate potential resistance mechanisms, target CD33 negative clones and/or be potentially combined with AMG 330.
Aims: These studies aimed to identify differentially expressed and restricted cell surface targets in AML and screen new BiTE® antibodies for efficacy *in vitro* and *in vivo*.
Methods: Next generation RNA sequencing analysis was used to screen AML patient samples, normal hematopoietic and non-hematopoietic tissues for differentially expressed and restricted targets then surface expression confirmed by flow cytometry. Human monoclonal antibodies to targets were generated in XenoMouse® and converted into BiTE® antibodies. An automated, robust high-throughput T-cell dependent cytotoxicity (TDCC) screening platform was developed using Steady-Glo® to quantify live luciferase-tagged AML cell lines after 48hrs incubation with non-activated human pan T-cells with an E:T ratio of 10:1. CHO cells expressing human and cynomolgus (cyno) targets were also screened with human TDCC assays. Cytokine release was measured using MSD V-plex assays and T-cell activation markers were assessed by flow cytometry. Activity was evaluated using a xenograft model with activated human T-cells mixed with luciferase tagged U937 cell line, implanted into athymic *nude* mice subcutaneously with daily BiTE® antibody administration at 500, 250, 100 and 50 µg/kg for 10 days. Tumors were measured by bioluminescence and caliper measurements.
Results: Two hematopoietic-restricted target RNAs were overexpressed in AML patient samples but were absent or low in essential normal non-hematopoietic tissues. Target surface expression was confirmed in AML cell lines and patient samples by flow cytometry. A high throughput TDCC assay was developed to screen BiTE® antibodies and was validated with a tool CD33/CD3 BiTE®. CD33 positive and negative cell lines were tested with the tool CD33/CD3 BiTE® and assays were reproducible and robust (signal/background 10 - 500 fold, Z'=0.75 - 0.85; EC_{50}=1 - 10 pM). Six Target A and nine Target B BiTE® antibodies were generated from human monoclonal antibodies and

several candidates bound human and cyno targets. In human TDCC assays with AML cell lines the potency of BiTE® antibodies to Target A were 0.42-4.9 pM (top candidates n=2) and Target B 0.64–929 pM (top candidates n=5). BiTE® antibody potency correlated with target expression levels and cyno T cells were also activated (potency shift<10 fold *vs*. human T-cells). Potency correlated with IFNγ and TNFα secretion and CD25, CD69, CD54 and CD71 expression on T cells. Little/no background activity was found in target negative cell lines suggesting BiTE® antibodies were specific. All doses of a Target A BiTE® completely inhibited U937 tumor growth in a xenograft model equivalent to the CD33/CD3 tool BiTE® and no evidence of tumor growth was identified out to 26 days.
Summary and Conclusion: Two differentially and overexpressed AML BiTE® targets have been identified and BiTE® antibodies to these targets are potent inducers of human and cyno T-cell activation, lyse AML cells in a target selective and proportional manner *in vitro* and a Target A BiTE® has efficacy *in vivo*. BiTE® antibodies to these targets may provide alternative therapeutic options in AML patients.

P804

A NOVEL CLEC12AXCD3 BISPECIFIC ANTIBODY EFFICIENTLY INDUCES T-CELL MEDIATED LYSIS OF CLEC12A+ AML BLASTS

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Background: Acute myeloid leukemia (AML) is the most common type of acute leukemia occurring in adults. Despite improvements in chemotherapy and supportive care, AML patients still have a very poor prognosis. Therefore, novel targeted therapies that can effectively eradicate both AML blasts and their progenitor cells are much needed. CLEC12A (also known as CLL1 and hMICL) is a myeloid differentiation antigen that is expressed on >90% of de novo and relapsed AML. Moreover, CLEC12A is selectively expressed on leukemic stem cells but not on normal hematopoietic stem cells. Therefore, CLEC12A is an attractive antigen for developing targeted therapeutic approaches for AML. Bispecific antibodies (BiAb) can be used to activate and redirect cytotoxic T cells to tumor cells to mediate efficient tumor cell killing. A novel T cell-redirecting full length human BiAb was generated, that binds CD3 on T cells with one arm while the second arm binds CLEC12A on AML tumor cells. The current studies describe the functional characterization of this CLEC12AxCD3 BiAb.
Aims: To evaluate the *ex vivo* functional activities of CLEC12AxCD3 BiAb in terms of target cell binding, target specific activation of resting T cells and target specific induction of T cell mediated lysis of CLEC12A$^+$ target cells.
Methods: The specific binding characteristics of CLEC12AxCD3 BiAb was evaluated by multicolor flow cytometry using T and AML cell lines, and healthy donor derived peripheral blood and bone marrow samples. The functional activity of CLEC12AxCD3 BiAb in terms of activation of resting T cells and induction of T cell mediated lysis of CLEC12A$^+$ target cells was evaluated by flow cytometery based cytotoxicity assays using resting T cells, purified from peripheral blood of either healthy donors or AML patients, as effectors and AML cell lines or patient derived primary AML blast samples as target cells. Cytokine levels in the supernatants of the cytotoxicity assays were analyzed by Luminex using the human 10-plex cytokine panel.
Results: The *ex vivo* functional characterization data revealed that CLEC12AxCD3 BiAb binds specifically to CLEC12A expressing myeloid cells and CD3 expressing T cells present in both peripheral blood and bone marrow samples, activates T cells (induction of CD25 and CD69 expression on CD4$^+$ and CD8$^+$ T cells) in a target cell dependent manner and efficiently induces T cell-mediated lysis of CLEC12A antigen expressing target cells. An initial version of the CLEC12AxCD3 BiAb with wild-type CH2 domain induced the release of non-specific pro-inflammatory cytokines such as IL-1β, IL-2, IL-6, IL-8, TNF-α and IFN-γ involved in Fcγ receptor interactions via the human IgG1 Fc-tail. To circumvent this, the CH2 domain of the heavy chain constant region was modified to abrogate Fcγ receptor binding while retaining its binding to FcRn for long *in vivo* half life. Analysis of this Fc-silenced CLEC12AxCD3 BiAb revealed that it completely abrogated the release of non-specific pro-inflammatory cytokines, while retaining its full capacity to induce antigen specific T cell proliferation as well as antigen specific redirected lysis of AML target cells. The CLEC12AxCD3 BiAb was able to activate and redirect AML patient-derived T cells to CLEC12A positive target cells as potently as that of healthy donor-derived T cells. Most importantly, co-incubation experiments of primary AML tumor cells with autologous resting patient T cells showed that Fc-silenced CLEC12AxCD3 BiAb can very effectively induce AML blast lysis (Figure 1).

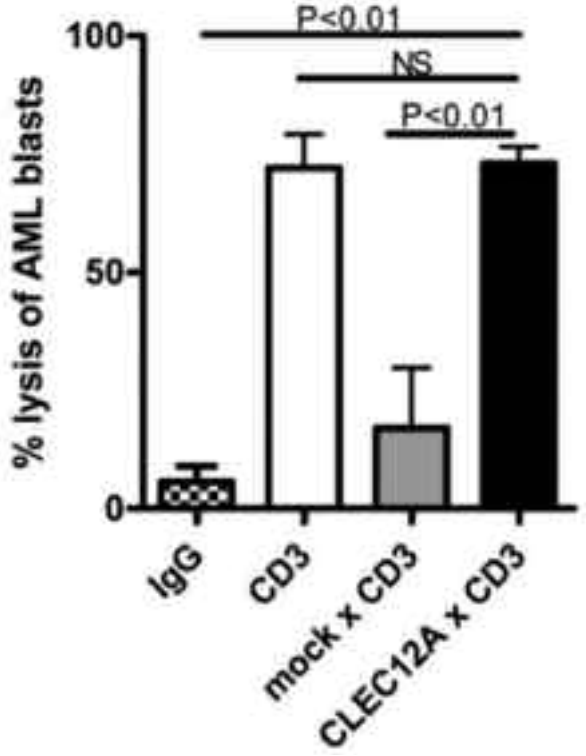

Figure 1. CLEC12AxCD3 BiAb induced specific lysis of AML blasts by autologous T cells.

Summary and Conclusion: The novel Fc-silenced CLEC12AxCD3 BiAb efficiently activates resting AML patient T cells and redirects them to specifically lyse AML blasts expressing the CLEC12A myeloid differentiation antigen. The specific and potent functional activities of this CLEC12AxCD3 BiAb warrant its further development for the treatment of AML.

P805

CATHECHOLAMINES DIFFERENTLY REGULATE HUMAN AML AND NORMAL HEMATOPOIETIC PROGENITOR CELL MOTILITY VIA MIR126 AND RGS16

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Background: Motility, proliferation, bone marrow (BM) retention and egress to the circulation of hematopoietic stem and progenitor cells (HSPCs) are key elements in normal hematopoiesis and also in the pathogenesis of acute myeloid leukemia (AML). HSPCs are tightly regulated in the BM niche by various molecules. We have previously found that normal human HSPCs functionally express beta2-adrenergic receptors (β2-AR) that are up-regulated by myeloid cytokines such as G-CSF. The catecholaminergic neurotransmitters, epinephrine and norepinephrine (NE), activate both Wnt and GSK3β signaling pathways via β2-AR, leading to enhanced HSPC proliferation, motility and BM repopulation (Spiegel et al, Nat Immunol 2007; Lapid et al, JCI 2013). These findings indicate HSPC regulation by dynamic interactions of the sympathetic nervous and hematopoietic systems. However, the role of catecholamines in regulation of AML remains elusive.
Aims: The main objective of this study is to determine the role of cathecholamines in regulation of HSPC and AML cell motility.
Results: We found that human primary AML cells and several human AML cell lines from different FAB subtypes express β2-AR. NE, a β2-AR activating ligand, increased β2-AR expression and the CXCL12-induced migration of human AML primary cells and cell lines. NE treatment significantly enhanced CXCL12 induced actin polymerization, which drives most of the cellular movements. Three to five daily injections of NE to NSG mice, previously engrafted with human AML cells, enhanced the egress of these cells from the BM to the peripheral blood. Looking for downstream effectors of β2-AR, we focused on RGS16, a G-protein signaling regulator, which negatively regulates CXCL12/CXCR4 axis (Berthebaud *et al.*, Blood 2005). We found that NE decreased RGS16 expression (both in protein and mRNA level) in monocytic AML cells concurrently with an increase in cell migration. However, no effect on either cell migration or RGS16 expression was observed in non-monocytic AML cells. One of the regulators of RGS16 levels is miR126, which is highly

expressed in AML and normal HSPCs and involves in mobilization and proliferation of normal HSPCs. Indeed, in search for the mechanisms underlying the above observed differences, we found that the enhancing effect of NE on CXCL12-induced migration of monocytic AML cells was accompanied by up-regulation of miR126 expression concomitantly with down-regulation of RGS16 expression, whereas in non-monocytic AML cells we observed the opposite effects, suggesting that NE differently regulates AML cells belonging to different FAB subtypes. Importantly, over-expression of miR126 by lentivirus transduction in several AML cell lines resulted in upregulation of their CXCL12-induced migration. Contrary to AML cells, normal HSPCs expressed low levels of β2-AR and NE did not affect either RGS16 expression or CXCL12-induced migration of both mononuclear and CD34+ cells derived from human cord blood and BM.
Summary and Conclusion: Our results demonstrate that while normal and AML cells share common mechanisms that govern their motility, there are unrevealed yet mechanisms, apparently cell-type dependent, which uniquely lead to opposite effects in normal HSPCs, monocytic and non-monocytic AML cells. Altogether, these findings suggest that targeting of miR126 and RGS16 pathways by specific agonists and antagonists may serve as a new approach for selective eradication of leukemia-inducing stem cells.

P806

CELL-TO-CELL COMMUNICATION IN ACUTE MYELOID LEUKEMIA BY TUNNELING NANOTUBES

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Background: Acute myeloid leukemia (AML) is a heterogeneous and aggressive blood cancer originating in the bone marrow. Conventional therapy is not well tolerated by elderly patients and the survival rate is limited. An improved understanding of cellular communication in AML could provide new information with respect to disease-linked mechanisms, progression, treatment and resistance. The tunneling nanotube (TNT) is a novel type of cell-to-cell communicator, 50-200 nm in diameter, F-actin containing structure connecting two or more cells. TNTs have been observed in a variety of cells including macrophages, B and T-cells, different cancer cells and osteoclasts. It has been shown to transport various cell components including mitochondria, in addition to spread pathogens like viruses and bacteria. TNT has also been suggested to have a role in transfer of multi-drug resistance genes. The exact molecular mechanisms behind TNT formation are still unclear, but the TNFαIP2 protein, found to be important in hematopoiesis, is one of the suggested molecules needed for TNT formation.
Aims: To identify and quantify TNTs in AML cell lines and AML patient cells and investigate the possible functional role of TNT following chemotherapy. Further map the molecular mechanisms responsible for TNT formation and regulation in AML.
Methods: The following cell lines were studied: OCI-AML3, NB4, HL-60, MV4-11 and MOLM-13. Primary cells: PBMCs from nine AML patients with more than 70% blasts in the blood and PBMCs from six healthy donors. The cells were investigated for TNT formation by fluorescence microscopy. For TNT verification the cell membranes were stained with lectin staining and the presence of F-actin by phalloidin staining. Both cell lines and primary AML patient cells were treated with cytarabine (AraC) alone (6 and 24h) or in combination with the two anthracyclines idarubicin and daunorubicin (4 and 24h). Also the effect of all-trans retinoic acid (ATRA) on TNFαIP2 (by immuno blotting) and TNT formation was investigated (24h).
Results: We found that all investigated cell types expressed TNT connections. The cell lines have small variation in percentage TNTs (0.3-7.8) as well as the PBMCs derived from six healthy donors (3.6-8.3) while the PBMCs derived from the nine AML patients showed greater variation (0-11.5). Treatment of cells with AraC resulted in a significant decrease in TNT numbers both in AML cell lines and in two of three investigated AML patients. Interestingly, idarubicin seems to have an antagonizing effect on AraC with more effect compared to daunorubicin (24h). Treatment with ATRA caused an increased expression of TNFαIP2 in MOLM-13, NB4 and HL-60, this correlated with TNT numbers in two cell lines. The level of TNFαIP2 seemed to correlate with TNT number in the cell lines investigated.
Summary and Conclusion: We demonstrated the existence of TNTs in both AML cell lines and primary AML cells. We observed a variance in TNT numbers between the different cell types and greatest deviation was observed in the patient samples. The chemotherapeutics daunorubicin and idarubicin demonstrated a tendency of TNT increase while AraC quenched TNT formation. The functional impact of TNT in bone marrow and the potential role in AML therapy together with the role TNT play in drug transfer will be further investigated.

P807

TIGAR COOPERATES WITH GLYCOLYSIS TO INHIBIT LEUKEMIA CELLS APOPTOSIS AND PREDICTS POOR PROGNOSIS IN PATIENTS WITH CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA

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Background: Recent studies has suggested that high levels of glycolytic metabolism in AML blasts were associated with resistance to chemotherapy and pateints's survival. TP53-induced glycolysis and apoptosis regulator (TIGAR) inhibits glycolysis and promotes tumor cells survival. Some studies have reported elevated expression of TIGAR in a few solid tumor types, suggestive of an important association.
Aims: The studies concerning mechanism of TIGAR and glycolysis inhibiting apoptosis and clinical implications of TIGAR expression in AML patients remained unknown.
Methods: Firstly we assessed expression level of TIGAR, by RQ-PCR, in the mononuclear cells from 116 patients with newly diagnosed cytogenetically normal AML (CN-AML) at diagnosis, and its clinical significance. All patients received cytarabine-based intensive chemotherapy. Patients with TIGAR expression values above the median of all patients were defined as high TIGAR expression ($TIGAR^{high}$), and all other patients were considered to have low TIGAR expression ($TIGAR^{low}$). Clinical and biological characteristics such as gene mutations were compared between $TIGAR^{high}$ and $TIGAR^{low}$ patients. Secondly we investigated the TIGAR functions in 3 leukemic cell lines and cells transducted with siRNA-TIGAR. CoCl2 and 2-DG were used to induce and block glycolysis, respectively. All study procedures and informed consent forms were approved by the institutional review board.
Results: There was no significant difference in most of the clinical characteristics between $TIGAR^{high}$ and $TIGAR^{low}$ patients. $TIGAR^{high}$ and $TIGAR^{low}$ patients had similar rate of complete remission (74.5% *vs* 72.7%). $TIGAR^{high}$ patients showed a trend towards a higher relapse rate than $TIGAR^{low}$ patients (29.1% *vs* 18.2%) but the difference was not significant. $TIGAR^{low}$ patients had significantly longer OS and DFS than $TIGAR^{high}$ patients (Fig1). Cumulative relapse incidence of $TIGAR^{high}$ patients was significantly higher than $TIGAR^{low}$ patients (P=0.044). Cox regression analysis showed that lower TIGAR expression was associated with a reduction of the risk of death (P=0.039), representing an independent prognostic factor. TIGAR mRNA expression and protein level were increased in all 3 leukemia lines. Treatment of leukemia cells with CoCl2 increased TIGAR expression, whereas inhibition of glycolysis with 2-DG decreased TIGAR expression. After siRNA-induced downregulation of TIGAR, PFKFB3 expression and ROS levels were elevated, while GSH content was reduced. Stimulation of glycolysis decreased PFKFB3 expression, while inhibition of glycolysis had the opposite effect. These data suggest that TIGAR could simultaneously inhibit glycolysis, decrease ROS and increase GSH levels in leukemia cells. Combining glycolysis inhibition and TIGAR knockdown yielded an extremely high level of apoptosis (88.0%). p53 only slightly upregulates TIGAR expression in leukemia cells, and that p53 activation promotes apoptosis, whereas TIGAR inhibits apoptosis.

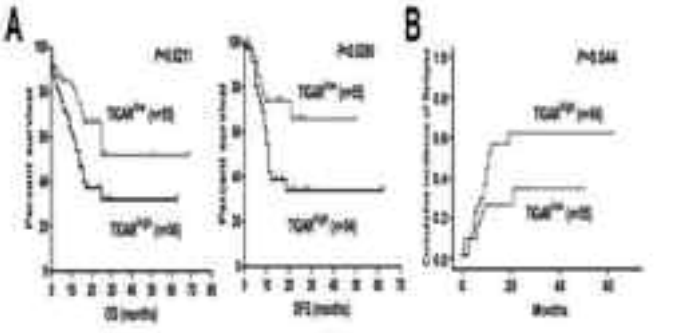

Figure 1.

Summary and Conclusion: High expression of TIGAR is associated with poor prognostic factor for OS and DFS in CN-AML patients. TIGAR was increased in leukemic cell lines and protected leukemia lines from apoptosis. TIGAR knowdown or block glycolysis alone could enhance leukemia cells apoptosis. Sustained TIGAR activation, uncoupled from p53, may support AML cell growth and survival. TIGAR in cooperation with glycolysis has a strong anti-apoptotic effect in AML cells, suggesting that combinations of TIGAR inhibitors with anti-glycolytic agents could prove to be powerful novel therapies for future clinical use.

P808

GLYCOLYSIS INHIBITION ON ACUTE MYELOID LEUKEMIA

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Background: Growing evidences show that upregulation of glycolysis occurs

also in leukemia cells. This metabolic peculiarity has been connected to signalling pathway alterations, especially those involving PI3K/Akt/mTOR, HIF1a, Ras/Raf/MEK/ERK. Thus, the current scenario depicts a crosstalk between proteins and metabolites that tends to the acquisition of a transformed metabolic profile conferring a proliferative advantage on leukemia cells. Therefore, targeting energetic pathways may represent a novel strategy for therapeutic intervention on leukemia.

Aims: Here we investigate whether (i) acute myeloid leukemia (AML) primary samples and cell lines are characterized by an increased glycolytic rate, (ii) the Pyruvate Dehydrogenase Kinase inhibitor, a pyruvate-mimetic molecule, the dichloroacetate (DCA), modifies glycolytic profile and (iii) affects proliferation and apoptosis of leukemic cells.

Methods: We investigated the glycolytic rate on resting and activated normal peripheral blood lymphocytes (NPBLs) and on AML cell lines. The effects of DCA exposure were evaluated on 4 human AML cell lines (U937, OCI-AML3, HL-60, MOLM13), on primary samples obtained from 4 AML patients and on NPBLs. Cell counts, apoptosis induction (AnnV) and changes in glucose and lactate culture medium levels (GEM4000, Instrumentation Laboratory, UK) were measured. Glucose consumption rate (GCR) and lactate production rate (LPR) were calculated according to Li *et al.* (Biotechnol. Appl. Biochem., 2005). Moreover, extracellular acidification rate (ECAR), directly related to the amount of lactate excretion, were obtained using an XF24 Analyzer (Seahorse Bioscience, USA), which allows real time analysis of metabolic fluxes.

Results: Resting NPBLs were characterized by a very low glycolytic rate, according to their quiescent state, while cultured phytohemagglutinin-activated NPBLs displayed a remarkable increase in glycolytic rate: the GCR calculated over 72 hours showed a 25 fold-increase, while LPR had a 10 fold-increase. All AML cell lines showed an even higher glucose catabolism when compared to activated NPBLs: at 24h the U937 cell line had a 6.7 fold higher GCR, while the OCI-AML3 cell line showed a 4-fold increase. XF24 real time metabolic measurement underlined differences in cell line glycolytic rates: after glucose injection (10mM), U937 showed doubled ECAR values as compared to HL60 (51±8,1mpH/min *vs* 25±6,3mpH/min, respectively). We then evaluated the effects of glycolysis inhibition on AML cells by DCA. By using XF Analyzer we demonstrated a 44% drop in ECAR following 5mM DCA injection in U937 cell line (from 107±10,2 mpH/min to 60±4,6mpH/min). Functional effect induced by DCA (1-7,5mM) showed cell growth arrest and apoptosis induction on 3 out of 4 AML models, in a dose- and time-dependent fashion. In fact, the IC50 of DCA exposure at 72h on U937, MOLM-13 and OCI-AML3 were 2,6mM, 5,1mM, 5,8mM, respectively. Conversely, HL-60 proved resistant (IC50 n.d.), as expected from the lower glycolytic rate characterizing this cell line. A similar pro-apoptotic activity was observed at 72h of DCA *in vitro* exposure on 4 out 4 primary samples: AnnV positive cells ranged from 14,1±8,9% (vehicle) to 25,3%±15,1% (1mM), 33±19,3% (2,5mM), 48,5±35,9% (5mM), 59,3±24,4% (7,5mM). Conversely, no significant effects were observed on both normal and activated NPBLs.

Summary and Conclusion: In this pre-clinical study, we documented that AML cells are characterized by higher glycolytic rates compared to normal cells. The DCA activity proved effective in modifying glycolysis rates and this effect is associated with apoptosis induction on primary AML and on cell lines.

P809

SK053 – AN ALLOSTERIC PROTEIN DISULPHIDE ISOMERASE INHIBITOR INDUCES DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Background: The use of differentiation-inducing agents including all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia was a remarkable therapeutic breakthrough resulting in cure rates exceeding 80%. However, there is no such significant progress in the treatment of other acute myeloid leukemia (AML) types. Thus search for new agents exerting anti-leukemic effects by targeting novel and unique cellular mechanisms is of utmost clinical importance. Numerous human proteins involved in tumor formation contain allosteric disulfide bonds that are cleaved by oxidoreductases or by thiol-disulfide exchange. Targeting of allosteric disulfide bonds is a novel promising strategy in cancer therapy. We have developed SK053, a small molecule inhibitor of enzymes involved in allosteric disulfide bonds formation such as protein disulfide isomerase (PDI).

Aims: The aim of the studies was to determine if targeting the formation of allosteric disulfide bonds with SK053 can induce antitumor effects in AML.

Methods: Precipitation of proteins binding to biotinylated form of SK053 (SK231) followed by mass spectrometry analysis was used to identify molecular targets in AML cells. Enzymatic assays with recombinant proteins was used to validate targeting activity of SK053. Trypan blue exclusion was used to determine cytostatic/cytotoxic effects of SK053 in AML cells. May-Grünwald-Giemsa staining, nitro blue tetrazolium (NBT) reduction assay and flow cytometry analysis of membrane differentiation markers were used to determine differentiation of AML cells incubated with SK053. Quantitative RT-PCR and immunoblotting were used to determine changes in gene expression and protein levels. Lentiviral transduction with shRNA targeting PDI was used to determine the role of these proteins in differentiation-inducing activity of SK053.

Results: SK231 precipitated PDI from human AML NB4 cells, and mass spectrometry analysis revealed that SK053 covalently binds to PDI. In a turbidimetric assay of insulin disulfide reduction SK053 inhibits the enzymatic activity of PDI with IC_{50} of 10 μM. Since PDI blocks translation of CCAAT enhancer binding protein alpha (CEBPA), a transcription factor involved in neutrophils maturation, we set out to evaluate the activity of SK053 in human AML cells to see whether it can induce differentiation and cytostatic/cytotoxic effects against these cells. We observed that SK053 exerts significant cytostatic/cytotoxic activity in various types of human AML cells (HL60, NB4, KG-1 and MOLM14), and induces differentiation of AML blasts into more mature myeloid cells as evidenced morphologically in May-Grünwald-Giemsa staining, nitro blue tetrazolium (NBT) reduction assay as well as by increased expression of cell membrane differentiation markers (CD11b, CD14 and CD15), measured with flow cytometry. Moreover, incubation of AML cells with SK053 induces expression of CEBPA and hexokinase 3 mRNA, decreases levels of SOX4 mRNA in quantitative RT-PCR and increases amount of CEBPA protein in nuclear fraction measured with immunoblotting. Moreover, differentiation of AML cells was associated with induction of ER stress. Finally, SK053 induces differentiation of primary leukemic cells freshly isolated from AML patients.

Summary and Conclusion: SK053 targets PDI and thioredoxin/thioredoxin reductase system, has significant anti-leukemic activity and induces differentiation of various types of human AML cells. Thus, targeting of enzymes involved in allosteric disulfide bonds formation with small molecule inhibitors presents a novel and promising therapeutic strategy in acute myeloid leukemia.

P810

THE ROS/SUMO AXIS IS INVOLVED IN ACUTE MYELOID LEUKEMIA (AML) CELLS RESPONSE TO CHEMOTHERAPEUTIC DRUGS AND CONSTITUTES A POTENTIAL TARGET TO OVERCOME CHEMORESISTANCE IN AML

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Background: Small Ubiquitin-related MOdifier (SUMO) is a post-translational modifier of the ubiquitin family that has recently emerged as a critical regulator of protein function and fate. Moreover, increasing evidence suggest SUMO has important roles in cancer.

Aims: The goal of this study was to determine the role of the SUMO pathway in the response of Acute Myeloid Leukemia cells to the chemotherapeutic drugs used in the clinic and the relevance of its targeting to overcome chemoresistance, which is responsible for the poor prognosis of this cancer.

Results: We report that cytarabine (Ara-C) and daunorubicin (DNR) induce a fast deconjugation of SUMO from its target proteins in AML cell lines and patient samples. This desumoylation is due to Reactive Oxygen Species(ROS)-dependent inhibition of the SUMO-conjugating enzymes through the formation of a disulfide-bond between E1 and E2 catalytic cysteines. One of the important roles of sumoylation is the control of gene expression. To identify genes induced by desumoylation, we carried out transcriptomic analysis in AML cells treated with anacardic acid, an inhibitor of the SUMO E1. Among the highly induced genes, we studied *DDIT3,* a gene known to induced by chemotherapeutic drugs and involved in AML apoptosis. We could show that genotoxics-induced desumoylation of the proteins present on its promoter participate in its transcriptional activation by Ara-C. Importantly, preventing desumoylation also delayed entry of the cells in apoptosis. In chemoresistant AML cells, chemotherapeutic drugs do not induce this ROS/SUMO axis. However, its reactivation by pro-oxidants or inhibition of the SUMO pathway with anacardic acid restores both *DDIT3* expression and apoptosis in chemoresistant cell lines and patient samples. In particular, anacardic acid target leukemic stem cells, which are resistant to Ara-C and thought to be responsible of patient relapse. Finally, inhibition of the SUMO pathway decreases tumor growth in mice xenografted with chemoresistant AML cells.

Summary and Conclusion: In conclusion our data show that the ROS/SUMO axis is involved in genotoxics-induced death of AML cells, in particular through the control of specific transcriptional programs. Moreover, targeting the ROS/SUMO axis might constitute a novel therapeutic strategy for AML patients resistant to conventional chemotherapies.

P811

ARSENIC TRIOXIDE AND FUCOIDAN SYNERGIZE TO INDUCE APOPTOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) represents a subtype of acute myeloid leukemia (AML) characterized by clonal expansion of malignant promyelocytes whose differentiation is blocked through t(15;17) translocation. Although arsenic trioxide (ATO) treatment has dramatically increased the survival rate of APL patients, its use and that of ATRA (another standard APL agent) can cause undesirable side effects such as the so-called differentiation syndrome characterized by fever, acute respiratory distress, acute renal failure and death. Therefore, the need to develop new less toxic strategies and/or use lower doses of ATRA and ATO is of great interest. Fucoidan is a sulphated polysaccharide which is extracted from brown seaweeds and has shown promising anti-tumor activities in various tumor cells. In the present study, we examined the anti-growth effect of fucoidan on a human APL cell line and also investigated the synergistic activity of fucoidan and ATO in APL, in order to reduce ATO doses and its related side effects.
Aims: 1. To determine the anti-growth effect of fucoidan on human APL cells. 2. To determine the probable synergistic effect of fucoidan with ATO on APL cells
Methods: Human APL cells (NB4 cell line) characterized by t(15;17) were treated with various concentrations of (i) fucoidan, (ii) both clinical and lower doses of ATO and (iii) a combination of the two agents. The proliferation rate, apoptosis, DNA content and DNA fragmentation were measured. Western blots were used to measure the expression level of apoptosis-related proteins. To determine whether the anti-growth effect of ATO and fucoidan is specific for APL cells, we treated t(8;21) translocation positive kasumi cell line with similar doses of fucoidan and ATO and measured the cells proliferation rate after 24, 48 and 96 hours.
Results: We found that fucoidan inhibited the growth of NB4 cells in a time and dose dependent manner. Cell cycle and apoptosis studies revealed increase of sub-G0/G1 population, DNA fragmentation and annexin V positive apoptotic. Activated caspase 3 and nuclear protein PARP (downstream target of activated caspase 3) increased in treated cells. In synergy studies, we found that the combination of fucoidan and ATO markedly decreased the amount of viable cells in the cytotoxicity assay compared to fucoidan or ATO only. These results were then confirmed with: elevated sub G0/G1 population in DNA content analysis, increased annexin V apoptotic cells and a significantly increased amount of fragmented DNA in cells treated with ATO+fucoidan compared to fucoidan and ATO only. The described effect was observed in cells treated with low dose ATO + fucoidan as well. To determine the specificity of ATO, we treated AML-M2 kasumi cells and observed that neither combined ATO/fucoidan nor single treatment with fucoidan and ATO decreased the proliferation of kasumi cells after 96 hours.
Summary and Conclusion: The results from our *in vitro* studies indicate that - Fucoidan induces a caspase-related apoptosis in APL cells - The *in vitro* combination of fucoidan and ATO (in both clinical and low doses) synergistically suppress the growth of APL cells - Such an effect is specific in APL cells as the two agents did not suppress the growth of AML1/ETO positive kasumi cells over 96 hours. Our data suggests that the combination of fucoidan and ATO should be investigated as a possible low-toxicity treatment of acute promyelocytic leukemia.

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P812

THE PROGNOSTIC RELEVANCE OF THE 2008 WHO CLASSIFICATION OF MYELOID NEOPLASMS IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background: In 2008, the World Health Organization (WHO) classification of myeloid neoplasms was revised combining morphologic, cytogenetic, and molecular data into a system relevant for prognosis. However, its prognostic relevance in pediatric population remains to be clarified.
Aims: Thus, we analyzed 453 pediatric patients categorized according to the 2008 WHO classification on a clinical trial for *de novo* acute myeloid leukemia (AML).
Methods: Between November 2006 and December 2010, 485 consecutive patients aged<18 years with suspected AML excluding acute promyelocytic leukemia, Down syndrome, secondary AML, myeloid/natural killer cell leukemia, and myeloid sarcoma were registered in AML-05 which was conducted by the Japanese Pediatric Leukemia/Lymphoma Study Group. The diagnosis according to the 2008 WHO classification was determined centrally by integrating morphologic, cytogenetic immunologic, molecular and clinical parameters. Although *FLT3*-ITD was prospectively analyzed for all patients, *NPM1* and *CEBPA* mutation was retrospectively analyzed using preserved samples.
Results: Of the 485 patients registered, 32 patients were excluded because of misdiagnosis or refusal by the guardians. Of the 453 eligible patients, 235 patients (48.5%) were identified as AML with recurrent genetic abnormalities, 93 patients (20.5%) as AML with myelodysplasia-related changes, and 111 patients (24.5%) as AML, not otherwise specified, and 7 (1.5%) as mixed phenotype acute leukemia. Among the 235 patients showing recurrent cytogenetic abnormalities, 126 patients had t(8;21)(q22;q22);*RNX1-RUNX1T1*, 32 had inv(16)(p13q22) or t(16;16)(p13;q22);*CBFB-MYH11*, 39 had t(9;11)(p22;q23); *MLLT3-MLL*, 30 had variant MLL translocations, 4 had t(6;9)(p23;q34); *DEK-NUP214*, 2 had inv(3)(q21q26), and 2 had t(1;22)(p13;q13);*RBM15-MKL1*. AML with mutated *NPM1* was found in 13 of 336 (3.9%) patients and AML with mutated *CEBPA* was found in 47 of 323 (14.6%). The 3-year event-free survival (3y-EFS) and 3-year overall survival (3y-OS) of patients with t(8;21)(q22;q22);*RNX1-RUNX1T1* was 67.5% and 91.2%, respectively. The 3y-EFS and 3y- OS of patients with inv(16)(p13q22) or t(16;16)(p13;q22);*CBFB-MYH11* was 76.7% and 96.9%, respectively. The 3y-EFS and 3y- OS of patients with t(9;11)(p22;q23); *MLLT3-MLL* was 58.6% and 71.1%, respectively. The 3y-EFS and 3y- OS of patients with variant MLL translocations was 49.2% and 65.2%, respectively. The outcomes for other subtypes of recurrent genetic abnormalities could not be determined because of the small number of patients. AML with myelodysplasia-related changes showed a significantly worse 3y-EFS compared with AML, not otherwise specified (37.1% *vs* 53.8%, p=0.02), but 3y-OS was not significantly different (56.8% *vs* 68.9%, p=0.05).
Summary and Conclusion: Almost half patients with pediatric AML were identified as AML with recurrent genetic abnormalities, while other half were identified as either AML with myelodysplasia-related changes or AML, not otherwise specified. The outcome for the core binding factor AML was excellent and the outcome for t(9;11)(p22;q23) was good as well. On the other hand, AML with myelodysplasia-related changes indicated a poorer outcome compared with AML, not otherwise specified. The outcomes for other AML subtypes should be determined in larger studies.

P813

REVISITING THE CLONAL DYNAMICS OF TRISOMY 21 AND MONOSOMY 7 MOSAICISM IN CONGENITAL NEUTROPENIA AML

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Background: It has been shown that congenital neutropenia patients enter a step-wise progression towards AML. Interestingly single gene mutations precede chromosomal abnormalities, while ploidy changes are foreshadowing

the acute stage of myeloid leukemia. The early detection of the latter is important as it can be of high prognostic value. The classical cytogenetic techniques, on which this knowledge has been based, have a known limited sensitivity and assay-to-assay variation.
Aims: To fully understand the time kinetics and the propensity of chromosomal changes, we decided to re-evaluate the ploidy mosaicism of CN AML samples with high sensitivity.
Methods: To this end we subjected bone marrow or peripheral blood of 59 CN patients to Affymetrix GenomeWideSNP arrays. Notable, eight individuals later progressed to AML OR MDS. First, to our surprise we failed to confirm chromosomal aberrations in prior cytogenetically verified samples with the Genotyping Console(TM) software. We therefore developed a custom workflow by comparing the signal of the copy number markers to the reference from the HAPMAP project and process the log2ratio in the R software package. The main statistical procedure was to recalibrate the signal intensities to autosomes and infere clonal ploidy mosaicism by fitting to exponential normal curves derived from XY chromosome log2ratio values from male and female samples.
Results: With this novel approach, we could confirm three previously characterized chromosomal changes in patients which later developed leukemia, and could detect a pre-leukemic clone earlier. In particular, one CN patient presented monosomy 7 (70% positive cells) already twenty month before AML M2/M4 diagnosis (pat#1), while in another case 68% of cells harboured this defect (pat#2) at AML M0 diagnosis. Additionally, the new method redated the occurance of trisomy 21 (14.9% positives) prior to AML M1 diagnosis eleven month before overt leukemia (pat#3). This is particular significant as this patient has been previously described by us as having developed monosomy 7 first, while we now identified trisomy 21 as the founding ploidy change prior AML with this more sensitive assay.

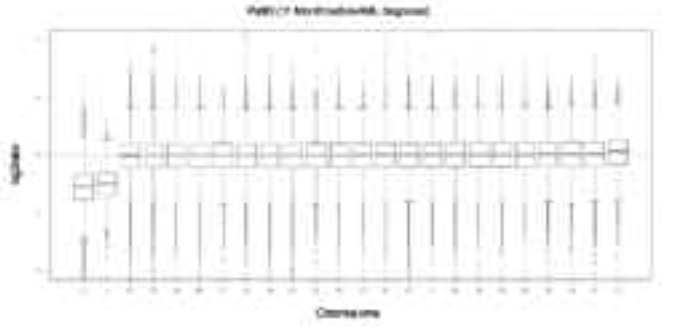

Figure 1.

Summary and Conclusion: Overall our results clarify the role of chromosomal abnormalities in CN and underscore its relevance in the dynamic towards AML progression. It is discussed that our statistical workflow is superior to conventional CNV calling algorithms to detect chromosomal cell clones prior overt leukemia. SNP Chip-based techniques should be considered as an high sensitive tool for ploidy monitoring in pre-leukemic syndromes in general.

P814

A SIMPLE AND EFFECTIVE STRATEGY TO DECREASE EARLY DEATHS IN ACUTE PROMYELOCYTIC LEUKEMIA USING A STREAMLINED SET OF GUIDELINES PAIRED WITH EXPERT SUPPORT

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Background: Large cooperative group studies have reported survival of acute promyelocytic (APL) leukemia at 90%, with low early mortality rates of approximately 5%. The recent Italian GIMEMA trial, in non-high risk patients, showed a survival rate of 98% in the ATRA/Arsenic trioxide arm. These spectacular results are not evident in studies from cancer registry data. The United States SEER data shows the relative survival rates in APL patients as 71% (one year) and 65% (five year). Studies from the Swedish registry, Brazil and other single institutions, show the early mortality as high as 30%. The common causes of death are hemorrhagic complications (HC), differentiation syndrome (DS), infection and multi-organ failure. We report our results showing early deaths can be decreased by using a set of streamlined treatment guidelines along with support from APL experts.
Aims: 1. To decrease early deaths in APL with a streamlined algorithm and expert support. 2. To improve overall survival in APL across the general population.
Methods: We performed a retrospective chart review of APL patients at Georgia Regents University Medical Center (GRU) and Emory University Hospital (EUH) between July 2005 and June 2009. Seven of 19 patients (37%) treated at GRU had died, an unusually high mortality rate. This high early death rate prompted the GRU group to develop simple treatment guidelines (1.5 pages) with emphases on quick diagnosis, prompt therapy, and proactive management of the major causes of death during induction. More importantly we made our treatment guidelines available to other leukemia treatment centers in Georgia and South Carolina and assisted treating oncologists with patient management during induction. We implemented this algorithm in 10/2010.
Results: From July 2005 through June 2009, a total of 59 patients, aged 18-89 years were seen; 19 at GRU and 40 at EUH. These included 21 high-risk and 38 low-risk patients. Patients were considered high-risk based on a presenting white cell count of >10,000. There were a total of 16 deaths (27%); ten in the high-risk group and six in the low-risk group. At Emory the death rate was 17.5%, with two of the nine deaths being patients transferred at a late stage of the illness. Nine deaths were secondary to hemorrhagic complications, five were DS related and two were from infection. Three patients died within five days of presentation to the hospital. From 11/2010 to 01/2014 the new algorithm was implemented. Ten patients were treated at GRU and 31 patients were at 10 other centers. All patients were either under direct care of an expert or co-managed with an APL expert. Age range was 21-73 years. 11 patients were high-risk and 30 low-risk. There were 2 deaths (4.8%) during induction both from hemorrhagic complications; one of these patients refused transfusions for religious reasons.

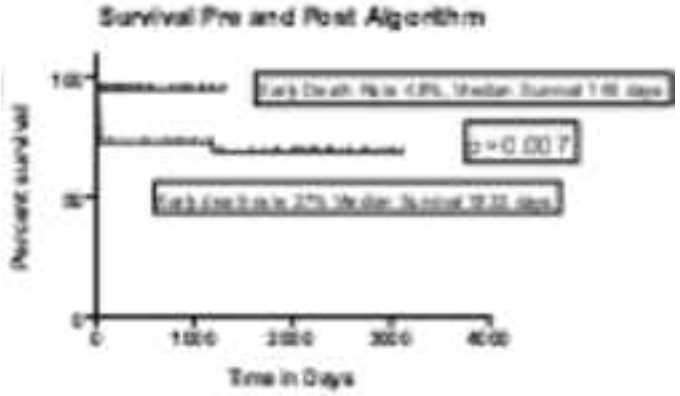

Figure 1.

Summary and Conclusion: Our own experience and multiple other studies show that early mortality is a significant problem globally in APL. We show that use of our streamlined treatment algorithm, developed independently due to poor outcome at GRU, along with support from experts will lead to better outcomes in this curable disease. Education and networking can lead to improved treatment outside of tertiary hospitals and prevent late stage transfers. A similar approach pioneered by investigators in Brazil shows this to be an effective model. We believe our experience warrants large scale implementation of our strategy to decrease early mortality in developed and emerging countries. We are recipients of a $1.68 million grant by the Leukemia Lymphoma Society to decrease early mortality in the states of Georgia and South Carolina with a population of 15 million over a 3-year period.

P815

INCIDENCE AND TYPE OF IDH1 AND IDH2 MUTATIONS IN AML-NK PATIENTS AND THEIR PROGNOSTIC IMPACT ON TREATMENT OUTCOME

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Background: The isocitrate dehydrogenase (*IDH)1* and *IDH2* mutations have been described as frequent recurrent aberrations in acute myeloid leukemia (AML), particularly in those with normal cytogenetics. Types of these mutations and their clinical impact on treatment outcome have not yet been fully understood.

Aims: In this study we performed an analysis of the clinical and prognostic impact of *IDH1* and *IDH2* mutations combined with other frequently occurred mutations.
Methods: In a total of 67 nomral karyotype acute myeloid leukemia (AML-NK) patients achieved complete remission (CR), candidate mutations in 21 genes were identified by whole exome sequencing which has 41-89X coverage and by single-nucleotide polymorphism array analysis using marrow mononuclear cells at diagnosis of AML-NK. Subsequently, mutation analyses of other genes (*i.e. NPM1, CEBPα* double mutation*, FLT3*/ITD*, DNMT3A, IDH1, IDH2*) were performed using Sanger sequencing in another subset of 527 AML-NK patients as a validation cohort.
Results: Of 594 patients in total (median age: 57, ranges: 15-85). *IDH1* and *IDH2* mutations were identified in 7.0% and 14.6% of the patients, respectively. *IDH2* mutation was associated with lower leukocytes counts at presentation of AML-NK (P<0.01). In *IDH1* mutation, R132C (3.4%) was the most frequent alteration. In addition, R132H (2.2%), R132S (0.5%), R132G (0.3%), K87K (0.2%), R82K (0.2%), P78H (0.2%) were detected. In *IDH2*, R140Q (11.3%), R172K (1.7%), R140W (0.5%), R140L (0.5%), I142L (0.2%), T146T (0.2%), G176G (0.2%) were identified. *IDH1* mutation was associated with higher incidence of *DNMT3A* mutation (46.3% *vs*. 29.3%, P<0.05). *IDH2* mutation was associated with higher rate of *NPM1* mutation (52.3% *vs*. 39.6%, P<0.05), whereas it also showed lower rate of *CEBPα* double mutation (4.7% *vs*. 12.4%. P<0.05). In 455 patients who received standard remission induction (RI) chemotherapy, *IDH1*^{R132} mutation was associated with higher rate of relapse (62.5% *vs*. 37.9%, P<0.05) and shorter leukemic-free surival (LFS) (median 12.5 ms. *vs*. *N.A*., P<0.01). On the other hand, *IDH2*$^{R140Q\ or\ R172K}$ mutation was an independent prognostic factor for longer LFS in univariate and multivariate analyses, adjusted by other variables including age, leukocyte counts at diagnosis, transplantation and other genetic mutations (P<0.05, Hazard ratio, HR 1.896, 95% confidence interval, 95% CI=1.040-3.455). Further, all patients divided into four groups according to the *IDH1*^{R132} and *IDH2*^{R140Q} or ^{R172K} mutation status - G1 (n=60): *IDH1*$^{R132-}$ & *IDH2*$^{R140Q+\ or\ R172K+}$, G2 (n=364): *IDH1*$^{R132-}$ & *IDH2* $^{R140Q-\&\ R172K-}$, G3 (n=31): *IDH1*$^{R132+}$ & *IDH2*$^{R140Q-\ \&\ R172K-}$, G4 (n=0): IDH1^{R132+} & *IDH2*$^{R140Q+\ or\ R172K+}$. G1 (27.3%) patients revealed lower rate of relapse than G2 (39.1%) or G3 (62.5%) (P<0.05). G1 patients also showed significantly better LFS than G2 (P<0.05, HR 1.933, 95% CI 1.058-3.532) or G3 (P<0.01, HR 3.157, 95% CI 1.460-6.830)(Fig. 1).

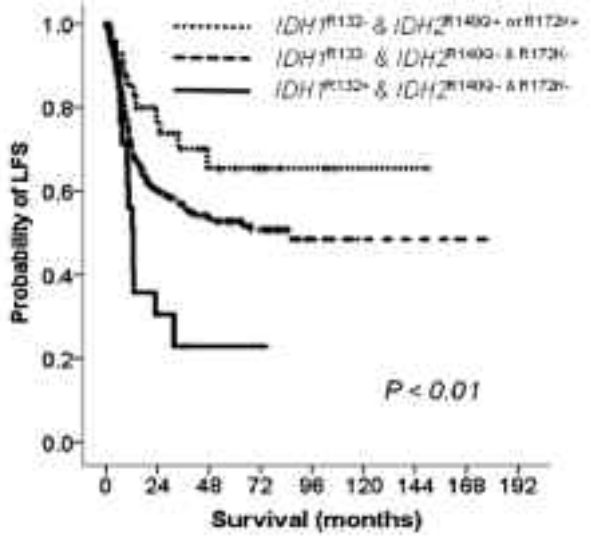

Figure 1.

Summary and Conclusion: Our study revealed that the incidence and characteristics of *IDH1* and *IDH2* mutations in AML-NK patients. We also found that *IDH1*^{R132} and *IDH2*$^{R140Q\ or\ R172K}$ had different prognostic effects on treatment outcome in these patients.

P816

SCREENING OF TP53 (TUMOR PROTEIN 53) MUTATIONS IN ADULT ACUTE MYELOID LEUKEMIA (AML) PATIENTS REVEALS A STRONG ASSOCIATION WITH COMPLEX KARYOTYPE AND POOR OUTCOME

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Background: AML is a heterogeneous disease with various chromosomal aberrations. The karyotype at diagnosis provides important prognostic information that influences therapy and outcome. The *TP53* gene is the most frequently mutated gene in human tumors. The reported *TP53* mutation rate in AML is low (2.1%). In contrast, the incidence of *TP53* mutations in AML with a complex aberrant karyotype (CK-AML) is higher (69-78%).
Aims: to investigate the frequency and the prognostic role of *TP53* mutations in adult AML patients (pts) focusing the screening on subgroups of pts with chromosome abnormalities.
Methods: 106 adult AML pts with FAB-M0, M1, M2, M3, M4, M5, miscellaneous cytogenetic abnormalities and normal karyotype (nK-AML, 14/106 pts) were examined. Forty-four pts (41.1%) showed 3 or more chromosome abnormalities (CK-AML), 42 (39.6%) presented one or two cytogenetic abnormalities (other-AML) and in 6 cases the karyotype was not available. Genomic DNA and/or cDNA were isolated from mononuclear AML blast cells. *TP53* mutation screening was performed on all 106 AML pts, in particular in 42 from exon (ex) 2 to 11, in 48 from ex 4 to 11 and in 16 pts from ex 2 to 8. Analysis was focused on coding sequences (RefSeq GRCh37/hg19 NG_017013.2).
Results: By PCR and subsequent Sanger sequencing, mutations of *TP53* were detected in 23 pts (21.1%). Seven pts revealed 2 mutations. 83% of all mutated pts had CK (19/23) by contrast the frequency of mutations was very low in "no CK-AML" pts (6.5%). Overall, mutated patients included 19/44 with CK-AML (43.2%); 1/6 (16.7%) with nK and 3/42 (7.1%) with other-AML. 26 *TP53* point mutations [19 missense, 1 silent, 5 intron and 1 3' untranslated region (UTR)] and 4 *TP53* deletions were found. Twenty-six out of 30 mutations/deletions (86.6%) were located in the DNA binding domain, 2 in the carboxyl-terminal tetramerization and regulatory domains and 2 in the transcriptional activation domain. All mutations in coding regions were classified by the IARC database (http://p53.iarc.fr/TP53GeneVariations.aspx) as deleterious.Of note, alterations of TP53 were significantly associated with poor outcomes in terms of overall survival and disease free-survival (P<0.0001).
Summary and Conclusion: Our data demonstrated that mutations of TP53 occur in 21.7% of AML with a higher frequency in the subgroup of CK-AML (p<0.0001–Fischer's exact test). Since *TP53* mutations have predicted to be deleterious and significantly correlated with prognosis, *TP53* mutation screening should be recommended in CK-AML pts. Supported by: EuropeanLeukemiaNet, AIL, AIRC, PRIN 2010-2011, Fondazione del Monte di Bologna e Ravenna, European Union Seventh Framework Programme [FP7/2007-2013],Progetto Regione-Università 2010-12 (L.Bolondi).

P817

ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WHO REQUIRE TWO COURSES OF INDUCTION THERAPY TO ACHIEVE COMPLETE REMISSION (CR) SHOULD UNDERGO ALLOGENEIC TRANSPLANT (ALLO-SCT) DURING THE FIRST CR

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Background: Although primary induction failure has been considered as one of the strongest negative prognostic factors and an indication for allo-SCT in adult patients with AML, the definition of this subgroup has not been clearly established. It was reported that AML patients who were unable to achieve CR after the second induction therapy should proceed to allo-SCT, whereas the allo-SCT indication for patients in CR after two courses of induction therapy has not been elucidated. Therefore, we conducted this large-scale retrospective study to address this unresolved issue.
Aims: The aim of this study was to clarify the clinical course and the appropriate therapeutic strategy of adult AML patients who obtained CR after two courses of induction therapy.
Methods: Among 310 consecutive AML patients who received anthracycline plus standard-dose Ara-C as the initial induction therapy between the years 1990 and 2010, 231 patients (median age, 50 years; age range, 15–65 years) who achieved CR within two courses of induction chemotherapy were included in this study. The Fisher's exact test was used for comparison of binary variables. The Mann-Whitney U-test was used for comparison of continuous variables. Overall survival (OS) rates were estimated by the Kaplan-Meier method and compared using the log-rank test. The Cox proportional hazards regression model was used for multivariate analysis. P<0.05 was considered to represent statistical significance.
Results: Of the 231 AML patients involved in this study, 186 achieved CR after the initial induction therapy (CR-1), and 45 achieved CR after the second induction therapy (CR-2). There were no significant differences in the baseline characteristics, including age, cytogenetic risk groups, white blood cell (WBC) counts at diagnosis, and the number of patients undergoing allo-SCT, when comparing the CR-1 and CR-2 groups. The 5-year OS rate of the CR-2 group was significantly inferior to that of the CR-1 group (33% *vs*. 52%, respectively; p=0.02). Of the 205 patients who did not undergo all-SCT during the first CR, patients in the CR-2 group had a tendency towards a higher relapse rate when compared with the CR-1 group (79% *vs*. 64%, respectively; p=0.09). Notably, a significant difference in the second CR rates was observed when comparing the CR-2 and CR-1 group (36% *vs*. 74%, respectively; p<0.01). Of the 45

patients (median age, 50 years; age range, 18–65 years) in the CR-2 group, 27 patients received a standard-dose Ara-C regimen as the second induction therapy, and 18 received a high-dose Ara-C regimen. In univariate analysis for OS in the CR-2 group, only allo-SCT was extracted as a significant prognostic factor (5-year OS rates with or without allo-SCT: 49% *vs*. 17%, respectively; p<0.01), while age, cytogenetic risk groups, WBC count, and the type of the second induction therapy did not reach the level of statistical significance. Furthermore, the prognostic significance of allo-SCT was also confirmed within multivariate analysis.
Summary and Conclusion: These findings suggest that the CR-2 patients have inferior outcomes when compared with CR-1 patients, possibly due to the higher relapse rate and lower second CR rate. Because CR-2 patients who underwent allo-SCT achieved excellent outcomes, donor research should be started for adult AML patients who have not achieved CR after initial induction therapy. If a suitable donor and good disease control are available, patients should undergo allo-SCT, preferably in the first CR. This strategy requires confirmation via appropriate prospective study.

P818

ALLOGENEIC TRANSPLANT FOR ACUTE MYELOID LEUKEMIA: CUT OFF LEVELS OF WT1 EXPRESSION AND PRE-EMPTIVE THERAPY OF RELAPSE

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Background: Leukemia relapse remains an unsolved problem for patients with acute myeloid leukemia (AML), undergoing an allogeneic stem cell transplant (HSCT). In a previous study (BJH 2013, 160:503) pre-emptive therapy with donor lymphocyte infusions (DLI) was triggered when the expression of WT1 on marrow cells, exceeded 180 copies x10^4 abl, with a marrow in remission. Survival was improved for patients receiving DLI, as compared to patients not receiving DLI (44% *vs* 14%, p=0.004). However, hematologic relapse was not significantly reduced for DLI patients (65% *vs* 74%). In the same study we also showed that 100 copies of WT1 was a better predictor of hematologic relapse.
Aims: Design of the study: We therefore asked whether pre-emptive therapy, triggered at a lower level of WT1 expression, (100 as opposed to 180 copies in the previous study) would be more effective in preventing leukemia relapse in AML post-HSCT.
Methods: Patients: 51 AML patients undergoing an allogeneic HSCT were studied: the median age was 43 years (17-74); the conditioning regimen was myeloablative in 92% of the patients. The donor was an identical sib (29%), a haploidentical family member (51%), an unrelated donor (13%) or an unrelated cord blood (7%). Patients were monitored for WT1 expression (x10^4 abl copies) on bone marrow cells, before and after transplant on days +30,+60, +90, +120, then every 2 months until 1 year and then at every outpatient visit. 2833). If WT1 expression exceeded 100 copies with a marrow in remission, the patient was eligible for pre-emptive therapy: this consisted of abrupt discontinuation of cyclosporine (CyA) and /or DLI.
Results: After HSCT WT1 expression was always<100 copies in 22 patients, and 4 relapsed (18%); it was between 101 and 179 copies in 19 patients, and 6 relapsed (31%); WT1 expression was over 180 copies in 10 patients, and 6 relapsed (60%) (p<0.001). The group we are interested is the one with WT1 expression between 101 and 179, since this was the group with the lower WT1 level triggering treatment.
Pre-emptive therapy: Of the 19 patients with WT1 101-179, 5 were non treated for several reasons: 4 relapsed. 14 patients received pre-emptive therapy (CyA discontinuation/DLI) and 2 relapsed (14%). We compared the relapse rate of these 14 patients treated at a WT1 cut off of 100, with the previous group treated at a WT1 cut off of 180 (BJH 2013): the relapse rate is significantly different (14% *vs* 64%, p=0.01) and survival is 86% *vs* 41% (p=0.01).
Summary and Conclusion: These data confirm our previous study, suggesting that WT1 levels post HSCT are predictive of hematologic relapse. In addition it seems that pre-emptive therapy triggered at a lower level of WT1 (100 copies) is more protective against leukemia relapse as compared to pre-emptive therapy triggered at a higher WT1 level (180 copies).

P819

THE PROGNOSTIC IMPACT OF CYTOGENETIC AND GENETIC ABNORMALITY FOR THE ACHIEVEMENT OF THE SECOND COMPLETE REMISSION IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Although, about 80% of patients with acute myeloid leukemia (AML) achieve first complete remission (CR1) with induction chemotherapy, after relapse, the probability of achieving a second complete remission (CR2) becomes lower. Concerning the allogeneic hematopoietic stem cell transplantation (Allo SCT) for patients with AML after first relapse, their prognoses are greatly different whether they are in CR2 or not. Several studies revealed that age, relapse-free interval, cytogenetic risks and previous allo SCT were independent prognostic factors for patients with AML after first relapse. Recently prognostically important mutations have also been identified, but the gene mutation affecting the CR2 rate is not still clear. In this study we analyzed the prognostic impact of gene mutations for the achievement of CR2 in adult patients with AML.
Aims: The purpose of this study is to clarify prognostic impact of gene mutations for the achievement of CR2 in adult patients with AML.
Methods: We retrospectively analyzed 64 patients of de novo AML who relapsed after treatment with conventional chemotherapy alone at Nippon Medical School Hospital and its affiliated facilities from 2000 to 2010. We excluded the patients received AlloSCT in CR1 and the patients with M3 in FAB classification. Bone marrow or peripheral blood samples containing 20% or more blast cells at diagnosis and first relapse were used for analyses. Mutation analyses were performed using PCR method for *FLT3-ITD*, *FLT3-TKD* and *MLL-PTD*, and direct sequence for *NPM1*, *C/EBPα*, *DNMT3A*, *IDH1/2*, *TET2* and *N/K-RAS*.
Results: The CR2 rate with the salvage chemotherapy for AML after first relapse was 30/64 (46.9%). The significant difference was not found in clinical and laboratory data such as age, white-cell count, percentage of blast cells, serum lactate dehydrogenase value at both initial diagnosis and first relapse between CR2 group and non-remission group. The patients with FAB classification M4E (5/5, 100%: p=0.02), inv(16) (4/4, 100%: p=0.04), and favorable karyotypes (14/19, 73.7%: p<0.01) attained significantly high CR2 rate. Although *FLT3-ITD* is known to be a poor prognosis, the CR2 rate was not significantly different whether the mutation was present or not at diagnosis. However the CR2 rate of the patients with *FLT3-ITD* at first relapse was significantly lower than the patients without it (1/11, 9%: p<0.01). Concerning the mutations of epigenetics modifying genes such as *DNMT3A*, *IDH1/2*, and *TET2*, the CR2 rate of the patients with *DNMT3A* mutation at diagnosis tend to be lower (2/8, 25%; p=0.07), Intriguingly, the CR2 rate of the patients with the mutations of epigenetics modifying genes at diagnosis as a whole was significantly lower than without them. (3/11, 27%; p=0.05).
Summary and Conclusion: Although our results showed *FLT3-ITD* at diagnosis could not predict the achievement of CR2, mutations of epigenetics modifying genes at diagnosis could predict it. We recently showed that the possibility that mutations in epigenetics modifying genes may cause genetic instability and induce *FLT3-ITD* mutations, leading to resistance to therapy and relapse (Leukemia. 2013;27(5):1044-52). Moreover, the 3 cases in which *FLT3-ITD* were observed only at relapse may have low level FLT3-ITD at diagnosis undetectable by standard method. Now we developed a highly sensitive mutation detection method known as Mutation Biased PCR (MB-PCR) and are now performing re-analyses of the relationship between *FLT3-ITD* at diagnosis and CR2.

P820

COMPREHENSIVE GENETIC CHARACTERIZATION OF AML CELL LINES AND CLINICAL SAMPLES USING A NOVEL AML TARGETED SEQUENCING STRATEGY

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Background: Next generation sequencing (NGS) has revealed much of the common genomic architecture of Acute Myeloid Leukemia (AML). However, the high cost of NGS and its interpretation limits specificity and coverage in most sequencing strategies. This prevents broad adoption of NGS for characterizing AML samples and cell lines. Instead, investigators use more limited assays that fail to fully characterize complex variants such as structural variants and internal tandem duplications in *FLT3*. Complete characterization of variants in AML cell lines and clinical samples is essential to understand disease development and progression. In cell lines, accurate mutation identification is critical for their use in modeling cancer pathogenesis, developing therapeutics, and standardizing diagnostic performance. Similarly, mutations and their consequences need to be precisely defined in clinical samples to correctly stratify disease types and select appropriate treatments or clinical trials. Furthermore, understanding mutations in the context of clonal architecture may prove crucial for personalized therapies.
Aims: To illustrate the increased capacity and resolution of a novel NGS AML targeting strategy for the comprehensive characterization of AML samples via a sequence analysis of the "AMLome" (a complement of 194 genes known or predicted to be involved in AML pathogenesis) of AML cell lines and clinical samples.
Methods: We targeted coding exons (from 171 genes in version 1; 194 genes in version 2) and potential genomic breakpoints within known somatic gene fusions (34 genes in version 1; 36 genes in version 2) comprising the MyAML™

gene panel. We sequenced target loci on the Illumina MiSeq platform to an average depth of coverage >350x for cell lines (using MyAML™ version 1) and >500x for clinical samples using (MyAML™ version 2). Using a custom bioinformatics pipeline, we performed thorough mutation detection analyses to identify single nucleotide variants (SNVs), indels, inversions and translocations. In addition, we calculated allelic frequencies to investigate potential aneuploidy and clonality.

Results: Analyses of targeted sequencing results from 12 AML cell lines identified the published genomic variants within MyAML™ targeted genes. These variants were more fully characterized for their precise genomic breakpoints and inserted sequence content. For example, in cell line MV4-11, both the t(4;11) translocation and 30bp *FLT3* internal tandem duplication were accurately detected and their sequences and breakpoints were fully characterized. Previously unreported genomic variants were detected that have the potential to significantly impact interpretations from experiments using these cell lines. These mutations include potential activating missense SNVs in oncogenes, damaging missense SNVs and frameshift indels in tumor suppressors and translocations creating possible fusion genes. Based on our results from AML cell lines, we extended our analyses to AML clinical samples. From these samples, we identified and characterized both previously reported and novel mutations.

Summary and Conclusion: By specifically targeting the AMLome using the MyAML™ gene panel, we can comprehensively characterize mutations within AML cells. Our results also suggest that this strategy can be utilized for the characterization of both AML cell lines and clinical samples with the potential to identify hitherto undetected therapeutic targets.

P821

PROGNOSTIC VALUE OF MINIMAL RESIDUAL DISEASE BEFORE ALLOGENEIC MARROW TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA BY COMBINEDWT1 EXPRESSION LEVELS AND FLOW CYTOMETRY ASSESMENT

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Background: Allogeneic bone marrow transplantation (BMT) offers the greatest chance of cure for most patients affected by acute myeloid leukemia (AML). Persistence of disease or high levels of pre BMT minimal residual disease (MRD) have been reported to predict disease relapse after BMT.WT1 expression levels and multicolor flow cytometry (MFC) are widely used as markers of MRD.We recently reported that combined evaluation of MRD by WT1 and MFC after induction therapy has a strong impact on relapse risk in AML patients.

Aims: The aim of the present study was to apply the same MRD assessment in pre BMT setting to evaluate its reliability in predicting relapse.

Methods: We retrospectively analyzed BMT outcome of 66 AML patients with both WT1-based and MFC-based MRD evaluation on bone marrow samples before transplant. Median age at transplant was 44 years. Forty-two patients were transplanted in first and 24 in second or subsequent complete remission. Induction regimens included fludarabine-containing regimens or standard "3+7" induction. Median follow-up was 24 months (range 1-117 months). Disease-free survival (DFS) was calculated from the time of transplantation until last follow-up or documented leukemic relapse. A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells /10^5 total events (threshold of $2.5x10^{-4}$ residual leukemic cells) at four-color flow-cytometry. Real-time PCR for WT1 was performed on DNA Engine 2 (Opticon®, MJ Research®). WT1 copy number/Abl copy number $1000x10^4$ was used as cut-off value for high WT1 expression.

Results: Twenty-five relapses (37.9%) were observed. Median DFS was 31 months. Our preliminary analysis shows that the probability of disease relapse was significantly influenced only by disease status (first or subsequent CR) and MRD status at transplantation. Specifically, MFC-MRD was the strongest predictor of longer disease free survival ($p<0.001$) since no relapses occurred in the eleven MFC-MRD negative patients. Among MFC-MRD positive patients a further stratification of risk is obtained by the evaluation of WT1 MRD status that was able toidentify patients with significantly worse DFS. ($p<0.01$, fig.1).The predictive value of MRD resulted independent from different induction schedules; furthermore undergoing BMT in second or subsequent remission did not affect the positive prognostic value of gaining a negative MRD status.

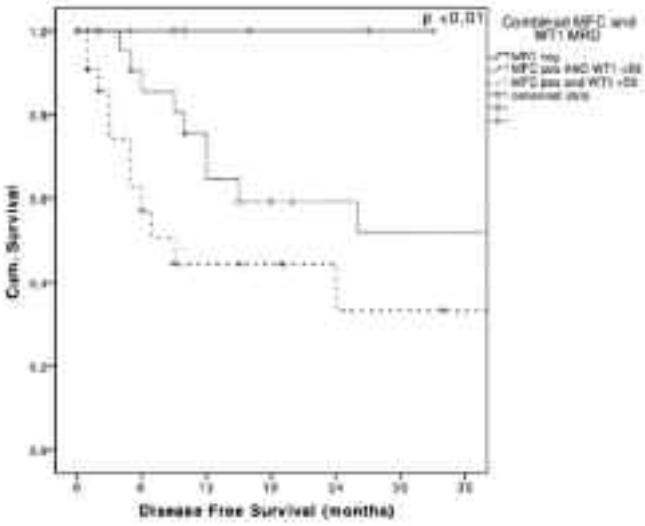

Figure 1.

Summary and Conclusion: Pre BMT evaluation of MRD by WT1 and MFCon bone marrow samples is a reliable predictor of relapse risk. Patients with negative pre-BMT MRD have a significantly longer DFS, while patients with both MRD markers display an higher risk of relapse.Identifyingpatients athigher risk may allow to modulate post BMT follow up, to detect earlier disease recurrence and perhaps to apply pre-emptive therapeutic strategies in order to delay or avoid AML relapse.

P822

VALIDATION OF SIE/SIES/GITMO "FITNESS CRITERIA" FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: APPLICABILITY AND OUTCOME ANALYSIS ACCORDING TO FITNESS AND TO TREATMENT RECEIVED

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Background: Acute myeloid leukemia (AML) is a disease of the elderly with a median age of 69-72 years (y) in population studies (SEER, 2012; Juliusson, 2009). Because of the adverse biologic features of leukemia and the high frequency of comorbidities that reduce patients (pts) tolerance it is difficult to find a balance between treatment efficacy and pts safety. In 2013, SIE/SIES/GITMO experts proposed objective criteria to define pts "fitness" to conventional intensive chemotherapy (i-CT) or non-intensive chemotherapy (ni-CT) as a tool for therapy decision making (Ferrara, Leukemia, 2013).

Aims: The applicability and the adherence to these "fitness criteria" in clinical practice as well as the outcome according to "fitness" and treatment received were evaluated in a consecutive series of AML pts (no M3).

Methods: From Jan 2008 to Sep 2012, 141 patients aged >65 y were diagnosed at our Institution. Median age was 74 y (range 65-88 y), 43% were >75 y. ECOG PS was evaluable in 130 pts (93%) and was >3 in 33 (23%). The classification according to "fitness criteria" was performed through review of electronic medical records. ELN prognostic criteria were also applied to 88 "de novo" AML: 15 (17%) favorable, 28 (32%) intermediate-I, 8 (9%) intermediate-II and 37 (42%) adverse risk. ELN criteria were not applicable in 16 pts (15%) with missing karyotype. Therapy-related or secondary AML (37 pts: 26%) were defined as adverse clinical risk. According to institutional policy, favorable and intermediate-I pts had received i-CT. Ni-CT with low-dose arac, azacytidine or experimental non-myelotoxic drugs, was given to the other pts. Pts with major comorbidities received best supportive care (BSC).

Results:

Table 1.

	i-CT	ni-CT	BSC
Total	30 (22%)	26 (19%)	82 (59%)
Fit (55 pts, 40%)	26 (47%)	12 (22%)	17 (315)
Unfit (67 pts, 49%)	4 (6%)	12 (18%)	51 (76%)
Frail (16 pts, 12%)	0	2 (12.5%)	14 (87.5%)

"Fitness criteria" could be retrospectively applied in 138/141 pts (98%): 55 pts (40%) were fit to i-CT (FIT), 67 pts (48.5%) unfit to i-CT (UNFIT), 16 (11.5%) unfit to ni-CT (FRAIL). Thirty pts (21.5%) had actually received i-CT, 26 (18.5%) ni-CT and 85 (60%) BSC. Treatment intensity distribution across different "fitness" groups is shown in table 1. Overall, median survival (OS) in FIT, UNFIT and

FRAIL pts was 11, 4 and 3.5 months (p 0.0001). Across all fitness groups, the higher the treatment intensity the longer was OS. In FIT pts treated with i-CT, ni-CT and BSC, median OS was 22, 9.5 and 4 months, (p<0.0001); in UNFIT pts 16, 9.5 and 2 months, respectively (p<0.0001). In FRAIL pts treated with ni-CT or BSC, OS was 9 and 2 months, respectively (p:NS) (Fig. 1). Considering adherence to "fitness" criteria, only 4 UNFIT (6%) and 2 FRAIL (12,5%) pts were actually overtreated, with i-CT or ni-CT. Their median OS (15 mo.) was better than expected. Four of them had ELN low-int-1 risk *vs* 5/22 treated adhering to fitness criteria (P<.05). Among FIT pts, 53% were actually undertreated (ni-CT 22% or BSC 31%); they belonged more often to ELN or clinical adverse groups (85% *vs* 10%; P<.001). Among undertreated pts OS was significantly better in pts receiving ni-CT than BSC, in spite of similar distribution among ELN risk groups. Among UNFIT pts, 76% were undertreated with BSC and fared worse than patients receiving ni-CT in spite of similar ELN risk.

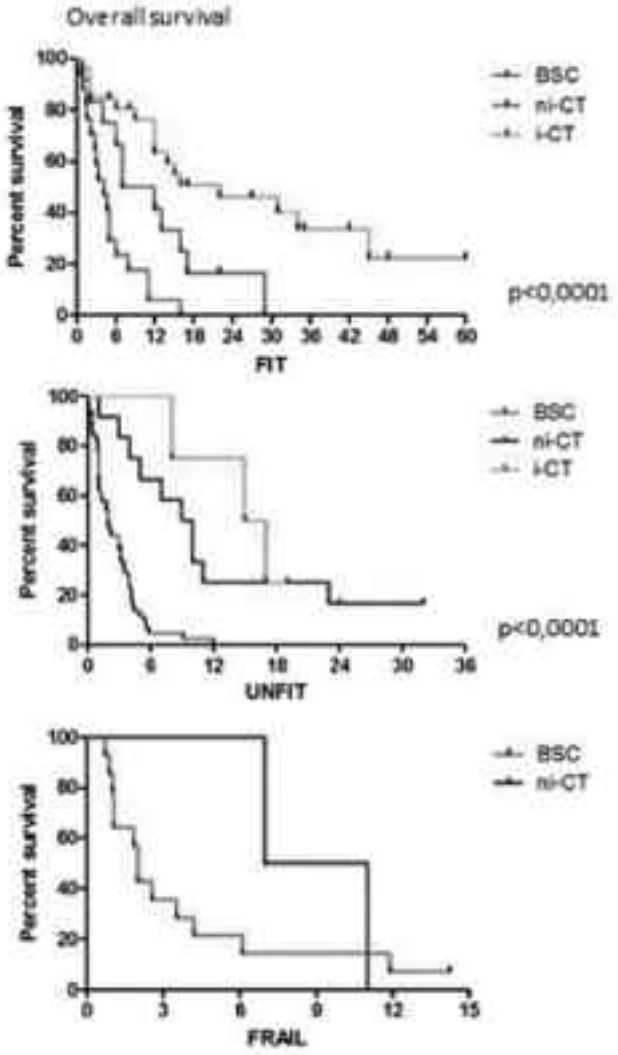

Figure 1.

Summary and Conclusion: The "fitness criteria" were easily applicable also retrospectively in the majority of AML pts. Intensity of care seems to favourably impact on outcome across all "fitness" categories. Particularly UNFIT ELN low-int risk patients should be offered i-CT, whereas undertreatment with ni-CT/BSC in FIT pts or BSC in UNFIT pts should be avoided. Further multicenter studies are needed to validate these data.

P823

C-KIT MUTATION IN CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA: PREVALENCE AND PROGNOSTIC ROLE IN TAIWAN. EXPERIENCE OF ONE SINGLE INSTITUTION

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Background: Core-binding-factor acute myeloid leukemia (CBF-AML) include those with t(8;21) and inv16, and is associated with a favorable prognosis. However, the up-to 40% of these cases harbor *c-KIT* mutations mainly involving exon 8 and 17, and are shown associated with a poor prognosis, and proper post-remission therapy in this group of patients is still not clear.

Aims: Current study is to examine the benefit of different post-remission therapy in patients with CBF-AML and c-KIT mutations.

Methods: For de novo AML patients diagnosed between 1999 and 2011 in Taipei Veterans General Hospital, those with cytogenetic abnormalities at diagnosis including (8;21) and inv16 and/or having fusion genes - AML1-ETO and CBFβ-MYH11 were identified, among whom those with archived DNA of bone marrow at diagnosis and relapse were enrolled. The data - clinical features, treatment modalities including post-remission therapy and outcome were collected. Mutation analysis of c-KIT gene was performed using exon-specific PCR and additional treatments by restriction enzymes (exon 17 D816 and N822). In addition, they were further validated by direct sequencing and cloning in selected cases. The impact of c-KIT mutations was analyzed comparing clinical features and outcome including overall (OS) and relapse free survival (RFS) of CBF-AML patients with wild *vs*. mutated c-KIT.

Results: There were 40 patients (male/female=27/13, median age=36 years) enrolled, including t(8;21)/AML1-ETO and inv(16)/CBF-MYH11in 31 (77.5%) and 9 cases (22.5%), respectively. At diagnosis, 12 patients (30%) had c-*KIT* mutation at diagnosis including 10 (32.3% of 31) and 2 (22.2% of 9) in patients with t(8;21)/AML1-ETO and inv(16)/CBFβ-MYH11, respectively. In the t(8;21)/AML1-ETO subgroup, the mutations involved exon 8 (2 cases, 6.4%), exon 17 (8 cases, 25.9%; included mutations at D816 [n=5] and N822 [n=3]) and, in the inv(16)/CBFβ-MYH11 one, they involved exon 8 mutation (2 cases) only. In terms of clinic-hematologic features at diagnosis, there was no significant difference comparing those with wild and mutated *c-KIT,* except for a male predominance in the c-KIT wild patients (*P*=0.027). 32 patients (80% of 40) received induction chemotherapy and 27 patients (84.4%) achieved complete remission. Post-remission therapy were usually given with 2-4 cycles of high-dose cytarabine, and in addition, hematopietic stem cell transplantation (HSCT) was further given in 14 patients, including autologous and allogeneic in 2 and 12 patients (sibling/unrelated donors=7/5), repectively. At the last follow, 20 patients were alive with median OS 23.0 months (range, 0.8 ~ 115 months). In terms of prognostic impact, the difference of OS and CR rate is not significantly different between patients with wild and mutated c-KIT at diagnosis. In patients who received induction chemotherapy and achieved CR, mutated *c-KIT* CBF-AML had a shorter RFS [median RFS: 74 months in wild type c-KIT, 6.6 months in exon 8 mutation, 5.4 months in exon 17 (D816) mutation and 2.5 months in exon 17 (N822) mutation, p=0.015]. For Patients with mutated *c-KIT* mutation, who received HSCT had a trend for longer RFS (9.2 months *vs*. 5.3 months, *P*=0.082), especially for those receiving allogeneic HSCT. In Cox regression analysis, Initial high white blood cell (WBC) count and mutated c-KIT were independent poor risk factors in terms of relapse free survival.

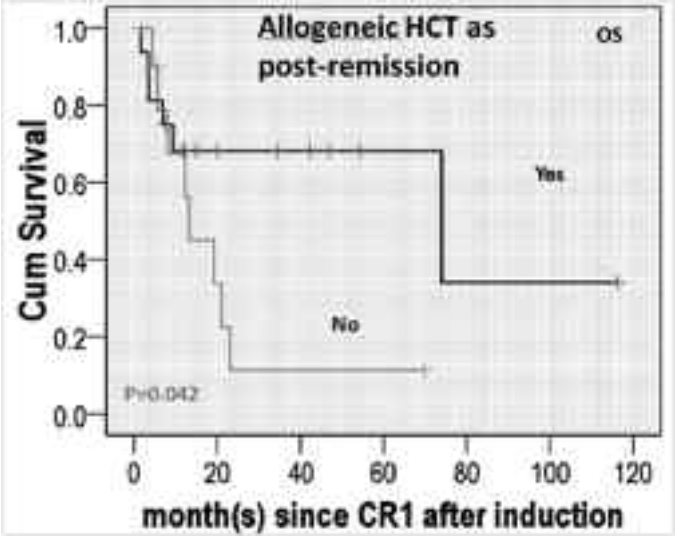

Figure 1.

Summary and Conclusion: CBF-AML with *c-KIT* mutations confers inferior RFS, which is possibly overcome by the use of HSCT as post-remission therapy, especially for allogeneic ones. The screen of *c-KIT* mutation status at diagnosis of CBF-AML is highlighted.

P824

ADVERSE PROGNOSTIC IMPACT OF IDH MUTATIONS ON OUTCOME IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE

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Background: Recent studies showed that the isocitrate dehydrogenase 1 (IDH1) and IDH2 mutations are associated with high risk of relapse and shorter survival in patients with acute myeloid leukemia with normal karyotype (AML-NK). They also confer adverse prognosis in the subset of AML-NK patients identified as NPM1-mutated/ FLT3 internal tandem duplication (ITD) negative.
Aims: To assess the incidence and clinicopathological features of *IDH1*/*IDH2* mutations, and to analyze the prognostic impact of IDH1/IDH2 mutations on overall survival (OS), complete remission rate (CR), early death (ED) rate and disease-free survival (DFS) in *de novo* AML-NK patients.
Methods: Diagnostic bone marrow samples from 110 adult *de novo* AML-NK patients were analyzed for *IDH1* and *IDH2* mutations by DNA polymerase chain reaction amplification/sequencing. All of the patients (median age 52 years, range 19-78; male/female 62/48; median follow-up 48 months) were managed at the Clinic of Hematology from 2009 to 2013 according to the Medical Research Council (MRC) 10 protocol. Statistical analysis included: the Fisher exact test, chi-square test and Kaplan-Meier method.
Results: *IDH* mutations were found in 26 (21.7%) patients: *IDH1*- 9 patients (7.5%); *IDH2* - 17 patients (14.2%). IDH1 mutation was associated with higher white blood cell (WBC) count (p=0.050), concomitant NPM1 mutation (p=0.050) higher ED rate (p<0.001) and lower CR rate (p=0.010). IDH2 mutation was associated with older age (p=0.050) and higher peripheral blood blast percentage (p=0.002). ED rate in our series was (20/108) 18.5%. Risk factors for ED by univariate analysis were higher WBC count (p=0.023), IDH1 mutation (p<0.001) and *IDH1*/*IDH2*+ status[1] (p=0.003). Multivariate analysis identified *IDH1*/*IDH2*+ status as independent prognostic factor for ED (p=0.001 HR 2.486 95%CI: 1.480-4.176). In addition, our *IDH1*/ *IDH2*+ patients had shorter OS (1 *vs* 9 months; p<0.001) and shorter DFS (11 *vs* 18 months; p=0.036) in comparison with patients without IDH mutations. Similarly, our *IDH1*/ *IDH2*+ patients had a lower CR rate compared to AML patients without IDH mutations (28% *vs* 63.9%; p=0.002). The subset of our patients identified as *NPM1*-mutated/*FLT3* ITD$^-$ with concurrent IDH1 mutation had lower CR rate compared to the counterpart without IDH1 mutation (12.5% *vs* 71.4%, p=0.024), higher ED rate (75% *vs* 16.7%, p=0.046), shorter OS (0 *vs* 6 months p=0.007) and shorter DFS (2 *vs* 7 p=0.036).
Summary and Conclusion: The study confirmed that *IDH1* and *IDH2* mutations confer poor prognosis in AML-NK patients regarding OS, DFS, CR and ED rate. In addition, IDH1 mutations confere poor prognosis even in the subset of the molecular low-risk group such as NPM1-mutated/FLT3-ITD negative patients.

P825

ROLE OF INDUCTION AND CONSOLIDATION CHEMOTHERAPY IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS: KOREAN SOCIETY OF HEMATOLOGY AML/MDS WORKING PARTY KAMS0119 STUDY

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Background: Diagnosis of acute myeloid leukemia (AML) at an elderly age generally predicts poor clinical outcomes. Elderly acute myeloid leukemia (AML) patients usually have a bad prognosis. Because the most treatment strategies are the result of came out from the data of studies in younger patients, those strategies cannot be directly applied to elderly patients. The exact benefit of induction and consolidation therapy in elderly AML patients has not yet been confirmed in elderly AML patients by randomized clinical trials.
Aims: This study aimed to find the role of induction and consolidation therapy in elderly patients.
Methods: We retrospectively collected data of 477 patients who were aged over 60 years at the time of AML diagnosis. Patients were divided into 2 groups.
Results: The induction group (n=266) included patients who received intensive induction treatment, and the best supportive care (BSC) group (n=211) received low-dose cytarabine or another conservative treatment. In the induction group, the complete remission (CR) rate was 58.3%, and treatment-related death was 15.4%. A factor associated with achievement of CR was good performance status (Eastern Cooperative Oncology Group [ECOG]<2) (hazard ratio [HR] 3.215; 95% confidence interval [CI], 1.508–6.853; *P*=0.002). The median overall survival was 339 days in the induction group and 86 days in the BSC group (*P*<0.001). In the induction group, failure to achieve CR (HR 4.059; 95% CI, 2.398–6.949; *P*<0.001) and poor performance status (ECOG >2) (HR 2.731; 95% CI, 1.073–6.949; *P*=0.035) were significantly associated with mortality. In the BSC group, bone marrow blasts (≥ 50%) (HR 1.965; 95% CI, 1.049–3.681; *P*=0.035) and poor risk karyotype were associated with mortality (*P*=0.004). Among patients who achieved CR, poor karyotype (HR 1.767; 95% CI, 1.018–3.067; *P*=0.043) and not receiving consolidation therapy (HR 2.313; 95% CI, 1.333–4.011; *P*=0.003) were related to mortality. At least more than 1 cycle of consolidation chemotherapy was associated with a better median OS (1 cycle: 471 days *vs*. >1 cycle: 847 days; *P*<0.001). In patients who failed to achieve CR, lack of salvage therapy was associated with mortality (HR 3.223; 95% CI, 1.426–7.285; *P*=0.005).

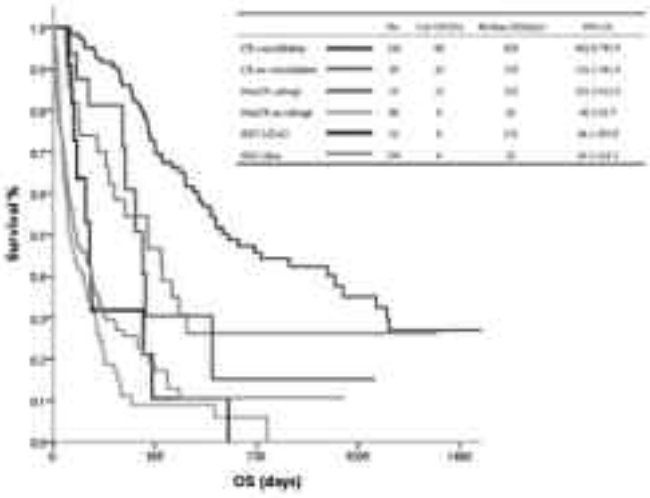

Figure 1.

Summary and Conclusion: In conclusion, appropriate induction chemotherapy for elderly AML patients with good performance status and more than 1 cycle of consolidation chemotherapy for patients who achieve CR are the best treatment strategies for improving OS in this patient population.

P826

COMBINED USE OF MULTI-COLOR FCM AND PCR ANALYSIS CAN DETECT LESS THAN 0.01% PATHOLOGICAL CELLS IN THE VAST MAJORITY OF DE NOVO AML CASES

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Background: A large proportion of acute myeloid leukemia (AML) patients achieve complete remission (CR); however, half of them ultimately relapse. Several retrospective studies show that monitoring minimal residual disease (MRD) can identify those patients at high risk of relapse. Both polymerase-chain reaction (PCR)- and flow cytometry (FCM)-based methods have been used to detect MRD. Although the recent introduction of multi-color (4–10 color) FCM has enabled more sensitive detection of AML than conventional 3-color FCM, it still cannot reproducibly detect MRD in the bone marrow of a significant proportion of patients, especially of those whose blasts lack CD34 antigen.
Aims: To develop strategies to detect MRD in the majority of AML patients.
Methods: PCR of disease-specific transcripts was used to detect MRD in acute promyelocytic leukemia (APL) and core binding factor leukemia (CBFL) patients. For other AML, two types of MRD assay were performed. One method detected leukemia-associated immunophenotypes (LAIPs). LAIPs were examined at the time of diagnosis using six or eight monoclonal antibody (MAb) panels comprising 26 or 32 different MAbs, respectively, and by 6-color FCM. For those patients with LAIPs, MRD was monitored using a patient-specific MAb panel. The second method involved PCR amplification of the nucleophosmin-1 (NPM-1) mutation. The presence or absence of the NPM-1 mutation was examined at the time of diagnosis and nucleotide sequencing was

performed if the mutation was detected. MRD was monitored in a real-time PCR assay using patient-specific probes. The limit of detection in above-mentioned MRD assays reached less than or equal to 0.01%.
Results: Between October 2010 and December 2013, 81 consecutive patients with de novo AML were tested for MRD. The median age of the patients was 63 years, and 19 (nine with APL and ten with CBFL) harbored disease-specific fusion transcripts. Of the remaining 62 patients, 53 (85.5%) had distinctive LAIPs. The most common LAIPs were characterized by abnormal antigen expression (lineage infidelity); for example, the expression of CD200, CD96, CD7, CD25 and CD56 on CD34-positive blasts. The NPM-1 mutation was detected in 19 out of 62 patients. Of 32 patients whose blasts expressed the CD34 antigen, 31 had LAIPs and one harbored the NPM-1 mutation. By contrast, of the 30 patients whose blasts lacked CD34, 22 had LAIPs and 18 harbored the NPM-1 mutation. When three MRD assays were combined, all patients but one harbored targets for MRD monitoring. A patient did not show disease-specific transcript, LAIPs nor the NPM-1 mutation. Morphological complete remission (CR) was achieved in 49 patients after induction chemotherapy. MRD assays using bone marrow cells performed at the time when CR had just obtained revealed residual AML cells in all patients. 19 patients harbored disease-specific transcripts and 13 patients showed mutated NPM-1 transcript. Multi-color FCM analysis of 26 patients who possessed LAIPs and entered in morphological CR revealed MRD in every patients. The limit of detection in multi-color FCM was confirmed to be 0.01%.
Summary and Conclusion: Combined use of PCR and FCM can enables us to detect MRD at a level of 0.01% in the vast majority of AML.

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INDOLEAMINE 2,3-DIOXYGENASE-1 (IDO-1) IS ASSOCIATED WITH HIGH INCIDENCE OF CHEMOREFRACTORY DISEASE IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Indoleamine 2,3-dioxygenase (IDO) is a heme-containing enzyme that catalyzes the first and rate-limiting step in tryptophan degradation along the kynurenine pathway. IDO is able to inhibit T-cell function and to induce the transformation of T-cells into regulatory T-cells. Several studies demonstrated that IDO expression is involved in immune tolerance induction during pregnancy, infection, transplantation, autoimmune diseases and neoplasias, including acute myeloid leukemia (AML). In particular, our and other groups demonstrated IDO expression in a significant proportion of AML patients and that it increases along with disease progression.
Aims: Here, we addressed the correlation between IDO expression by AML cells, risk factors at diagnosis and patients' outcome.
Methods: Adult AML patients from the Hematology Institute "L. and A. Seràgnoli" in Bologna were analyzed for risk characteristics at diagnosis and for IDO expression by RT-PCR and by Western-Blot analysis. Patients were stratified according to age at diagnosis, d*e novo* or secondary disease (pre-existing myelodysplastic syndrome or radio-chemotherapy), leucocytosis, cytogenetics (on the basis of cytogenetic characteristics patients were divided into low, intermediate and high risk groups) and FLT3 and NPM mutational status.
Results: Fifty-two patients with AML at diagnosis were analyzed for IDO expression both at gene and protein level. According to IDO transcript levels, the patients were divided into IDO-negative (21%) and IDO positive (79%). Positive patients were further subdivided into three different subgroups according to the IDO level: IDO-low expression (78%), IDO-intermediate expression (10%) and IDO-high expression (12%) patients. When IDO protein was assessed, we found a correlation between IDO mRNA level and the detection of IDO protein. In particular, IDO protein was detectable only in IDO-high-expressing patients. No statistically significant differences in the recurrence of prognostic characteristics at diagnosis between the groups considered were observed, even though IDO-negative and IDO-low expressing patients showed a higher median age at diagnosis than those expressing IDO at low and intermediate level and an increased frequency of high-risk cytogenetics was found in IDO-high expressing patients. Response to induction chemotherapy regimen was then analyzed among the four groups of patients. Only patients who received cytotoxic chemotherapy were evaluated for response. Intriguingly, we found that refractory patients were 60% among patients who express IDO at high level and 27% among IDO-negative patients.
Summary and Conclusion: In conclusion, IDO-high expressing patients show an increased proportion of refractory disease than IDO negative patients. To support our preliminary findings, a multivariate analysis on a larger cohort of patients is currently ongoing.

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MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA DETECTED BY WILMS TUMOR GENE 1 EXPRESSION AND MULTIPARAMETER FLOW CYTOMETRY

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Background: Evaluation of minimal residual disease (MRD) in acute myeloid leukemia (AML) is an important strategy to asses therapy response. Several techniques have been investigated for an accurate MRD detection. Recently, beside multiparameter flow-cytometry (MFC), Wilms Tumor gene 1 (WT1) expression was suggested to reflect MRD. However, despite its potential role, at the moment there is no consensus on its clinical application.
Aims: Aim of this study was to investigate the role WT1 expression and leukemia associated immunophenotype (LAIP) in MRD assessment after intensive induction chemotherapy.
Methods: A total of 137 clinically and molecularly well characterized consecutive AML patients were treated from 2008 to 2013 with intensive induction chemotherapy according to age, performance status and co-morbidities in a single hematology center. Therapy response was defined according to ELN criteria. WT1 expression by real time polymerase chain reaction (RT-PCR), using the standardized European LeukemiaNet method, and LAIP using a six (19 patients) or eight (118 patients) colour multi parameter flow-cytometry (MFC) were performed on bone marrow samples at diagnosis and after induction chemotherapy. LAIP was defined as positive when ≥ 1 leukemic cell per 10^4 of normal cells was detected. Both parameters were compared with clinical and therapy related variables of the patient cohort. Disease-free survival (DFS) was studied by Kaplan-Meier analysis and the statistical difference between the curves was quantified by the Wilcoxon test.
Results: Ninety-seven patients (71%) achieved a complete remission after induction chemotherapy, whereas 34 patients (25%) were non responders and 6 (4%) died. WT1 expression at diagnosis was determined in the bone marrow of all patients with a mean value of 12952 copies (range 2-83200 copies) and repeated after induction chemotherapy in 94 patients (69%) with a mean value of 1601 copies (range 0-29274 copies). Twenty-two patients with high WT1 expression (≥250 copies) after induction chemotherapy showed a significantly reduced DFS with a median of 36 months (range: 2-44) compared to 64 patients with low (<250) WT1 expression showing a median DFS of 57,9 months (range: 3-63), p=0.00057. Moreover, after induction chemotherapy a MFC was performed in 96 patients resulting in 77 assessable LAIP; 57 becoming negative and 20 remaining positive. Next, the level of LAIP was compared with DFS. Fifty-seven patients with positive LAIP after induction chemotherapy had a significantly shorter DSF than patients with a negative LAIP with a median of 18,5 *vs* 36,3 months, respectively (p=0,04).
Summary and Conclusion: In conclusion, the patient subgroup that failed to obtain a significant reduction of bone marrow WT1 expression after induction chemotherapy showed a significantly shorter DFS than the subgroup with a negative post-treatment WT1 level. Similar results were achieved measuring bone marrow LAIP expression after induction therapy. Patients with a still positive LAIP had a significantly shorter DFS than patients becoming negative, although the statistical significance was not that strong as in the analysis of WT1 expression.These results may encourage the consideration of WT1 expression beside LAIP in the bone marrow as an important MRD tool in therapy response evaluation and therefore risk stratification of AML patients.

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INTEREST OF CYTOGENETIC AND FISH EVALUATION FOR PROGNOSIS EVALUATION IN 198 PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION IN A SINGLE INSTITUTION

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Background: The prognostic interest of cytogenetic remission (CyCR) and Fluorescence *in situ* (FISH) evaluation in patients treated for Acute Myeloid Leukemia and who reached complete remission have been poorly studied.
Aims: To evaluate the prognostic value of CyCR and FISH evaluation in AML patients who reached cytological CR after one induction cycle.
Methods: 198 intensively treated AML patients with abnormal karyotype, who reached cytological CR and were evaluated with conventionnal G banding cytogenetic method after one course, have been identified in our institution between 1989 and 2012. 61 were also evaluated with FISH. T(9 ;22)(q34 ;q11.2) and promyelocytic leukemia cases were excluded. Most patients were treated according to EORTC AML10, AML12 or AML13 protocols. Probabilities of DFS and OS according to CyCR and FISH CR were estimated with the Kaplan-Meier method, and differences between survival distributions were evaluated by the log-rank test. A Cox proportional hazards model was constructed to determine if CyCR was associated with outcome, when adjusting for other prognostic variables. Median follow-up was 52 months for patients still alive.
Results: In the 198 patients who reached cytological CR, median age was 48 (range 17-81). Sixty-two (31%) patients harbored favorable karyotype (*i.e.* core-binding factor AML), 84 (42%) harbored unfavorable karyotype, and 52 (26%) intermediate karyotype. One hundred and seventy four patients (88%) reached CyCR, when 24 (12%) did not. In univariate analysis, reaching CyCR wasn't associated with Disease-Free Survival (25+/-9% *vs* 39+/-4% after 5 years ; p=0.17) and Overall Survival tended to be higher in patients who reached CyCR than in other patients (51+/-4% *vs*. 27+/-10% at 5 years; p=0.08). In multivariate analyses including all variables with p value<0.1, only cytogenetic group was an independent factor for OS, when reaching CyCR was not (p=0.14). As nearly all patients with favorable karyotype reached CyCR, a subgroup analysis was performed in the 136 patients with intermediate or unfavorable karyotype. In this subgroup, reaching CyCR wasn't associated with DFS or OS in univariate analyses. In multivariate analyses, reaching CyCR was not associated with DFS or OS. In order to evaluate if FISH could provide more prognosis information than conventional cytogenetic after induction, we focused in patients who reached cytological CR and CyCR, and who were evaluated with FISH (52 patients). 32 of the 52 patients (61%) reached FISH CR after induction, when 20 (39%) did not. In univariate analysis, reaching FISH CR tended to be associated with better DFS (45+/-9% *vs*. 21+/-9% at 5 years; p=0.08), and was strongly associated with better OS (67+/-9% *vs*. 25+/-10% at 5 years, p=0.004). When focusing in the 36 patients with intermediate or unfavorable karyotype, reaching FISH CR was associated with a trend to better OS (47+/-12% *vs*. 29+/-11% at 3 years, p=0.08) and no significant difference for DFS (41+/-12% *vs*. 23+/-10% at 3 years, p=0.23).
Summary and Conclusion: Cytogenetic remission didn't significantly impact prognosis in 198 AML patients who reached cytological Complete Remission after one induction cycle. Evaluation with FISH at the time of cytological CR seems to be able to provide prognosis information, and might be useful for MRD detection. Larger prospective studies are needed to confirm these data.

P830

ACUTE MYELOID LEUKEMIA IN PATIENTS OLDER THAN 75: PROGNOSTIC IMPACT OF FLT3-ITD AND NPM1 MUTATIONS

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Background: The benefit associated with chemotherapy in older patients with acute myeloid leukemia is debated. The prognostic impact of molecular mutations in these patients is unknown.
Aims: To evaluate the outcome of AML patients aged more than 75, to identify prognosis factors, and to evaluate if *NPM1, CEBPA* and *FLT3-ITD* status have prognostic value in intensively treated patients.
Methods: We conducted a retrospective analysis of 79 consecutive AML patients aged more than 75 years admitted in our unit between 1998 and 2012. APL were excluded. Patients received either intensive regimens or supportive care only. Cytogenetic analysis at the time of diagnosis was performed in most patients. *FLT3-ITD, NPM1* and *CEBPA* mutations detection were retrospectively performed from frozen cells from diagnosis.
Survival probabilities were estimated with the method of Kaplan and Meier, and compared using the log-rank test. A Cox model was constructed to determine if *FLT3-ITD* was associated with outcome, when adjusting for other prognostic variables. Median follow-up time was 87 weeks for patients still alive.
Results: The median age at diagnosis was 78 years (range 75-92). Median OS was 13.8 weeks. 81% of patients died in the first year following diagnosis (23% during the first hospitalization following diagnosis). Overall, 42 patients (53%) received chemotherapy. Treated patients were significantly younger than the overall sample (p=0.008), and had significantly lower ECOG PS (p=0.004). Patients with poor cytogenetic were less likely to be intensively treated (p=0.006). Median overall survival of treated patients was 36.3 weeks *vs*. 5.7 weeks for other patients. OS at 1 year was 33 % +/- 7 % for treated patients, *vs*. 7 % +/- 4 % for others (p<0.001). Among the 42 intensively treated patients, 19 achieved CR (45 %). Achievement of CR was significantly related to OS (p<0.001). Early death (during the first 30 days) occurred in 8 (19 %) of the 42 treated patients. In univariate analyses, age, ECOG PS, prior myelodysplasia, ABCB1 activity, WBC, or cytogenetic group did not influenced CR and OS in this group. *FLT3-ITD* was analyzed in 35 treated patients, *NPM1* status in 33 of them and *CEBPA* in 18. 11 patients (31%) harbored *FLT3-ITD* and 7 patients (21%) harbored *NPM1* mutation. No *CEBPA* mutations were identified. In univariate analyses, *FLT3-ITD* and *NPM1* status were not associated with OS or DFS. *FLT3-ITD* did not influence CR rate, when *NPM1* mutations tended to be associated with higher CR rate (p=0.12). A multivariate analysis for OS was performed. CR achievement (p=0.0003) and *FLT3-ITD (*p=0.04) were independently associated with OS. To evaluate more accurately the influence of *FLT3-ITD* and *NPM1* mutations on disease evolution and prolonged survival, we performed a second analysis excluding patients who experienced early death. *FLT3-ITD* was identified in 10/28 (35%) patients and was associated with worse survival (p=0.036). *NPM1* mutations tended to be associated with CR (p=0.09). Patients harboring *FLT3-ITD* without *NPM1* mutation (5 patients on 26) had significantly worse OS than other patients (p=0.02)
Summary and Conclusion: Age should not be a limitation for intensive treatments in elderly AML people, as reaching CR is associated with longer OS. A proper selection of patients, with adapted evaluation of comorbidities and disease characteristics should be performed before treatment to avoid early death and to maximize CR reaching chances. *FLT3-ITD* or *NPM1* status might be useful to predict survival in elderly patients. Larger studies are needed to conclude.

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MOLECULAR RELEVANCE OF THE NERVE GROWTH FACTOR RECEPTOR GENE (NTRK1) EXPRESSION IN AML-RELATED MYELOID SARCOMA

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Background: Myeloid sarcoma (MS), also called granulocytic sarcoma, is a rare condition characterized by the occurrence of tumor masses constituted by immature precursors from the granulocytic series in an extramedullary localization. MS may be diagnosed *de novo*, although most often coincides with different malignant blood diseases involving the granulocytic series, acute myeloid leukemia (AML) being the most frequent. The most common localization of MS is in the paravertebral region, subperiosteal space of the skull, peritoneum, and pelvis. Approximately 10% of t(8;21) AML patients will develop MS, as a consequence, the relative RUNX1-RUNX1T1 transcript represent the most common cytogenetic and molecular abnormalities. The t(8;21) and the other core-binding factor related leukemias were frequently

associated with KIT mutations. Moreover, the murine model showed that RUNX1-RUNX1T1;c-Kit^{D814V} coexpression resulted in AML of relatively short latency and frequent MS was observed.
Aims: To understand the molecular basis for the MS prevalence in RUNX1-RUNX1T1 expressing human AMLs, we examined the tyrosine receptor kinase A (TRKA) nerve growth factor (NGF) receptor gene (NTRK1) expression as a target gene up-regulated by the expression of RUNX1-RUNX1T1, allowing nerve growth factor-induced CD34$^+$ cells expansion. NGF is normally expressed by bone marrow stromal cells, whereas TRKA is expressed in hematopoietic progenitor cells.
Methods: To evaluate the clinical and molecular relevance of these findings in MS development, we analysed NTRK1 expression in 32 primary AML samples by qualitative and quantitative RT-qPCR.
Results: We observed strong NTRK1 expression only in the 23 samples derived by AML patients associated with MS at a prevalent paravertebral region localization.

Table 1.

Summary and Conclusion: In summary, our data identify a functionally relevant target gene overexpressed in AML clinically positive for paraspinal or ileal MS localization and not expressed in AML without MS. These observations suggest a possible MS-promoting activity of the NGF/TRKA signaling in AML cells competent to respond to NGF ligand.

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ELN FAVORABLE ACUTE MYELOID LEUKEMIA SHARE THE SAME GOOD PROGNOSIS ACROSS CYTOGENETIC/MOLECULAR SUBGROUPS: PRELIMINARY RETROSPECTIVE REAL-LIFE ANALYSIS OF THE RETE EMATOLOGICA LOMBARDA (REL)

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Background: The European Leukemia Net (ELN) stratification classifies as favorable prognosis four subgroups of acute myeloid leukemia (AML) with specific cytogenetic/molecular abnormalities. These forms are potentially curable with standard chemotherapy, even though a substantial proportion of patients still relapse. Several clinical trials report a relapse rate (RR) of about 30% and an overall survival (OS) of about 60% at 5 years.
Aims: The purpose of this study was to assess in a real life setting the response rate and OS of newly diagnosed AML patients with t(8;21)(q22;q22)/*RUNX1-RUNX1T1*, inv(16)(p13q22) or t(16;16)(p13q22)/*CBFbeta-MIH11*, normal karyotype and mutated *NPM1* and negative *FLT3*-ITD or double mutated *CEBPA* (*CEBPA*dm). Data were collected from hematological centers of the regional network REL (Rete Ematologica Lombarda).
Methods: We analyzed adult patients diagnosed with AML with favorable prognosis at 6 REL centers between 2007 and 2013. All patients received standard induction chemotherapy followed by consolidation cycles and/or autologous transplant. Clinical and molecular data were analyzed at diagnosis, after induction, and at the end of treatment. The Kaplan-Meier product-limit method was used to estimate survival curves, and the log-rank test was adopted to evaluate differences between groups of patients.
Results: We studied 148 AML patients (81 males and 67 females) with a median age of 55 years (range 20-80): 24 with t(8;21) (group 1), 28 with inv(16) or t(16;16) (group 2), 85 with normal karyotype and *NPM1*mut *FLT3*-ITDneg (group 3), and 11 with normal karyotype and *CEBPA*dm (group 4). After induction chemotherapy, 8% of patients received no consolidation cycles, while the remaining received a median of 3 cycles (range: 1-5), which were based on high dose cytarabine in 74% of cases. Complete remission (CR), evaluated in 142 patients, was achieved in 130 (92%): 21(91%), 25(93%), 74(91%), 10(91%) respectively in group 1, 2, 3, 4. Post-induction and post-consolidation molecular assessment were available in 115 (78%) and 131 patients (89%) respectively. Molecular CR was obtained in 63% of responders. Half of them achieved molecular CR already after induction, with a significant difference between group 3 and combined group 1 and 2 (57.5% *vs* 25%, p=0.019). However, this difference was no longer significant after consolidation (68% *vs* 62%, p=0.53). With a median follow up of 1.6 years, the RR was 40% without any difference between groups, whereas three years disease free survival (DFS), event free survival (EFS) and OS were respectively: 48%, 40% and 69% with no significant difference between groups. Age (<60 years) was the only parameter that affected EFS (p=0.038) and OS (p<0.001).
Summary and Conclusion: This study, conducted in a non selected population of favorable risk AML patients, confirms high CR rate and the prognostic value of age, favoring patients younger than 60 years. RR is higher than reported in literature, but the OS is comparable. Earlier molecular response was obtained in *NPM1* mutated patients, but the incidence of relapse and survival did not differ from the other groups. In conclusion, although data collection is not yet complete and follow up is short, these results confirm that the ELN classification identifies a clinically homogeneous group of good risk AML patients that share comparable outcome, with a significant proportion of long-term survivors with chemotherapy alone even outside clinical trials.

P833

A POST-REMISSION MAINTENANCE TREATMENT WITH LOW-DOSE CHEMOTHERAPY + RETINOIDS AND DIHYDROXYLATED VITAMIN D3 MAY IMPROVE THE SURVIVAL OF POOR-PROGNOSIS AML/MDS PATIENTS

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Background: Acute myeloid leukemia (AML) patients over 60 or with secondary / therapy-related disease still have, despite aggressive treatments, a poor outcome, only some 5-15% of these patients becoming long-term survivors. The same is true for high risk myelodysplastic syndrome (MDS) patients ineligible to allogeneic stem cell transplantation (Juliusson G. *et al.*: Blood 2009; Bello C. *et al.*: Cancer 2011; Kayser S. *et al.*: Blood 2011).
Aims: On the basis of our previous encouraging experience with a combination of low dose chemotherapy + retinoids and dihydroxylated vitamin D3 on MDS and AML patients uneligible to standard chemotherapy (Ferrero D. *et al.*: Leuk Res 1996; Haematologica 2004), we wished to evaluate the same approach as a maintenance treatment on AML/MDS who had obtained a complete remission (CR) after intensive chemotherapy but had poor prognosis features (age over 60 and/or secondary AML, therapy-related AML, previous relapse, high risk MDS).
Methods: From January 1997 to December 2012, 261 high risk (according to the above described criteria) AML or MDS patients were treated in the two hematology wards of the same hospital, with CR- aimed induction chemotherapy +/- consolidation. Overall, 169 (64.8%) of these patients obtained a CR that was maintained for at least 2 months (mo) in 161 (61.7%). Fifty-three patients underwent an allograft and 14 received other different maintenance treatments, without differentiating agents. Forty-five patients (35 AML and 10 MDS, median age 64, 87% with documented intermediate/ high-risk caryotype) received, in one of the two hematology wards, the maintenance therapy based on 2 alternated schedules: a) 6-thioguanine + 13-cis retinoic acid + dihydroxylated vitamin D3; b) low-dose cytarabine + 6-mercaptopurine + all-trans retinoic acid + dihydroxylated vitamin D3. The treatment was continued until relapse or 3.5 – 4 years of continuous CR. Three patients in first CR who later found a donor and underwent allografting were censored at that time. We retrospectively compared, at a median follow up of 57 months, the outcome of patients who received the maintenance therapy, to that of a matched population of 49 patients treated in the same years in the other hematology ward who stopped treatments after consolidation. Minimal residual disease was evaluated before and during maintenance in 8 patients by WT-1 or CBFβ-MYH11 transcript.
Results: Maintenance therapy was generally well tolerated. Main toxicities included mild oral and cutaneous dryness, occasional nausea /vomiting and mild to moderate cytopenia (11 patients). Only two patients discontinued

treatment because of these effects and no therapy related deaths were observed. Maintenance group had a lower relapse incidence (70.3% *vs*. 86.4% at 5 years p=0.007) and a longer disease-free survival (median 21.2 *vs*. 8.7 months, p=0.017). The relapse reduction improved overall survival: median 40.4 months (34 % at 5 years) for maintenance group *vs*. 15.8 (14.2 % at 5 years) for controls (p=0.005). At multivariate Cox analysis both cytogenetic and maintenance therapy resulted independent outcome predictors for overall survival. Maintenance treatment also reduced minimal residual disease (detected by WT1 and CBFβ-MYH11) in 5 of 8 evaluable patients.
Summary and Conclusion: Although maintenance has never been regarded as a standard strategy in AML treatment, some randomized studies reported a significant benefit with ARA-C employed as a long-term maintenance (Löwenberg B. *et al.*: J Clin Oncol 1998; Büchner T. *et al.*: J Clin Oncol 2003). The present results, although obtained in a non-randomized study, suggest that our strategy of low-dose chemotherapy + retinoids and dihydroxylated vitamin D3 as maintenance therapy might improve the outcome of poor risk AML/MDS patients.

P834

MDRO INFECTION THE ‘’MAJOR HURDLE TO ACUTE MYELOID LEUKEMIA INDUCTION THERAPY’’ EXPERIENCE OF A TERTIARY CARE COMPREHENSIVE CANCER CENTER FROM INDIA

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Background: Infection and febrile neutropenia remains a major challenge in patient undergoing intensive chemotherapy for AML in developing countries.
Aims: To review the cultures for multidrug resistance organism (MDROs) in our AML induction patients and their morbidity and mortality.
Methods: We analyzed our 44 consecutive AML patient who underwent 7+3 Induction chemotherapy. Fecal Surveillance cultures were taken in 29 patients prior to chemotherapy and were carried out by a method based on Landman D et al(J Clin Microbiology 2005). During febrile periods multiple blood, urine, secretions cultures were taken.
Results: From Sept 2012 to Jan 2014, information on AML patients who underwent standard 7+3 Induction was reviewed. 44 patients[27 males and 17females] of median age 37 years[range 16yr-63yr] were treated. The median WBC count at diagnosis was 13500 cells/micL[range 800-1,34,000 cells/micL] and the peripheral blood blast was 47%[range 10%>96%].The cytogenetic was done in 42 patients and 27/42[64%] had intermediate risk. Surveillance culture done in 29 [55%] patients and MDRO were detected in 23[79%] patients.The pattern of resitance shown in table1. During febrile episodes organism isolated in 31[70%] patients. Postive blood cultures 27[87%],positive sputum or ET aspirate culture 6[19%], and positive Pus cultures in 5[16%]patients.The pattern is shown in table2.The median duration of hospital stay was 26 days[range 10day-51days].20[47%] patients required intensive care support, 21[47%] patients needed ionotropic support for septic shock and 14[31%] invasive ventilation. Post induction Day+10 or +14 bone marrow showed<5% blast in 41[93%] patients and 31[70%] patient had complete remission at day+28. 9[20.5%] patient died during induction chemotherapy. In 7/9 patient the cause of death was attributed to MDRO septicemia and related organ failure.

Table 1. Resistance pattern for survillace culture isolates.

Isolates	Meropenem	Colistin	Oxacillin	Linezolid
Ecoli (20)	10	1	NA	NA
Klb.Pneumonae(8)	7	NIL	NA	NA
Enterococcus(5)	NA	NA	5	NIL
Crysobact(1)	1	1	NA	NA

Table 2.

Isolates(No)	Meropenem	Colistin	Tigecycline	Amikacin
Kleb.Pneumonae(17)	15	4	1	3
Pseudomonas(3)	1	nil	nil	1
Acenobacter(2)	2	nil	nil	nil
Strephomonas(2)	nil	nil	nil	nil
Chrysobacterium(1)	1	1	nil	nil
Proteus(1)	nil	1	nil	nil
Isolates(No)	Oxacillin	Linezolid	Vancomycin	
Staphylococcus(7)	5	nil	1	
Eentrococcus(2)	2	1	1	

Summary and Conclusion: The high level of antimicrobial resistance in enteric flora in newly diagnosed AML patient is a concern at diagnosis and the major cause for mortality and morbidity in AML patient in developing world is MDROs.

P835

ACQUIRED IDH1 AND IDH2 MUTATIONS IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKAEMIA: THEIR PREVALENCE AND ASSOCIATION WITH OTHER GENE MUTATIONS – A SINGLE CENTRE EXPERIENCE

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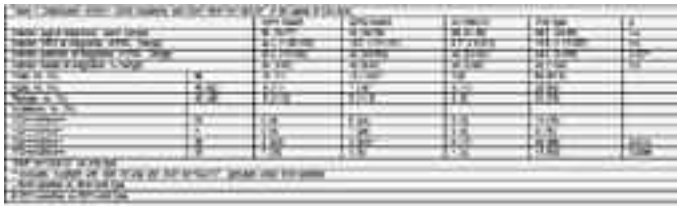
Background: In recent years, the prognosis of patients with acute myeloid leukaemia (AML) and cytogenetically normal (CN)-AML has been refined by the identification of genetic alterations. Recently, mutations in Isocitrate dehydrogenase (IDH) genes, first reported in malignant gliomas, have been evidenced in AML by whole genome sequencing. Recurring mutations in IDH genes occur in approximately 15%>20% of patients with newly diagnosed AML, and are associated with the normal karyotype.
Aims: In this study, we assessed the prevalence of IDH1 and IDH2 mutations including the IDH1 SNP rs11554137 in bone marrow and peripheral blood specimens collected from CN-AML patients during 2009-2012. We also compared the patient characteristics and other genetic alterations in relation to IDH mutations.
Methods: Exons 4 of IDH1 and IDH2 genes were directly sequenced in 89 CN-AML patients and 20 healthy donors. FLT3/ITD and NPM1 mutation status were also determined in the AML patients.
Results: A total of 23 IDH mutations were identified among the 89 CN-AML patients. The prevalence of IDH1 and IDH2 mutations were 11% (10/89) and 14.5% (13/89) respectively. IDH1 SNP rs11554137, a polymorphism which had been reported to be an adverse prognostic factor, was detected in 7 patients, one of whom also had an IDH2 R140Q mutation. This polymorphism without the presence of IDH1 or IDH2 mutation was also detected in 2 healthy donors. The 10 IDH1 mutations detected were R132H (n=4), R132C (n=3), R132L (n=2) and R132G (n=1). R140Q was the predominant IDH2 mutation detected in this cohort of patients (12/13), the other mutation was R140W (n=1). The relation between the IDH mutations and various patient characteristics were determined by the Student *t* test, equal variances not assumed (continuous variables) and the Fisher exact test (categorical variables) (details in Table 1). The platelet counts were significantly lower in the SNP-positive patients than in the patients with no IDH mutation or SNP (*p=0.027*). Other clinical parameters showed no significant difference between the IDH- or SNP-positive groups and the wild type group. In this study, all 10 IDH1 mutations were found in patients who were $FLT3/ITD^{neg}$ while all but 3 were NPM1 mutant-positive. IDH1 mutations were significantly associated with the low risk group $ITD^{neg}/NPM1^{mut}$, as compared to the IDH1-wild type group (*p=0.005*). The association of the IDH2 mutations with ITD/NPM1 mutation status was less defined. In contrast, SNP rs11554137 appeared to be associated with $ITD^{neg}/NPM1^{wt}$ status (6/7) and one such patient also had a R140Q mutation. This association was determined to be statistically significant (*p=0.014,* SNP-positive *vs* SNP-wild type).

Table 1.



Summary and Conclusion: Conflicting findings regarding the impact of IDH mutations on clinical outcomes had been reported. One study found IDH mutations to be a poor prognostic factor in CN-AML with $NPM1^{mut}/ITD^{neg}$ while another suggested worse outcome in patients with $NPM1^{wt}$ without FLT3/ITD. There was yet another study which suggested IDH1 single nucleotide polymorphism rs11554137 to be associated with an inferior outcome in adult CN-AML, most pronounced in NPM1/ITD high risk group while the IDH1 (R132) mutations were not. As the prognostic implications remain controversial, it would be important for our centre to continue this study with a larger patient cohort and perform a longer follow up to further correlate IDH mutation status, particularly IDH1 R132 and SNP rs11554137 as they appeared to be highly associated with the low risk and intermediate groups respectively, with other genetic alterations and prognosis.

P836

CLINICAL USEFULNESS OF WT1 MRNA EXPRESSION IN BONE MARROW DETECTED USING A NEW WT1 MRNA ASSAY KIT FOR MONITORING ACUTE MYELOID LEUKEMIA: A COMPARISON WITH PERIPHERAL BLOOD WT1 MRNA EXPRESSIONS

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Background: The Wilms' tumor gene 1 (WT1) is highly expressed in acute myeloid leukemia (AML). Many studies have reported the usefulness of WT1 mRNA expression as a monitoring marker of minimal residual disease (MRD). We previously assessed the clinical usefulness of WT1 mRNA expression in the peripheral blood (PB) of 191 patients using the WT1 mRNA assay kit and reported that WT1 mRNA expression in PB is useful as an MRD monitoring marker in AML patients. However, the usefulness of WT1 mRNA expression in the bone marrow (BM) has been investigated only in a small number of subjects. Since the WT1 mRNA Assay Kit has been newly developed, we decided to investigate the clinical usefulness of WT1 mRNA expression in BM by comparing the expression with that in PB in a larger number of subjects.
Aims: This study aimed to assess the performance of the newly developed WT1 mRNA assay kit and to evaluate the clinical usefulness of WT1 mRNA expression in BM.
Methods: The new kit is based on one-step multiplex quantitative RT-PCR. Thus, the component reagents are limited to 2 types in order to simplify the measurement operation, and the RT and PCR reaction are performed continuously in one step to amplify GAPDH (Internal standard) and WT1 in the same tube. Table 1 compares the new and former kits used in this study. Informed consent was obtained from 164 blood disease patients including 118 AML patients. Using the new and former kits, WT1 mRNA expression in BM and PB was determined to assess the performance of the new kit in comparison with the former kit. Additionally, the clinical usefulness of WT1 mRNA expression in BM was assessed on the basis of comparison with the expression in PB. In addition, human genomic DNA was applied as the sample instead of RNA, and WT1 mRNA and GAPDH mRNA were determined by both kits to evaluate cross-reactivity with human genomic DNA.
Results: The results showed a favorable correlation between the new and former kits, with r=0.9849 and y=0.9260x + 0.1119 in 163 PB samples and r=0.9568 and y=0.9035x + 0.2396 in 158 BM samples, demonstrating that the performance of the new kit is equivalent to that of the former kit. As for the usefulness of the new kit in investigating the clinical usefulness of WT1 mRNA expression in BM on the basis of comparison with the expression in PB, correlation between WT1 mRNA expression in BM and PB was investigated in 115 AML patients from whom both BM and PB were collected. The results showed a favorable correlation, with r=0.9048 and y=0.7378x + 1.4041. As observed in PB, WT1 expression in BM was significantly lower (P<0.01) in the remission stage compared to the levels in 3 disease stages (pretreatment, post-treatment relapse, and refractory stages) (Fig. 1). In AML patients from whom it was possible to collect multiple samples during the course of the study, changes in WT1 mRNA expression in BM reflected the disease status and demonstrated fluctuations mostly similar to those in the case of PB. No cross-reactivity with human genomic DNA was detected in both kits.

Table 1. Compearison of the new kit with the formerly used kit.

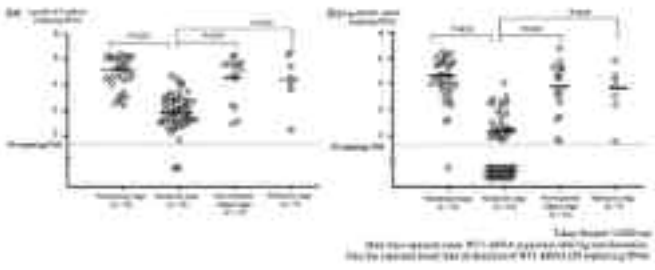

Figure 1. Compearison between WT1mRNA expression levels in BM and PB in each AML disease stage (a) in BM at each disease stage, (b) in PB at each disease stage.

Summary and Conclusion: The new kit demonstrated a performance equivalent to that of the former kit. WT1 mRNA expression in BM was highly correlated to that in PB and demonstrated changes that reflected the AML status, as observed with WT1 mRNA expression in PB, indicating that WT1 mRNA expression in BM is useful as an MRD monitoring marker.

P837

MENINGIOMA 1 (MN1) EXPRESSION: REFINED RISK STRATIFICATION IN ACUTE MYELOID LEUKEMIA WITH NORMAL CYTOGENETICS

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Background: Prognostic stratification of cytogenetically normal acute myeloid leukemia (CN-AML) is a point of intensive research.
Aims: was to determine the prognostic importance of the meningioma 1 (MN1) gene expression levels in CN-AML.
Methods: One Hundred patients with AML were diagnosed, MN1 expression were analyzed using quantitative real time (QRT) PCR.
Results: High expression was detected in 48 (48%) patients (range: 2.35-31.99, mean: 13.9 ± 8.49) in comparison to 52 (52%) patients with low expression (range: 0.02-2.3, mean: 0.68 ± 0.77). The course of the disease in patients with high MN1 expression was unfavorable. Patients with high MN1 expression was associated with significant low complete remission (CR) rate (62.5% *vs*. 88.4%, high *vs*. low MN1, P=0.001) and high mortality rate (75% *vs* 46.1, P=0.03).AML patients with high MN1 expression tended to be refractory (37.5% *vs* 19.2, P=0.00) and relapse risk (54.1% *vs* 23%, P=0.02). Multivariable analysis confirmed high MN1 expression as an independent risk factor for disease free survival (DFS) and overall survival (OS).
Summary and Conclusion: MN1 is one of the most potent hematopoietic oncogenes. MN1 promote self-renewal and proliferation; block differentiation and is associated with repression of genes associated with differentiation and cell-cycle arrest. **Heuser et al** found that MN1overexpression is associated with treatment failure, specifically a significantly worse day 15 response rate; higher relapse rate, and shorter relapse-free and overall survivals. MN1 over expression independently predicts bad clinical outcome in CN-AML patients. MN1 over expression is associated with poor induction response, shorter relapse-free survival, and shorter overall survival. This leads to improve risk stratification of this heterogeneous group of patients with AML.

P838

THE TREATMENT'S OUTCOMES OF THE SECONDARY ACUTE MYELOID LEUKEMIA– RETROSPECTIVE MULTICENTER PALG STUDY

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Background: The secondary, to the previous treatment of the other cancers, acute myeloid leukemia (sAML) is still a clinical challenge. The prognosis in this group of patients is particularly bad.
Aims: The goal of this retrospective, multicenter PALG study was assessment of the outcomes of the secondary AML treatment in Poland.
Methods: Eighty six patients, 58% female with median of age 59 (18-84) diagnosed on 2004-2013 in 6 PALG centers were included to the study. Median time from the diagnosis of primary tumors to the development of sAML was 4 years. Median WBC at the diagnosis of sAML was 14,55 G/l. Cytogenetic risk

was low in 10,5%, intermediate 30%, high in 15%, unknown in 44.5% of patients. According to FAB classification they were M0 in 6%, M1 in 24%, M2 in 30%, M3 in 2%, M4 in 30%, M5 in 6%, M6 in 2%. In 70% of cases the diagnosis of sAML was preceded by the solid cancers (the most frequent was breast cancer in 32%), in 30% hematologic neoplasms. In 78% chemotherapy, in 49% radiotherapy, in 37% both before diagnosis of sAML were given. In 63% younger patients the intensive induction chemotherapy (group 1: DAC – daunorubicin/araC/cladribine 3+7+5 or DA - daunorubicin/araC 3+7), and in the other 37% palliative chemotherapy (group 2: low dose cytarabine – based) were given. In 8 patients intensively treated after complete remission (CR) assessment allogeneic hematopoietic stem cell transplantation (alloHSCT) was carried out.
Results: After induction CR was obtained in 53% *vs*. 11% patients in group 1 *vs*. 2 respectively, p<0.001. Eleven percent of patients in both groups expired during post induction pancytopenia. Median progression free survival (PFS) was reached after 12 *vs*. 3 months, and 33% *vs*. 29% patients were PFS after 4 years, respectively, p=0.23. Median OS was reached after 6 *vs*. 3 months, and 4% *vs*. 3% patients were alive in group 1 *vs*. 2 respectively after 4 years, p=0.11.
Summary and Conclusion: Secondary AML incidence increases because of the more effective treatment of the other cancers. The diagnosis of the secondary AML is burdened with a poor prognosis. In our study effective induction chemotherapy and using alloHSCT does not improve survival in the secondary AML patients in comparison to low dose chemotherapy supplemented by supportive care despite of the higher percentage of CR. This observation requires further studies.

P839

TIME FROM DIAGNOSIS TO TREATMENT (TDT) INITIATION AFFECTS PROGNOSIS OF ACUTE MYELOID LEUKEMIA (AML) IN YOUNGER ADULTS

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Background: Starting intensive chemotherapy immediately after a diagnosis of AML is believed to be crucial to improve outcome. This recommendation is included in the recent *ELN* guidelines, on the basis of a single study (Sekeres 2009) in which a TDT ≥ 5 days had an unfavorable impact on complete remission (CR) rate and overall survival (OS) in patients (pts) ≤ 60 years. This strategy was recently put in doubt by a study reaching opposite conclusions (Bertoli 2013) and arguing that a longer TDT is necessary to stabilize the pts and to allow treatment to be tailored to genetic results.
Aims: To observe the effect of TDT on CR rate and OS of AML pts ≤ 60 years.
Methods: We analyzed 304 consecutive pts aged ≤ 60 years, treated with an uniform regimen from May 1998 to May 2013. We evaluated the influence of the following variables on CR rate and OS: TDT (<*vs* ≥ 5 days), age (≤ *vs* >40 years), secondary *vs de novo* AML, genetic risk group and white blood cell count (WBC) at diagnosis (≤ *vs* >$50x10^9$/L).
Results: Median age was 47 (14-60), 250 pts had *de novo* AML; among the 165 cases with a complete genetic characterization, 33% were favorable, 56% intermediate and 10% unfavorable; 31% had a WBC >$50x10^9$/L. The median TDT was 4 days (1-49), and 57% of the pts had a TDT<5 days. The CR rate was 76%, higher for *de novo* AML (80% *vs* 56%, *p*<0.001) and for the favorable genetic group (98% *vs* 74%, *p*<0.001). The remaining variables did not influence the CR rate. With a median follow-up of 62 months, OS was 32% at 5 years (median 16 months; CI 12.0-19.9). In multivariate analysis, a negative impact on OS was found for a TDT ≥ 5 days (HR 1.34; *p*=0.04), for age >40 years (HR 1.45; *p*=0.02), for secondary AML (HR 1.82; *p*<0.001) and for unfavorable genetics (HR 3.71; *p*<0.001).
Summary and Conclusion: A TDT ≥ 5 days has an independent negative impact on OS for younger pts. In this population, delaying chemotherapy should be an exception.

P840

THE IMPACT OF MOLECULAR MINIMAL RESIDUAL DISEASE MONITORING IN PEDIATRIC PATIENTS WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA ENROLLED IN THE AIEOP AML 2002/01 PROTOCOL

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Background: Acute Myeloid Leukemia (AML) accounts for 15% of pediatric leukemia. Improvements of prognosis achieved in the last twenty years are also due to a better stratification of patients into risk groups that allowed to deliver tailored treatment. The AIEOP LAM 2002/01 protocol classified patients with t(8;21)(q22,q22)*AML1-ETO* and inv(16)(p13;q22)*CBFB-MYH11,* who responded to induction therapy as belonging to the Standard-Risk (SR) group. These patients were considered at good prognosis; unfortunately, although they all reached morphological complete remission (CR) after the first induction course, they showed a high incidence of relapse (24%) (Pession A. *et al*., Blood 2013). At present, little is known about the kinetics of relapse or the development of resistance mechanisms; moreover, in many studies post-remission therapy in AML does not take into account the level of residual leukemia.
Aims: Here, we evaluate the prognostic impact of molecular minimal residual disease (MRD) levels in terms of onset of relapse. We studied bone marrow of 49 and 27 patients carrying either t(8;21) or inv(16) abnormalities, respectively, at time of diagnosis, and at the end of first and second course of induction therapy (ICE).
Methods: MRD was evaluated as number of fusion transcripts by quantitative RT-PCR using absolute quantification, and calculated as logarithmic (Log) disease reduction after the first and second ICE course as compared to diagnosis. The prognostic impact was assessed by the calculation of the cumulative incidence of relapse (CIR) according to the different MRD levels.
Results: Results revealed that after I ICE, 21 out of 49 t(8;21)-rearranged patients (43%) showed a low MRD reduction (<2 Logs), and ten of them remained low responders also at the end of second course (20.5%). We found that a reduction of MRD lower than 2 Log conferred a higher CIR after I and II ICE (50% at 10 years). The *CBFB-MYH11* patients achieved higher MRD level reduction since the end of I ICE course (always >2 Logs). Inv(16) rearranged patients were all alive at last follow-up, and CIR never showed statistically significant differences for MRD levels in inv(16)-rearranged patients after the induction therapy. The impact of copy numbers (*i.e.* transcript levels) at diagnosis and after induction courses on relapse risk never showed significant results in both subgroups of *CBF*-rearranged patients.
Summary and Conclusion: In conclusion this is the first study for MRD evaluation performed by absolute quantification of fusion transcripts in pediatric AML. Results revealed that MRD evaluation using quantitative PCR is an important diagnostic tool that permits the assessment of response to CR and the identification of patients at greater risk of relapse after induction therapy for *AML1-ETO* rearranged patients. We propose that at the end of induction therapy the *cut-off* of MRD<2 Log to be used to guide therapeutic decisions for this subgroup of SR patients. On the contrary, MRD levels of *CBFB-MYH11* rearranged patients do not have prognostic value on CIR after induction therapy, this suggesting that novel molecular features should be investigated for this subgroup of AML.

P841

NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS CAN BENEFIT FROM A SIGNIFICANT BETTER SURVIVAL WHEN INCLUDED IN CLINICAL TRIALS

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Background: Advances in chemotherapy have improved the prognosis of patients with acute myeloid leukemia (AML), however, with the fast emergence of new molecules and treatment strategies, these advances can be open only to a proportion of patients who are included in clinical trials waiting for the availability/validation of the new therapy.
Aims: The aim of this study is to evaluate the impact of being included in a clinical trial on survival outcome of newly diagnosed AML patients.
Methods: Between July 2007 and September 2013, 572 consecutive newly diagnosed AML patients were included in this study; there were 311 (54%) males and 261 females with a median age of 63 years (range: 20-92), 249 (44%) were less than 60 years old, 216 (38%) were between 60 and 75 years, and 107 (18%) were older than 75 years; 406 (71%) were *de novo* AML and 166 (29%) secondary AML. The patients were divided into risk groups according to both cytogenetic and molecular biology data as proposed by the European LeukemiaNet (Dohner *et al.* Blood 2010). Accordingly, 335 (59%) patients were unfavorable, 83 (15%) favorable, 48 (8%) intermediate I and 106 (18%) were in intermediate II category. 186 (32%) were included in a clinical trial (CT patients) and the rest of patients were treated outside clinical trials [No CT patients, N=386 (68%)]. In CT patients, the treatment procedure consisted on induction chemotherapy followed by consolidation, among them, 58 (31%) received allogeneic hematopoietic stem cell transplantation (allo-HSCT). No CT

patients, received in majority of cases (N=222, 58%) induction chemotherapy + consolidation treatment and in the rest of cases they received other chemo/palliative treatment; in this group, 87 (23%) received allo-HSCT.
Results: We have compared the overall survival (OS) of CT and No CT patients after stratification on age and cytogenetics/molecular biology risk groups. After a median follow-up of 22 months (range: 1-77) for CT patients and 10 months (range: 0.03-77) for No CT patients, the 2-years OS probability of overall survival (OS) for CT unfavorable risk patients all age included was 44.4% *vs*. 25% for the same population but No CT (p=0.002) and it was 95% *vs*. 80% for CT and No CT patients with favorable risk all age included respectively (p=0.002) (Figure). Accordingly, this was still valid when we studied the group of patients aged less than 60 years with 2 years OS for CT unfavorable risk patients of 62.3% *vs*. 45% for the same population but No CT (p=0.007). OS outcome was better in the other groups of age within the CT arm and stratified according to risk group but was not statistically different. In addition, allo-HSCT was well distributed between CT and No CT patients, thus it did not influence on the outcome.

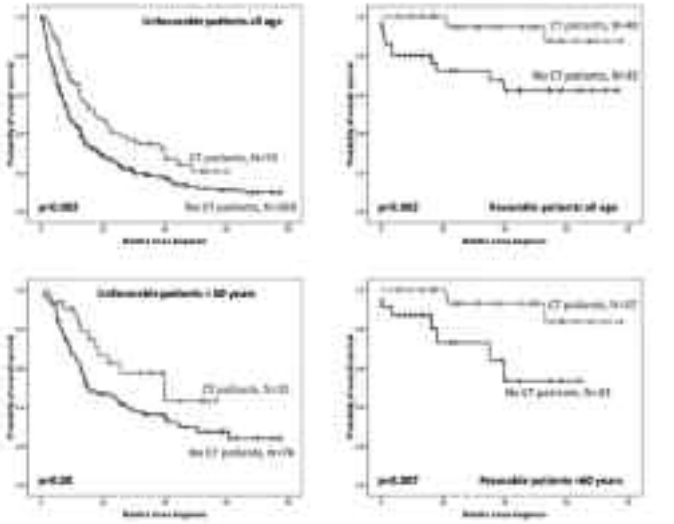

Figure 1.

Summary and Conclusion: We showed that newly diagnosed AML patients can benefit from a better overall survival when included in clinical trials; this can essentially be due to the emergence of new anti-leukemic molecules and to the strict follow-up and adherence to the protocol not only by the patient but also the by the medical team.

P842

CHARACTERIZATION OF FLT3 AND NPM1 MUTATIONS IN AML SAMPLES USING A HIGH THROUGHPUT AMPLICON SEQUENCING ASSAY

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Background: Acute Myeloid Leukemia (AML) is the most common acute leukemia in adults. In Europe the yearly incidence is approximately 3 cases per 100,000 individuals, while in the US approximately 18,860 new cases are expected to be diagnosed in 2014. Mutations in fms-related tyrosine kinase 3 (*FLT3)* and nucleophosmin (*NPM1)* are observed in over one third of all AML patients. These mutations include *FLT3* internal tandem duplications (ITD) within the juxtamembrane domain (15-30% of patients) and substitutions within the tyrosine kinase domain (TKD; 5-10% of patients), as well as 4bp insertions (15-30% of patients) within the C-terminal domain of *NPM1*. These mutations have significant impacts on prognosis; patients with *FLT3*-ITDs have poor prognosis while patients with *NPM1* mutations without an associated *FLT3*-ITD mutation have better long-term outcomes. Characterization of these mutations is critical for accurate therapeutic decisions. In addition, as new therapies arise, such as tyrosine kinase inhibitors targeting constitutively active *FLT3*, accurate mutation detection and characterization is critical to identify presence of resistance mutations and to stratify patients for clinical trials.
Aims: To develop a deep sequencing assay targeting the exons of *FLT3* and *NPM1* corresponding to regions with mutations known to be critical for stratifying patients with AML and potentially containing novel resistance mutations to FLT3 targeted therapies.
Methods: We designed an assay that combines amplicons targeting exons 14, 15, 16, 17 and 20 in *FLT3* and exon 12 in *NPM1* that identifies the major AML related mutations in these two genes. Following multiplex PCR amplification, amplicons were normalized and pooled. These pools were then sequenced on the Illumina MiSeq platform. Coupled with a multiplex PCR step, 24 samples were tested per run. Each MiSeq run of the 24-sample pool generated a minimum depth of coverage of 50,000x for each targeted region. Custom bioinformatics was used to identify *FLT3*-TKD1 & TKD2 region mutations, *FLT3*-IDT mutations, and insertion mutations within *NPM1*.
Results: Accurate detection of *FLT3* and *NPM1* mutations was obtained from positive and negative cell lines and known clinical samples as confirmed by parallel testing using PCR capillary electrophoresis and Sanger sequencing. These analyses correctly characterize each of the known mutations, including an *FLT3*-ITD insertion as large as 192 bases. Allelic ratios were also determined in each case. Unlike capillary electrophoresis methods, this assay provides the exact inserted sequences for *FLT*-ITD and *NPM1* mutations. Furthermore, serial dilutions of the positive controls with negative control DNA showed sensitivity down to 1%. From these analyses, we determined that our novel amplicon based sequencing assay was able to accurately identify *FLT3* and *NPM1* mutations in these samples.
Summary and Conclusion: A novel high throughput NGS assay that utilizes multiplex PCR amplification of exons in *FLT3* and *NPM1* was developed to identify AML mutations. This assay detects *FLT3*-TKD1 and TKD2 region single and multiple nucleotide substitutions, as well as *FLT3*-ITD and *NPM1* insertions. The additional information provided by this assay allows mutations to be further characterized by exact location and sequence. Lastly, due to the extensive depth of coverage provided by this assay, our results indicate that the mutation detection sensitivity can accurately distinguish mutations at a frequency as low as 1%. In conclusion, this assay provides a robust and accurate method for characterizing *FLT3* and *NPM1* mutations in AML cells.

P843

COMPARATIVE ANALYSIS OF FOUR COURSES OF STANDARD-DOSE CONSOLIDATION WITHOUT MAINTENANCE *VERSUS* THREE COURSES OF LOW-INTENSITY CONSOLIDATION WITH MAINTENANCE IN ADULTS WITH ACUTE MIELOID LEUKEMIA

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Background: The key problem in the treatment of adult patients with acute myeloid leukemia (AML) is to prevent disease recurrences. Although intensive consolidation therapy has proven useful, the practicability of maintenance therapy remains controversial.
Aims: To examine the efficacy of standard-dose consolidation *versus* low-intensity consolidation with maintenance therapy for patients with AML.
Methods: A total of 198 patients with median age 43.9 years (range, 15-64) with de novo AML which did not receive allogeneic hematopoietic stem cell transplantation (HSCT) were enrolled in this report. Of these, 97 patients during 2000-2009 were assigned to receive 2 cycles of induction "3+7" (daunorubicin 45 mg/m^2 on days 1-3; cytarabine 100 mg/m^2 every 12 hours [q12h] on days 1-7) and consolidation of 3 cycles "1+5" (daunorubicin plus cytarabine) following by maintenance chemotherapy also cycles for 2 years (local trial AML-2000). Other 101 patients during 2007-2012 were treated 2 cycles of induction "3+7" or "3+7" plus HAM (cytarabine 3 g/m^2 per q12h on days 1-3; mitoxantrone 10 mg/m^2 on days 3-5) if the complete response (CR) was not documented after the first cycle. Then there were 4 cycles of consolidation HiDAC (3 g/m^2 per q12h on days 1-3) without following maintenance (local trial AML-2007).
Results: In total, 57.1% of patients achieved CR. The 5-year overall survival (OS) rate was 20.4 ± 3.3%, and the relapse-free survival (RFS) rate for the 113 patients who achieved CR was 36.3 ± 5.0%. No statistical difference was observed either in the 5-year OS rate (21.2 ± 4.3% *vs*. 22.2 ± 5.2%; P=0.184) or in the 5-year DFS rate (33.7 ± 6.5% *vs*. 44.3 ± 6.8%; P=0.781) between the two trials. However, the incidence of late recurrence was higher for trial AML-2000 (26.0% *vs*. 8.6%; P=0.047). The median length of follow-up of surviving patients was 3.1 and 9.4 years respectively. The probability of achieving CR (42.6% *vs*. 74.0%: HR 0.26; 95% CI 0.11-0.61; P=0.002) and 5-year OS rate (24.0 ± 9.8% *vs*. 37.7 ± 8.4%; P=0.054) were lower for patients ≥ 46 *versus*<46 years. Furthermore 5-year OS rate was worse for patients achieve CR after two cycles of therapy *versus* one cycle of therapy (17.3 ± 9.0% *vs*. 44.1 ± 9.4%; P=0.007). Standard-dose consolidation compared with low-intensity concolidation was accompanied by a higher frequency of adverse events III/IV degree, including neutropenia (100% *vs*. 68.9%; P<0.001), thrombocytopenia (100% *vs*. 55.2%; P=0.012) and enteropathy (29.4% *vs*. 0%; P=0.001).
Summary and Conclusion: Both the concept of post-remission therapy for adults with AML were demonstrated an equivalent efficacy. Maintenance therapy is justified at least for patients who did not receive intensive consolidation and allogeneic HSCT.

P844

MN1 OVEREXPRESSION IS A POWERFUL PREDICTOR OF FAILURE OF INDUCTION CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIAS SUCH AS CONVENTIONAL CYTOGENETICS AND AGE

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Background: Acute myeloblastic leukemia (AML) is a heterogeneous group of conditions characterized by the proliferation of hematopoietic progenitors committed to the myeloid lineage. For the last 30 years, induction therapy, is the combination of cytarabine and an anthracycline, being well established poor prognosis conferred by this treatment failure in overall survival (OS) of patients, therefore given the morbidity and mortality associated with this therapeutic approach has identified several variables such as age, cytogenetics (CG) able to predict this failure to make a proper allocation of appropriate therapy for each patient.
Aims: Identify clinical or biological variables that are associated with higher failure rate to induction therapy and compared with established factors such as age and CG.
Methods: We analyzed 20 variables in a cohort of 120 patients with AML who received induction chemotherapy with the intention of complete remission (CR). We assume treatment failure not achieve CR after the first cycle. To define CR modified Cheson guideline were used and we assign CG risk according to the MRC (1999) and 2010 European Leukemia Net (ELN) criteria. The variables analyzed were: *FLT3* mutation, *NPM1* mutation, *DNMT3A* mutation, *CEBPA* mutation, total leukocyte count, hemoglobin, platelets, blasts in peripheral blood, bone marrow blasts percentage, CD34 expression on blasts, LDH, creatinine, overexpression of genes *EVI, BAALC, WT1, MN1*, cytogenetic risk according to MRC and ELN, age and sex.
Results: In our cohort of 120 patients, 62% were male in 38% of women. The mean age at diagnosis was 54 years, with 59.3 for those with persistent disease and 47.5 years for reaching CR (t-student p=0.000064). In bivariate analysis the relative risk (RR) was significant for known variables such as age, considered as dichotomous in>55 years (RR=2.62 (1.59 to 4.29) χ^2 p=0.000024) and>65 years (RR=2.69 (1.81 to 3.98) χ^2 p=0.0000019) and CG (adverse risk against good risk group, RR=7.00 (1.93 to 9.58) p=χ^2 0.009), and Intermediate risk group as compared to the good ELN criteria, RR=4.46 (1.92 to 6.80) χ^2 p=0.002). Of the other variables analyzed reached statistical significance only mutated *NPM1* as a protective factor (RR=0.47 (0.23 to 0.97) χ^2 value p=0.017)) and overexpression of *MN1* (RR=4.71 (1.84 to 12.03) χ^2 value p=0.000017)). In multivariate analysis, all three variables confirmed and maintained their statistical significance (Odds Ratios (OR): CG: 18,479, 95% CI (1.148 to 297.516), Age: 1,058 95% CI (1.017 to 1.101), *MN1*: 9,411, 95% CI (2.645 to 33.482).
Summary and Conclusion: In our series consisted of 120 patients, confirmed the prognostic value of age (>55 years CR rate: 35%, RR=2.62 (1.59 to 4.29),>65 years CR rate: 21% RR=2.69 (1.81 to 3.98)) and risk CG (CG adverse CR rate: 30%, RR=7.00 (1.93 to 9.58)) have for failure to induction therapy and also confirm previous findings of other cooperative groups as CALGB, wherein overexpression of MN1 (CR rate: 40%, RR=4.71 (1.84 to 12.03) were associated with an increased risk of failure with conventional chemotherapeutic schemes.

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P845

A PHENOTYPIC STUDY OF MONOCLONAL B-CELL LYMPHOCYTOSIS, WITH EMPHASIS ON BIPHENOTYPIC CASES

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Background: Monoclonal B-cell lymphocytosis (MBL) has attracted a significant research interest as the prelude of chronic lymphoproliferative disorders. Recently, technical advances and the wide application of flow cytometry have allowed for the identification of the disorder in an increasing number of cases. Immunophemotypically, the MBL cases are usually classified according to the expression of CD5 and CD23. However, data from a complete immunophenotypic analysis and the comparison of phenotypic profiles with those of clinical lymphoproliferations are available for a limited number of cases only.
Aims: In this study, we present the immunophenotypic profiles of 70 MBL cases, analyzed with multi-parameter flow cytometry. In addition, we have applied interphase FISH (i-FISH) for the investigation of chromosomal aberration frequently observed in chronic lymphoproliferations.
Methods: The study included 49 men and 31 women with MBL, according to the currently established diagnostic criteria. Multi-parameter flow cytometry was performed on a peripheral blood sample, with the use of a wide panel of antibodies for surface and cytoplasmic markers, currently applied in the characterization of B-CLL and related low-grade B-cell lymphomas. i-FISH was also performed in all samples, for the detection of del(13q14), +12, -11/11q-, 17/17p-, -6/6q-, t(11;14)(q13;q32) and rearrangement of BCL2 and IGH genes, regardless of the morphological features or the phenotype. In cases with a B-cell count<15% of total WBC, the i-FISH study was performed on highly purified B-cells, after immunomagnetic separation targeting CD19.
Results: Our cases were classified into 4 main categories: i) CD5+CD23+ (35 cases), ii) CD5+CD23- (12 cases), iii) CD5- (18 cases) and iv) MBL with two distinct monoclonal B cell populations (5 cases). 23/35 cases in the first category had a typical B-CLL phenotype, while 7 and 5 cases presented a phenotypic profile closely related to the features of LPL and MZL respectively. In 21/35 (60%) cases, a single cytogenetic abnormality was identified by i-FISH: 16 cases showed del(13q14) (hemizygous in 12 and homozygous in 4) and 5 cases had +12. All 12 cases of the second category were found with a MCL phenotype and in half of them (6) t(11;14)(q13;q32) was found as the sole aberration. CD5- MBLs was the most heterogenous group with immunophenotypic feautures ranging from LPL, to MZL and atypical B-CLL. FISH testing showed del(13q14) in 5/18 CD5- MBL cases. Lastly, the analysis of the biphenotypic cases revealed an interesting immunophenotypic pattern with the coexistence of a $CD5^-CD20^+$ MZL-like population and a $CD5^+CD20^-$ B-CLL-like population in 4 cases, expressing the same light chain isotype in three of them and a different one in the fourth. The fifth case in this group comprised an IgG+ HCL-like and an IgMD+ MZL-like population, with the same light chain restriction. No cytogenetic aberration was identified among the bipenotypic cases and, therefore, a separate clonal origin of the two distinct could not be verified.
Summary and Conclusion: Our results indicate that MBL is a highly heterogenous disorder, and that its phenotypic features may correspond to various chronic lymphoproliferations. The presence of t(11;14) among cases with a consistent phenotype is perhaps equivalent to the diagnosis of the recently described “indolent” MCL. Long term follow up of MBL cases, with a phenotype deviating from that of typical B-CLL, could reveal a preclinical stage in other chronic lymphomas, as well. Moreover, it could prove informative for possible phenotypic changes during disease progression. The presence of biphenotypic MBL cases leads to the assumption that different subsets of normal B cells in the same individual may be targets for oncogenetic events involved in lymphomagenesis. An extensive genetic study and follow-up of these individuals will hopefully allow for the distinction between biphenotypic and biclonal cases and help in our understanding of the pathogenetic mechanisms in B-cell lymphoproliferative diseases.

P846

TARGETED ARRAYCGH USED FOR DETERMINING MINIMALLY DELETED REGION ON CHROMOSOME 6Q IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: Cytogenetic abnormalities such as del(13q), del(11q), trisomy 12, del(17p), 14q32 translocations or a complex karyotype and the mutational status of *TP53, SF3B1, NOTCH1*, and *BIRC3* have improved present-day risk stratification in chronic lymphocytic leukemia (CLL). Recurrent cytogenetic abnormalities also include chromosome 6q deletion. It occurs at a relatively low frequency (3-6%) and its prognostic significance remains controversial; the same holds true for the extent of deletion and the candidate genes in the deleted region.
Aims: The objectives were to analyze a group of CLL patients with chromosome 6q deletion using arrayCGH, to determine the minimally deleted region (MDR) and the candidate genes located within the region, and to try to determine their relative gene expression, and evaluate some clinical characteristics.
Methods: The peripheral blood/bone marrow samples from a group of 1055 CLL patients in three hematology centers in the Czech Republic (Olomouc, Plzen and Brno) were analysed using conventional cytogenetics and FISH, and 6q deletion was found in 70 (6.6%) of the patients. ArrayCGH with chromosome 6 specific and oligonucleotide microarrays was performed in 52 patients (36 M/16 F; median age 61 years, Binet stage A (n=19), stage B (n=18), stage C (n=15), 25 pts at diagnosis, 27 pts in the course of the disease; 29 untreated and 23 treated; unmutated *IGVH* 41 pts). *FOXO3, NF-kB*, *TBX21*, *IL-2* and *BCL10* gene expression was assessed by quantititative RT-PCR in peripheral blood mononuclear cells obtained from CLL patients with/without 6q deletion (n=17/n=30) and in healthy controls (n=19); PGK1 as a normaliser.
Results: 6q deletion, as a single aberration, was observed in 9 (17%) patients and in 20 (38.4%) patients as part of complex karyotype. In one patient, chromothripsis of chromosome 6 was observed. arrayCGH confirmed high heterogeneity of the range of 6q deletion and MDR of 1.4 Mb was determined in the q21 region spanning eight genes (*FOXO3, SOBP*, *SCML4*, *SEC63*, *OSTM1*, *NR2E1*, *LACE1*, and *ARMC2)*. Expression profiling of *FOXO3*, a regulator of cell cycle and/or apoptosis, revealed a lower number of *FOXO3* transcripts in CLL patients with 6q deletion than in those without 6q deletion (p=0.03) and healthy subjects (p<0.0001). Of the genes (*NF-kB*, *TBX21*, *IL-2* and *BCL10*) possibly influenced by *FOXO3* levels, increased mRNA expression of *NF-kB* was detected in CLL patients with 6q deletion compared to patients without 6q deletion (p=0.03) and healthy controls (p<0.0001). Cytokine *IL-2* expression was lower in patients with 6q deletion, but reached significance only between CLL patients with 6q deletion and controls (p=0.0003). The clinical evaluation of the deletion showed that 6q deletion was more prevalent in males; the patients had unmutated *IGVH* and were in more advanced clinical stages (Binet B and C).
Summary and Conclusion: In our CLL patients with 6q deletion, we determined MDR of 1.4Mb involving eight genes including *FOXO3*. Deletion of 6q was more frequently observed in males, patients with unmutated *IGVH* and in more advanced stages of the disease. The observed low mRNA expression of *FOXO3* and high expression of *NF-kB* in CLL patients with 6q deletion demands further investigation. This work was supported by the grants: IGA MZ ČR NT 13576 and IGA-LF-2014-001.

P847

INCORPORATION OF NEXT-GENERATION SEQUENCING INTO ATM GENE ANALYSIS IN PATIENTS WITH CLL AND MCL

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Background: ATM kinase plays a key role in p53 activation after dsDNA breaks. Germinal *ATM* inactivation leads to neurodegenerative syndrome Ataxia telangiectasia and heterozygous mutations to predisposition for breast cancer and lymphoid tumors, particularly chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). In CLL, *ATM* defects (11q- and/or mutation) are at diagnosis the most frequent aberrations (20% of patients) associated with negative prognosis. Regarding MCL, hallmark translocation t(11;14) is commonly accompanied by *TP53* mutation and even more frequently by *ATM* inactivation (40-56% of patients), but information about clinical impact is still limited.
Aims: Our aims were: (1) to introduce next-generation sequencing (NGS) mutation analysis of *ATM* in CLL and MCL samples, (2) to correlate disclosed mutations with the ATM functional status in CLL.
Methods: The following samples were selected for NGS analysis: (a) 16 CLL samples according to the ATM functional status (determined by functional test reported by us previously) including 15 dysfunctional or borderline samples and one sample with normal ATM function; (b) 14 initial MCL samples that were chosen randomly. Three of the 16 CLL samples had known *ATM* mutation status and served as controls. Primers for all coding exons (n=62) and adjacent splicing sites were designed for multiplex PCR setting. Some exons had to be divided to obtain amplicons of similar length (350±50 bp). The amplicon library per sample was prepared through 15 multiplex PCR reactions, purification and pooling. Nextera XT kit (Illumina) was used for library tagmentation and normalization and sequencing ran on MiSeq instrument (Illumina). Final data was analyzed by software CLC Genomic Workbench and Annovar. The median coverage was 4025 and cut-off value for variant frequency was set to 5%.
Results: We identified 5 CLL patients with presumably pathogenic *ATM* mutations. Two of these samples were positive controls, in which NGS confirmed mutations previously identified by Sanger sequencing and moreover detected one additional minor mutation. The third mutated sample harbored frameshift alteration p.2613_2616del (allelic frequency (AF) 83%) and the next one manifested non-synonymous variation p.1570A (AF 33%) together with silent alteration V245V (AF 18%) leading to partially aberrant skipping of exon 10. The last mutated sample exhibited missense and frameshift mutation leading to substitution p.I323K (AF 88%) and p.A1211fs (AF 5%), respectively. All remaining samples were wild-type according to NGS output. Importantly, all three new samples with *ATM* mutation(s) showed dysfunction in functional test. On the other hand, there were 9 samples with predicted ATM dysfunction that do not harbor any pathogenic alteration in analyzed region. Concerning MCL samples, we identified 6 *ATM* mutations in 4 patients, namely p.Q2522H (AF 41%), p.Y2833fs (AF 38%), p.Q2730L (AF 32%), p.R2871T (AF 32%), p.E1724G (AF 15%), p.E590fs (AF 7%). In most of samples we detected several common polymorphisms that should not significantly affect ATM stability and function.
Summary and Conclusion: We implemented NGS analysis enabling effective and sensitive *ATM* mutation detection. ATM dysfunction can be connected with mutations in coding region but possibly with other defects in non-coding regions or in some other part of ATM-p53 pathway as well. Supported by grants NT/13493-4, NT/13519-4, CEITEC CZ.1.05/1.1.00/02.0068 and SuPReMMe CZ.1.07/2.3.00/20.0045.

P848

NOD/SCID GAMMA XENOGRAFT MODEL OF B-CELL MALIGNANCIES USING TP53- AND/OR ATM-DEFICIENT CELL LINES

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Background: Xenograft models represent very promising biological tools to study the molecular basis and pathogenesis of many hematological malignancies. To establish a reliable and appropriate *in vivo* model of human leukemia we used immunodeficient NOD/SCID gamma (NSG) mice. These mice lack a functional receptor for interleukins and do not have functional T, B and NK cells, in contrast to NOD/SCID mice that retain NK cells. Here we report a study concerning mouse xenograft models using TP53- or ATM-deficient B-cell lines MEC-1, SU-DHL-4, JEKO-1, GRANTA-519 and REC-1.
Aims: The present study was designed to compare engraftments in NSG mice of TP53- or ATM-deficient B-cell lines. Our goal was to create a mouse xenograft model by transplanting cells of five cell lines, including chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) into NSG mice. We would then analyze the engraftment potential of these cell lines. The current study shows that B-cell lines-engrafted NSG mice can be successfully used for investigating the pathogenesis of hematological malignancies with potential for application in tailored therapy.
Methods: In total, 30 six to eight-week-old non-irradiated mice (n=6 per experimental group) were injected with 5x10e6 malignant cells in phosphate-buffered, saline (PBS). The injections were given intraperitoneally (IP, n=3) or subcutaneously (SC, n=3). Six control mice (one per group) were injected with PBS only. All animals were monitored individually throughout the experiment. After 4 weeks, all recipient mice were sacrificed by cervical dislocation and peripheral blood (PB), bone marrow (BM) and tissue samples (spleen, liver, tumor) were analyzed by histology, immunohistochemistry and flow cytometry analysis.
Results: All malignant cell lines except SU-DHL-4 injected IP or SC into NSG mice successfully engrafted. Three to four weeks after SC injection, four cell lines produced rapidly growing subcutaneous tumors in both male and female mice. Immunophenotype of all engrafted cell lines corresponded to human mature B cells expressing CD10-19+20+HLA-DR+ except for SU-DHL-4 and REC-1 cells which were moreover CD10+ and HLA-DR- respectively. In two lines, MEC-1 (CLL in transformation) and GRANTA-519 (MCL), we observed highly efficient engraftment of leukemic cells with very aggressive growth. MEC-1 IP injection led to significant weight loss (P=0.03), mild hepatomegaly and huge splenomegaly and death in one case. In GRANTA-519, there was huge tumor found in abdominal cavity or subcutaneously in all mice. Intraperitoneal tumor filled always the entire abdominal cavity. Immunohistological and flow cytometric analysis revealed a massive infiltration of liver, spleen and bone marrow by human CD19 and CD20 positive cells with high proliferative index. Noteworthy, only with GRANTA-519 it was observed that transplantation of

human tumor cells resulted in prominently higher infiltration of the bone marrow regardless of administration route. It might be due to wild-type TP53 status in combination with mutated ATM. In the other cell lines tested, the mutation status of ATM is unknown while TP53 is mutated.
Summary and Conclusion: According to our results, GRANTA-519 and MEC-1 cell lines appears to be the most suitable for xenograft model of human B-cell malignancies and would be a criterion for future use of selected lymphoid xenotransplant for chemical agent-based treatment experiments. Supported by research grants MSMT MSM0021622430, IGA-MZ-CR NT11218-6/2010, IGA-MZ-CR NT13493-4/2012, MSMT CZ.1.07/2.3.00/20.0045, VaVPI CEITEC CZ.1.05/1.1.00/02.0068 and Czech Leukemia Study Group - For Life.

P849

COPY NUMBER ALTERATIONS AND NOTCH1 MUTATIONS PREDICT THE OUTCOME OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA AND NORMAL FISH

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Background: Fluorescent in-situ hybridization (FISH) is the gold-standard method for cytogenetic assessment in chronic lymphocytic leukaemia (CLL), although commercial FISH panels only screen four specific aberrations (trisomy 12, deletions of 13q14, 11q22-q23*/ATM* and 17p13/*TP53*). Patients whose tumour cells harbour none of these aberrations are considered to have an intermediate-favourable outcome, but there are significant differences among them. On the other hand, copy number (CN) arrays scan unbalanced aberrations of the whole genome and could be a suitable alternative for FISH. Conventional cytogenetics after incubation with novel mitogens (DSP30 + IL-2) has also proven useful in these patients.
Aims: The purpose of this study was to analyse through CN arrays all consecutive patients from our database who had a normal FISH result at diagnosis and DNA available for analysis.
Methods: We identified 79 consecutive patients with CLL and a normal FISH result at diagnosis. Conventional cytogenetics was performed on Giemsa-banded chromosomes obtained after a 72-hour culture and stimulation with tetradecanoyl-phorbol-acetate (TPA). FISH studies for 11q, 13q and 17p deletions and trisomy 12 were performed using the Vysis CLL probe kit. *IGHV* mutational status and *TP53* mutation analysis were performed by Sanger sequencing. Exon 34 of *NOTCH1* and exons 14, 15, 16 and 18 of *SF3B1* were also sequenced. Copy number alterations (CNAs) were evaluated using high resolution CN array platforms (Affymetrix 6.0). Maximally selected rank statistics (maxstat) were used to identify the best cutpoint for CNAs. Time to first treatment (TTFT) was evaluated using cumulative incidence curves and considering death without therapy as a competing risk, while overall survival (OS) was evaluated using Kaplan-Meier plots and the log-rank test. Multivariate modeling of TTFT and OS were performed using Fine & Gray and Cox regression methods. Both outcomes were evaluated from the time of DNA sampling.
Results: Of all 79 patients with normal FISH results, 42% had at least one alteration: 14% had 1, 10% had 2, 9% had 3 and 9% had 4 or more CNAs. The most common losses were 18p, 14q, and 13q (each detected in 3 patients); and the most common gain was 2p (3 patients). Moreover, 18% patients had an abnormal karyotype by CBA, 40% had unmutated *IGHV* genes, 2/53 (4%) had *TP53* mutations, 6/51 (12%) had *SF3B1* mutations and 5/65 (8%) had *NOTCH1* mutations. Maxstat analysis revealed that the best cutpoint for genomic complexity was 1 (*i.e.* 0-1 *vs*. 2 or more). Univariate analysis revealed that the following covariates were associated with TTFT: genomic complexity (p<.001), unmutated *IGHV* (p<.001), high ZAP70 expression (p<.001), high CD38 expression (p<.001), *TP53* mutations (p<.006), *NOTCH1* mutations (p=.018); while there was a trend toward a shorter TTFT for patients who had abnormal cytogenetics (p=.10). Multivariate analysis confirmed that genomic complexity and *IGHV* mutational status both had an independent impact on TTFT (p=.044 and p=.003, respectively). In terms of overall survival, the covariates with a significant effect by univariate analysis were *NOTCH1* mutations (p=.001), genomic complexity (p=.039) and *TP53* mutations (p=.38), while there was a trend for *IGHV* mutational status (p=.052). Multivariate analysis showed that presence of *NOTCH1* mutations was the only factor independently associated with OS (p=.003).
Summary and Conclusion: The outcome of patients with CLL and normal FISH results is modulated by genomic complexity as determined by copy number arrays, as well as other molecular abnormalities such as *NOTCH1* mutations and the *IGHV* mutational status. These results may be taken into account when designing risk-adapted therapeutic approaches.

P850

Abstract withdrawn

P851

TAP63 IS EPIGENETICALLY REGULATED AND HIGHLY OVEREXPRESSED IN AGGRESSIVE CLL STEREOTYPED SUBSET #8

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Background: CLL stereotyped subsets #6 (IGHV1-69/IGKV3-20) and #8 (IGHV4-39/IGKV1(D)-39) both express unmutated BCRs yet exhibit different clinical behavior with subset #6 being less aggressive compared to subset #8, which exhibits the highest risk for development of Richter's syndrome among all CLL. No explanation is available for this difference, thus prompting investigation into the underlying mechanisms.
Aims: Taking the above into consideration and also given our recent finding that subsets #6 and #8 display differential DNA methylation profiles, we here explored the impact of this phenomenon on gene expression with the aim of obtaining insight that would assist in unraveling their pathobiology.
Methods: Five genes with the most striking differences in methylation status were chosen for comparative gene expression profiling in 8 subset #6 *versus* 10 subset #8 cases: ZNF300, EP400, PRDM16, TP63, HOXA5. TP63 isoform expression was assessed by RT-PCR with appropriate primers in a cohort of 64 cases, while determination of the Tap63 isoform in the same cohort was performed by RQ-PCR. Quantitative measurements were performed on negatively selected CD19+ B cells from peripheral blood samples obtained at diagnosis or before any treatment. TP53 mutational screening was performed by bidirectional Sanger sequencing.
Results: Differential DNA methylation between the two stereotyped subsets was linked to differential gene expression. Among the differentially expressed genes, TP63 showed the highest difference (FD=52.5, p<0.001) and was overexpressed (while in parallel hypomethylated) in subset #8 *vs* #6. This initial study concerned total mRNA *i.e.* could not discriminate between TP63 isoforms. Therefore, in order to identify which p63 isoforms are expressed in CLL, we screened by RT-PCR with appropriate primers 64 CLL cases, of whom 41 carried unmutated IGHV genes (U-CLL, including 10 subset #8 and 8 subset #6 cases), while 23 carried mutated IGHV genes (M-CLL). We detected mRNA of the TAp63 isoform only, whereas the ΔNp63 isoform was not expressed in any case. This is noteworthy, given recent reports that TAp63 not only directly affects cell survival but also influences the migratory and invasive properties of CLL cells to the bone marrow, indirectly regulating their survival as well. In order to investigate potential correlations with immunogenetic features of the leukemic clones, we quantitated TAp63 mRNA expression in the same cases and observed significantly higher TAp63 mRNA levels in U-CLL *versus* M-CLL (p<0.01). Interestingly, subset #8 showed significantly (p<0.001) higher TAp63 expression compared to all other subgroups under study, including the remaining U-CLL. Considering that mutant p53 binds to, and inhibits the activity of TAp63, we also performed TP53 mutational analysis and found only 4/55 cases bearing mutations; of note, none of these mutations located in the region responsible for the formation of aggregates with TAp63.
Summary and Conclusion: In conclusion, differential DNA methylation underlies varying expression of the TP63 gene, more specifically its TAp63 isoform, in CLL subgroups with distinct immunogenetic profiles. Overexpression of Tap63 in CLL subset #8 may represent a novel relevant pathomechanism of aggressiveness for this particular subset.

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TARGETED NEXT-GENERATION SEQUENCING FOR MUTATIONAL SCREENING IN CHRONIC LYMPHOCYTIC LEUKEMIA: A HIGH-THROUGHPUT YET TAILORED APPROACH WILL FACILITATE IMPLEMENTATION WITHIN A CLINICAL SETTING

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Background: Recently, next generation sequencing (NGS) studies have revealed a number of novel recurrent mutations in CLL, the prime examples being mutations within NOTCH1, SF3B1 and BIRC3, with higher frequencies in patients with a more aggressive disease and a poor clinical outcome. Considering the increasing number of prognostically relevant genes and the relatively high cost and laborious nature of Sanger sequencing, we aimed at exploring targeted NGS as a novel strategy to assess the mutation status of several genes with prognostic potential. Major advantages with this approach revolve around its capacity to screen a large number of genes and patient samples simultaneously (currently up to 96 samples). Aside from the ability to multiplex both genes and patient samples, the high sequence depth achievable also enables tracking minor subclonal populations over time. Since subclonal dynamics are partially shaped by the treatment regimen received, this will become important as new therapies enter the clinic. An additional favorable attribute of targeted NGS is the ability to analyze all coding exons within a gene regardless of length. This is noteworthy since the size of several prognostic genes, such as ATM, would hinder comprehensive gene analysis within a clinical setting.
Aims: To this end, we designed a HaloPlex gene panel (Agilent Technologies) focusing on 10 genes (mean coverage 99%); TP53, ATM, SF3B1, NOTCH1, BIRC3, MYD88 and POT1 have been linked to CLL prognosis, while XPO1, KLHL6, LRP1B are less characterized but were observed in various NGS studies.
Methods: A total of 168 high risk CLL patients were investigated (unmutated IGHV, n=119; IGHV3-21 subset #2, n=49). Sequencing libraries were run on the Illumina 2000 HiSeq instrument and a mean read depth of ~1500 reads/base within the regions of interest was obtained. Data were analyzed using our in-house bioinformatics pipeline that required the following conditions to be met for a variant to pass all filtering steps: (i) exonic or in a splicing region; (ii) non-synonymous or resulting in a frameshift; (iii) not listed in dbSNP137; (iv) variant allele frequency >0.1; and, (v) variant allele read depth >10.
Results: Using the conservative cut-off of 10% for the mutant allele, we found that 105/168 (62.5%) patients carried at least one mutation; ATM (n=33; 20%), BIRC3 (n=5; 3%), NOTCH1 (n=28; 17%), SF3B1 (n=34; 20%) and TP53 (n=17; 10%). Collectively, mutations within these 5 genes accounted for 131/171 (77%) of all mutations observed. Fifty-three patients had more than 1 mutation and 45/53 harbored mutations within more than one gene. We selected 63 mutations for validation, with mutant allele frequencies ranging from 0.10-0.98, and were able to confirm all of them by Sanger sequencing (Table 1).

Table 1.

Gene	No. of cases with mutation	No. of mutations	Selected for validation	No. validated
ATM	33	43	0	0
BIRC3	5	6	4	4/4
KLHL6	1	1	0	0
LRP1B	18	19	0	0
MYD88	2	2	0	0
NOTCH1	28	29	17	17/17
POT1	7	8	0	0
SF3B1	34	35	29	29/29
TP53	17	18	13	13/13
XPO1	9	10	0	0
Sum		171	63	

Summary and Conclusion: With the prognosis of CLL becoming increasingly dependent on an understanding of the molecular landscape of the disease, mutational screening within clinical practice will soon become routine. However, given the large number of potentially significant genetic mutations increasingly being identified, it is apparent that a high-throughput tailored approach is vital in order to efficiently acquire and utilize this data. This study demonstrates the applicability of targeted NGS as a new approach for mutational screening within clinical routine. In the future the need to confirm NGS results by Sanger sequencing will decrease dramatically thereby significantly reducing the amount of labor and costs for clinical laboratories wishing to use NGS technologies.

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CYTOGENETIC EVOLUTION PATTERNS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Among the various prognostic factors reported as relevant in chronic lymphocytic leukemia (CLL), specific cytogenetic abnormalities have a prominent role and can be correlated to distinct clinical course and response to treatment.
Aims: The importance of the emergence of new chromosomal aberrations in different disease timepoints is still under investigation.
Methods: We studied 88 CLL patients with available cytogenetic data at both diagnosis and disease progression. Cohort characteristics were as follows: male/female: 67/21 | Binet stage at diagnosis A/B/C: 60/19/6 | unmutated IGHV genes: 50/87 (57.4%) | CD38 positivity: 39/84 (46.4%) | ZAP-70 positivity: 22/44 (50%). Cytogenetic abnormalities detected by fluorescence *in situ* hybridization (FISH) at diagnosis were: 13q deletion: 30/65 (46.1%), 11q deletion: 6/63 (9.5%), 17p deletion: 9/65 (13.8%), trisomy 12: 9/59 (15.2%). 41/88 (50%) patients had abnormal karyotype at diagnosis. Secondary malignancies were reported in 12/88 (13.6%) of the cases. Patients were divided in two groups, regarding the presence (32/88, 36%, group A) or not (56/88, 64%, group B) of additional cytogenetic aberrations in the karyotype obtained at disease progression.
Results: The additional aberrations observed in group A patients concerned 20 translocations, 19 additions, 21 deletions and 36 multiple numerical aberrations; 19 patients had ≤2 new aberrations and 12 had ≥3, the latter being considered as the emergence of a complex karyotype. Most common breakpoints involved chromosomes 14 (8 breakpoints), 17 (6 breakpoints), 18, 11 and 1 (5 breakpoints). Additional chromosomes involved mostly chromosomes 6 and 21 (4 and 3 cases respectively), whereas chromosome monosomies concerned chromosomes 17 (3 cases) and 9, 10, 13, 16, 18 (2 cases each). No statistically significant difference was observed between the two groups concerning stage at diagnosis, gender, IGHV mutational status or CD38 positivity. On the contrary, group A cases had significantly increased ZAP-70 expression (p=0.03). No correlation was noted concerning the presence of 11q deletion, 13q deletion, 17p deletion or *TP53* mutation and *NOTCH1* mutation at diagnosis. However, patients with trisomy 12 at diagnosis had significantly more frequent occurrence of additional cytogenetic abnormalities at disease progression (p=0.02). Within group A patients, the emergence of a complex karyotype (≥3 abnormalities) at disease progression was identified as prognostically adverse, decreasing overall survival (5-year survival 78% *vs* 91%, p=0.024). In addition, group A patients had a remarkably higher prevalence of secondary malignancies (7/32, 21% *vs* 3/56, 5.3%, p=0.02), regardless of follow up duration. More specifically, in group A, 3 patients developed a second hematologic malignancy (2 acute myeloid leukemia, 1 polycythemia vera) and 4 solid organ malignancies (stomach, bile duct and 2 lung cancer cases), whereas in group B had only 3 cases with skin, prostate and lung cancer, respectively. Survival analysis showed similar survival rates in both groups, (5-year survival 87.5% *vs* 84.1%) irrespectively of CLL-related death or not.
Summary and Conclusion: In conclusion, clonal evolution at disease progression of CLL can provide prognostic information in selected patients *i.e.* those with karyotype complexity and may be associated with genomic instability and intensified cell signaling, as reflected by the high frequency of secondary malignancy and ZAP-70 expression, respectively.

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CHARACTERIZATION AND SIGNIFICANCE OF RARE BALANCED IMMUNOGLOBULIN TRANSLOCATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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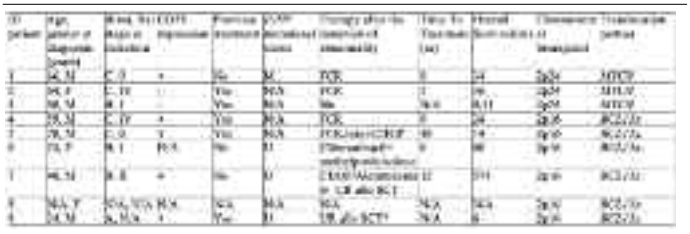
Background: The type of cytogenetic aberrations can predict outcome in chronic lymphocytic leukemia (CLL). Recent studies suggested that translocations may have a negative impact on response to therapy and survival, especially when unbalanced. Balanced translocations involving immunoglobulin (IG) genes are commonly observed in B-cell non Hodgkin lymphoma, and in only 5-7% of CLL cases. In this entity, their exact characterization seems important since the outcome may vary according to the partner gene.
Aims: To characterize two partners (*BCL11a* and *MYCN*) in unbalanced translocation involving IgH.

Methods: The Belgian Cytogenetic Group for Hemato-Oncology (BCGHo) and the Groupe Francophone de Cytogénétique Hématologique (GFCH) retrospectively reviewed files of patients referred for cytogenetic characterization of CLL between 2004 and 2012. Inclusion criteria were (i) morphological and immunophenotypical (defined as a Matutes score ≥ 3/5) diagnosis of CLL, (ii) a karyotype showing a t(2;14) and (iii) involvement of *MYCN* or *BCL11a* confirmed by fluorescence *in situ* hybridization (FISH). FISH was performed using (i) a probe containing LSI N-MYC [Abbott, labeled in spectrum orange (SO)] and 3 pooled bacterial artificial chromosomes (BAC) located on *IGH* [labeled in spectrum green (SG), *i.e.* RP11-1087P08, RP11-346I20, and RP11-675H01], (ii) a 632 kb break-apart probe containing BACs located at the 3' (RP11-604J13 and RP11-785E21) and at the 5' (RP11-573O13 and RP11-418N22) end of *BCL11a* and (iii) commercially available probes to investigate the common "CLL loci".

Results: Nine patients were collected, 3 with a translocation involving *MYCN* and 6 with a translocation involving *BCL11a*. Their median age was 59 years with a male/female sex ratio of 6/3. Except for one patient, all cases with available data needed therapy. The time to treatment was relatively short (0 to 48 months) in most of the patients. In 2 cases with *MYCN*, there was an associated del(13q). Three of 6 cases with *BCL11a* displayed trisomy 12. Most *BCL11a* abnormalities were associated with a non-complex karyotype.

Table 1: Clinical patient characteristics at the time of the detection of the IG abnormality



ID:identification, M: male, F: female, (m): months, FCR: fludarabine, cyclophosphamide, rituximab, CHOP: cyclophosphamide, adriamycine, vincristine, prednisone, UR: unrelated, allo SCT: allogeneic stem cell transplantation, *: scheduled, N/A: not available, †: dead.

Summary and Conclusion: Among *IG* translocations in CLL, we identified a novel rare partner, *MYCN*. Due to the limited amount of cases, it is impossible to draw conclusions on its prognostic significance at the present time. Another recurring IG translocation partner is *BCLL11a*, associated with an unmutated *IGVH* and expression of CD38. This work is supported by the Salus Sanguinis Foundation.

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IMPACT OF TWO CYTOGENETIC LESIONS ON THE OUTCOME OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in western country and is characterized by heterogeneous clinical and biological features. The most important prognostic factors are represented by mutational status of immunoglobulin heavy chain variable region gene (IGHV) and genomic aberrations evaluated by fluorescent *in situ* hybridization (FISH), *i.e.* deletion of 11q (11q-), 13q (13q-), 17p (17p-) and 12 trisomy (+12). These chromosome lesions provide prognostic information when classified according the model proposed by Döhner *et al.* (2000). These authors analyzed patients with none, defined as "normal", and 1 genetic lesion; in particular, those harboring 11q- or 17p- had an unfavorable prognosis. However, the daily application of FISH to clinical practice shows that a subset of patients harbors more than one recurrent abnormality.

Aims: The aim of this study was to define the impact of two cytogenetic lesion on CLL patients' outcome.

Methods: In this study we retrospectively analyzed 727 CLL patients referred to the Hematology and Clinical Immunology Unit of Padua University Hospital since 1988. Criteria for inclusion were i) diagnosis of CLL according to iwCLL/NCI criteria and ii) cytogenetic analyses with FISH on bone marrow or peripheral blood sample before starting treatment. Patients with 11q- or 17p- were grouped together. Test *t* Student was used to compare continuous variables. Chi square and Fisher exact tests were used to compare categorical variables. Log-rank test was used to compare survival. Cox proportional hazards model was used to identify independent prognostic factors.

Results: 422 patients fulfilled all inclusion's criteria and were recruited in this study; 124 (30%) had a normal FISH (group 0), 255 (60%) had one FISH aberration (group 1) and 43 (10%) had two cytogenetic lesions (group 2). With respect to patients belonging to the first two groups, group 2 patients had a shorter treatment free survival (TFS, p<0.0001) and overall survival (OS, p<0.0001) in univariate analyses, and a higher risk to underwent treatment (HR 1.00 *vs* 1.15 *vs* 2.52, for group 0,1 and 2 respectively) and death (1.00 *vs* 1.25 *vs* 3.18) in multivariate analyses. Moreover, differences in outcomes among the three groups were also consistent with the expression of unfavorable clinical and biological features at presentation. In particular, patients with two cytogenetic abnormalities were at a more advanced Rai (p<0.0001) and Binet (p=0.0392) stage at diagnosis, had a higher white blood cell count (WBC) and absolute lymphocyte count (ALC) at initial presentation (p<0.0001), and lower percentage of mutated IGHV gene (p<0.0074) than the other two subsets. Furthermore, we characterized patients with 2-FISH lesions and we defined as 13q HR (high-risk) patients harboring 13q- and 11p- or 17p- (*i.e.* high-risk cytogenetic lesions), and +12 HR those carrying +12 and a high-risk deletion. 27 patients, *i.e.* 6.4% of the cohort and 12% of all 13q- subjects, were re-classified as 13q HR. Their median TFS and OS were intermediate between patients harboring only 13q- and those harboring high-risk cytogenetic lesions, 13.82 *vs* 2.50 *vs* 0.81 (p<0.0001) and not reached *vs* 14.76 *vs* 8.62 (p<0.0001), respectively. In addition, they had three times higher risk of treatment (p<0.0001) and death (p=0.0038), presented with a more advance stage (Rai p=0.0200, Binet p=0.0681), higher male/female ratio (p=0.0134), WBC (p=0.0005), ALC (p<0.0001 and percentage of unmutated IGHV (78% *vs* 26%, p<0.0001) that 13q- patients. Considering +12 HR patients, who account for 1.4% of the cohort and for 10% of all subjects with 12 trisomy, are characterized by disease aggressiveness intermediate between those harboring +12 and 11q- or 17p- patients (p<0.0001 for TFS and p=0.0124 for OS). +12 HR patients have a similar risk to underwent treatment, but four times higher risk of death than +12 (p=0.0410); no differences were found among clino-biological features.

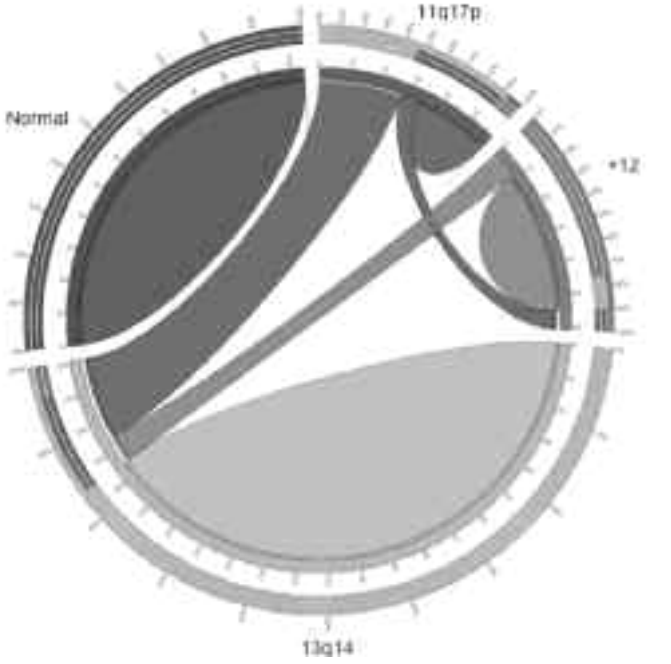

Figure 1.

Summary and Conclusion: This study i) defines new cytogenetic classes, ii) identifies subsets of patients with a more aggressive disease, iii) discloses the prognostic implications of 2 cytogenetic lesions and iv) allows a better prediction of CLL patients' prognosis and outcome.

P856

SF3B1 MUTATIONS IN CLL ARE ASSOCIATED WITH A DEFECTIVE DNA DAMAGE RESPONSE

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Background: Mutations or deletions in *TP53* or *ATM* are well-known determinants of poor prognosis in Chronic Lymphocytic Leukemia (CLL), but

only account for approximately 40% of chemo-resistant patients. Genome-wide sequencing has uncovered novel mutations in the splicing factor sf3b1, that were in part associated with *ATM* aberrations, suggesting functional synergy.
Aims: To uncover the mechanism underlying the contribution of SF3B1 mutation to CLL pathogenesis.
Methods: Detailed genetic and sequence analyses were done in a CLL cohort (n=105) containing *ATM*, *SF3B1* and *TP53* gene defects. Functional analyses were performed: a) p53/ATM target gene induction by multiplex assay, b) apoptosis responses to irradiation and chemotherapeutics, and c) γH2AX focus formation as marker for DNA damage by FACS and microscopy.
Results: There was considerable overlap between *ATM* and *SF3B1* lesions; 18/29 of *SF3B1* mutated cases carried concurrent 11q deletion and/or *ATM* mutation. Combined *TP53* and *SF3B1* mutations were found in 3/29 cases. In this report, we focus on the ten patients where single *SF3B1* lesions were identified. Functionally, the single *SF3B1* (sSF3B1) mutated samples resembled *ATM* mutated CLL in displaying defective ATM/p53 transcriptional and apoptosis response to various DNA-damaging regimens (Fig1A). In *ATM* mutated cases, sensitivity to fludarabine can be restored by nutlin-3a. This was also found for *sSF3B1* mutations (Fig1B), although ATM kinase function remained intact. Finally, γH2AX formation was increased both at baseline and upon irradiation in *SF3B1* mutated cases. The observed effects could not be explained by defective splicing of ATM in *SF3B1* mutated cases.

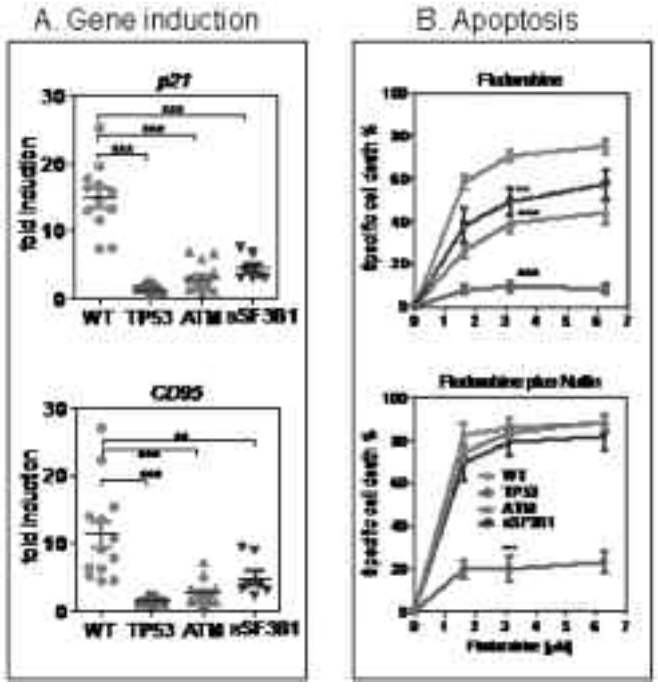

Figure 1.

Summary and Conclusion: Our data demonstrate that single mutations in *SF3B1* are associated with increased DNA damage and/or aberrant response to DNA damage. Combined, our observations suggest an explanation for the poor prognosis of affected patients, and may lead to new treatment strategies.

P857

SYSTEMATIC DRUG SENSITIVITY SCREENING IN LYMPHOID MALIGNANCIES IDENTIFIES VULNERABILITIES OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH HIGH RISK ABERRATIONS

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Background: The impact of mutations and pathway deregulation on drug sensitivity in cancer is only partly understood.
Aims: To identify relevant pathway dependencies that are exploitable by targeted treatment approaches we systematically investigated heterogeneity of drug response and their association with genetic lesions in primary lymphoid malignancies.
Methods: Primary leukemia / lymphoma cells obtained from peripheral blood were characterized with an *ex vivo* high-throughput drug screening platform. Cell viability was assessed by colorimetric quantification of ATP (CellTiterGlo®) at 48 hours of drug exposure. Heat inactivated human serum was supplemented to mimic micro-environmental conditions. In an initial screen 2000-3000 different compounds were tested for cytotoxicity on a cohort of 36 patients (27 chronic lymphocytic leukemia (CLL), 6 T-cell prolymphocytic leukemia (T-PLL), 3 non-CLL B-Non-Hodgkin-Lymphoma (B-NHL)). 67 promising compounds targeting different pathways were further characterized on an extended cohort (n=111; 97 CLL, 5 T-PLL, 6 non-CLL B-NHL, mononuclear cells of 3 healthy donors). To understand heterogeneous pathway dependencies, drug sensitivity was assessed for relations with somatic genetic variants. Genetic characterization was performed by FISH, targeted sequencing of recurrent aberrations (*BRAF, MYD88, NOTCH1, SF3B1, TP53*) as well as whole exome sequencing.
Results: Compounds dependent on functional p53 (Nutlin-3 and fludarabine) induced cell death more efficiently in CLL cells with wild-type (n=80) than with mutated p53 (n=17; Nutlin-3 10μM: 51±18 *vs* 80±18; fludarabine 10μM: 40±36 *vs* 67±23 [% viability of untreated control], both p<0.001). In addition, compounds with related targets, *e.g.* in the B-cell receptor pathway, showed similar response profiles (Fig. 1a). Based on the sensitivity data, we derived a hierarchy of compounds with preferential sensitivity for specific genetic subgroups. We identified compounds with increased activity in groups with high risk aberrations (*e.g. TP53* mutation, 11q deletion, unmutated *IGHV*; Fig. 1b). Validation of the top hit with increased sensitivity in *TP53*-mutated CLL (wt *vs* mut*TP53*: 58±31 *vs* 37±18 [% viability of untreated control], p<0.01) confirmed genotype-specific cytotoxicity across concentration ranges (0.1-10μM). Other compounds inhibiting the same target support our observation. The effect was reproducible in co-culture of CLL cells with HS-5 stroma cells (66±16 *vs* 32±13 [% viability of untreated control], p<0.05).

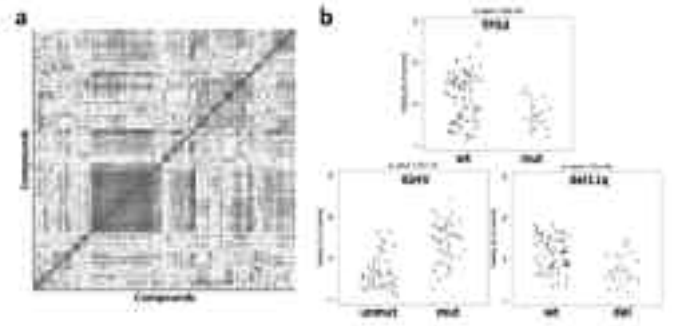

Figure 1.

Summary and Conclusion: Our data provide a comprehensive map of biologically determined drug sensitivity in lymphoid malignancies. The work offers a novel functional classification of lymphoma based on drug sensitivity and identifies potential vulnerabilities of high risk CLL.

P858

TRISOMY 12 IS ASSOCIATED WITH A DISTINCT PATTERN OF LYMPHOCYTOSIS DURING TREATMENT OF CLL WITH IBRUTINIB, WHICH DOES NOT AFFECT RESPONSE DEPTH OR DURATION

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Background: Treatment with ibrutinib (Ibr) results in transient lymphocytosis, which likely relates to disruption of CLL cell-microenvironment interactions, including integrin-mediated adhesion. Mutated *IGHV* gene and 13q deletion were reported by Woyach *et al.* (*Blood* 2014) to be associated with prolonged lymphocytosis, but not associated with survival endpoints. It is unclear whether this phenomenon correlates with clinical efficacy.
Aims: We sought to identify associations between patterns of lymphocytosis in Ibr-treated patients (pts) and baseline prognostic factors, which may provide insight into the mechanism of action of Ibr.
Methods: 89 pts were treated with Ibr monotherapy on investigational protocols at MD Anderson Cancer Center between June 2010 and August 2013. 40 of

89 were treatment-naïve. We evaluated time-to-peak (TTPeak) absolute lymphocyte count (ALC), % rise in ALC from baseline (%rise), time to 50% reduction in ALC from baseline (TT50%) and time to normalization or nadir of ALC (TTNadir), according to baseline prognostic variables.
Results: Median TTPeak was 28 days, median %rise was 108%, median TT50% was 16 weeks and median TTNadir was 24 weeks for the total cohort (Table).

Table 1.

* IQR=interquartile range.

Pts with trisomy 12 (T12) showed a distinct pattern of lymphocytosis (Figure), independent of additional chromosomal abnormalities (17p- was also present in 10/16 cases). A brief and modest rise in ALC was seen (median TTPeak 7 days, p=0.02, median %rise of 56%, p=0.02) followed by a rapid reduction to<50% of baseline at a median of 6 weeks, p<0.001 (Figure). There was no difference in response rates or progression-free survival in T12 *versus* other FISH categories. Pts with 11q- showed delayed TTNadir (51 weeks *vs* 24 weeks, p=0.002), while pts with fludarabine-refractory disease showed a trend toward delayed TTPeak (49 *vs* 28 days, p=0.051) and more prolonged lymphocytosis (median TT50% 32 weeks *vs* 16 weeks, p=0.052). Pts with mutated *IGHV* gene showed a trend toward delayed TT50% (16 *vs* 24 weeks, p=0.086), while no difference in any analyzed parameter was seen for pts with 13q-.

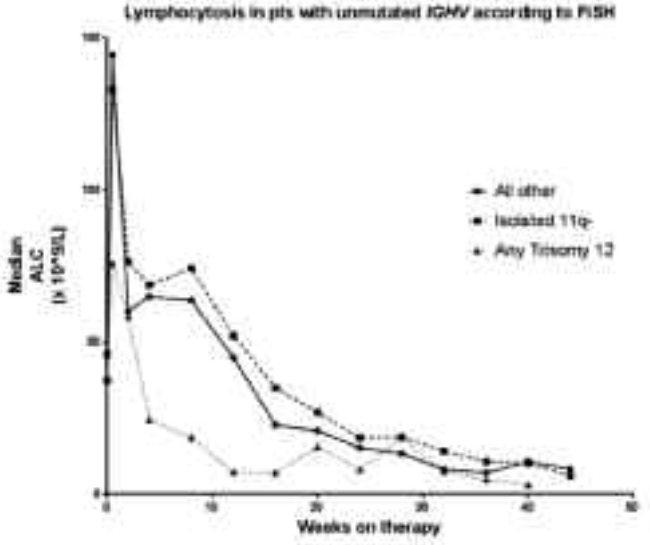

Figure 1.

Summary and Conclusion: Trisomy 12 CLL was associated with a unique pattern of lymphocytosis during ibrutinib monotherapy. The mechanism of this is unclear. However, T12 CLL cells universally overexpress CD49d, the α-4 subunit of the α4β1 integrin heterodimer, (Zucchetto, A *et al. Blood* 2013;122:3317), which is important in adhesion to VCAM-1 and fibronectin in the tissues. This overexpression may confer relative resistance to mobilization. The equivalent response in T12 pts to those without, despite the attenuated lymphocytosis, suggests that reduction in proliferation and/or induction of apoptosis within tissue sites may significantly contribute to therapeutic efficacy.

P859

INTEGRATION OF NOVEL GENE MUTATIONS INTO KARYOTYPE-BASED SUBGROUPS AS A PROGNOSTIC RISK STRATIFICATION TOOL IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Cytogenetics with novel mitogens has allowed to reveal chromosomal aberrations in regions uncovered by standard fluorescence *in situ* hybridization (FISH) and to identify novel genetic subgroups with prognostic relevance among patients with chronic lymphocytic leukemia (CLL) (Rigolin et al, Blood 2012). Whole exome sequencing (WES) approaches have identified previously unknown gene mutations with prognostic impact in CLL patients (*i.e. NOTCH1*, *SF3B1*, *BIRC3*) and have allowed to refine the FISH-based prognostic categories (Rossi et al, Blood 2013). Little is known about the integration of novel gene mutations and FISH into karyotype-based subgroups.
Aims: 1) To identify in CLL patients karyotype-defined categories using novel mitogens; 2) to screen the novel gene mutations into karyotype-defined categories; 3) to identify specific CLL subgroups defined by a combined FISH, molecular and cytogenetic approach, with the aim of further improving the prognostic stratification currently in use.
Methods: The study has so far included 153 CLL patients at diagnosis. FISH and cytogenetics with novel mitogens have been performed as previously described (Bardi et al, J Biomed Biotechnol 2011). The mutational screening of *NOTCH1*, *SF3B1*, *BIRC3* and *TP53* were performed by Sanger sequencing, as described (Rossi et al, Blood 2013).
Results: FISH was evaluable in all 153 cases. 17p- (5, 3.3%) showed adverse mutations in 80% of cases, 11q- (13, 8.5%) in 38.5% of cases, +12 (19, 12.4%) in 36.8% of cases, normal FISH (46, 30%) in 17.4% of cases and del13q- only (70, 45.8%) in 11.4% of cases. Karyotype with novel mitogens was performed in 146 cases and evaluable in 144 (98.63%). A normal karyotype was present in 40 cases (27.8%), del13q- only in 33 (22.9%), one lesion (other than 13q-) in 28 (19.4%), two lesions in 24 (16.7%), ≥3 lesions in 19 (13.2%). Low risk (normal karyotype and 13q-), intermediate risk (1 or 2 lesions) and high risk (≥3 lesions) accounted for 50.7%, 36.1% and 13.2% of cases, respectively. *NOTCH1* (n=12), *SF3B1* (n=13), *BIRC3* (n=4) and *TP53* (n=4) mutations distributed across the three karyotypic categories, with the exception of *TP53*, more frequently mutated in the high risk group. Overall, there was an increase in the incidence of unfavorable mutations between low risk (11/73, 15.1%), intermediate risk (13/52, 25%) and high risk (9/19, 47.4%), significant only between low and high risk (p=0.0047). Integrating FISH, mutations and karyotype, we showed that the best CLL prognostic category, *i.e.* CLL with 13q- only by FISH and wild-type (WT) for *NOTCH1*, *SF3B1*, *BIRC3* and *TP53* (n=59), showed additional karyotypic lesions in 32.2% of cases (1 additional lesion in 9, 2 lesions in 8, ≥3 lesions in 2 cases). Moreover, CLL with normal FISH and WT for mutations (n=34) showed karyotypic lesions in 32.3% of cases (1 lesion in 5, 2 lesions in 3, ≥3 lesions in 3 cases). Analogous figures were demonstrated in an independent cohort of 82 CLL in first progression. The assessment of the prognostic impact of this refined classification is ongoing.
Summary and Conclusion: FISH, gene mutations and karyotype using novel mitogens provide complementary prognostic information in CLL patients. Karyotype may further refine patients prognostic stratification in one third of cases belonging to good prognosis categories. A more refined prognostic risk assessment and a personalized management is becoming a realistic upcoming prospect for CLL patients.

Chronic lymphocytic leukemia and related disorders - Clinical 2

P860

CD4+CD16- LGL LEUKEMIA SHOWED DISTINCTIVE BIOLOGICAL AND CLINICAL FEATURES

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Background: T-cell Large Granular Lymphocyte Leukemia (T-LGLL) is a chronic lymphoproliferative disorder characterized by the clonal expansion of CD3+ Large Granular Lymphocyte (LGL). Most patients have an indolent course with neutropenia present in about 50-60% of patients, representing the major feature of the disease. Recently, somatic STAT3 mutations conferring constitutive activation of JAK-STAT pathway have been discovered in approximately 30% of patients with T-LGLL. Patients generally express CD3, CD8, CD16 and CD57 but rare forms of CD4+ LGLL are well known and characterized by CD4+/CD8- or dim/Vb13.1 expression, and a pathogenetic relationship with CMV.
Aims: Aim of this study is to identify different biological and clinical subsets of T-LGLL patients through immunophenotype analysis and STAT-3 mutational analysis of T-LGLs.
Methods: LGLs in 41 patients with more than 50% clonal LGL in PBMCs were analysed by flow cytometry using antibody for CD3, CD4, CD8, CD16, CD56, CD57, Vb repertoire, CD158a, CD158b, CD158e, CD94, CD159a. DNA samples from these 41 patients were available for STAT-3 mutation analysis through Sanger sequencing and PCR ARMS for Y640F and D661Y mutations.
Results: By FACS analysis we found 14/41 patients CD4+ with 100% CD56+/CD57+ double positive; among them, 11 were CD4+/CD8+, while 3 patients were CD4+/CD8-. CD16 expression was present in 22/41 patients; 20/22 were also CD3+/CD8+/CD57+ while only 2/41 were CD3+/CD4+/CD8+/CD57+, these last were excluded from CD4+ and CD16+ subgroup. Vb13.1 expression was found in 4/12 CD4+CD16- patients (33%) but in any of CD4-CD16+. KIRs and Lectin type receptors expression was found in 60% and 70% of CD4-CD16+ LGLs, respectively (with CD158b expressed in half of cases and CD94 in 60% of cases), but only in 17% and 33% of CD4+CD16-LGLs, respectively. Neutropenia (Absolute Neutrophil Count, ANC,<1500/mm^3) was present in 21/41 patients (51%), with 14/41 (29%) experienced severe neutropenia (ANC<500/mm^3). Among CD4-CD16+ patients, 90% experienced neutropenia while CD4+CD16- patients did not; consistently, CD4-CD16+ patients had ANC level significantly lower than CD4+CD16- patients (mean ANC of CD16+: 690/mm^3; mean ANC of CD4+ 2817/mm^3; p=0,05). Finally we investigated somatic STAT-3 mutation through Sanger sequencing and ARMS PCR for Y640F and D661Y mutations in these 41 patients. We identified 9/41 (22%) patients mutated by sequencing (6 with Y640F and 3 with D661Y) and 11/41 (27%) by PCR ARMS (7 with Y640F and 4 with D661Y). All mutated patients were CD4-/CD16+ while in samples from CD4+CD16- patients of T-LGLL no one mutation was found.
Summary and Conclusion: In this cohort, a high frequency of CD4+CD16- type of T-LGLL was recognized, characterized by reduced NK receptor expression, low incidence of neutropenia and absence of STAT3 mutations. This subset of patients is clearly separated by the more classical CD4-/CD16+ subgroup of T-LGLL, characterized by high incidence of neutropenia, skewed expression of NK receptor and presence of somatic STAT-3 mutations. These data suggest different pathogenetic mechanisms and clinical courses of these two types of T-LGLL and identify in CD4-/CD16+ LGLL type a subset with different biology and more aggressive course as compared to the CD4+/CD16- LGLL subgroup.

P861

DEVELOPMENT AND VALIDATION OF A NOTCH CUSTOM NGS ASSAY FOR IDENTIFYING NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA AND OTHER LYMPHOID MALIGNANCIES

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Background: NOTCH1 is known to be activated by oncogenic mutations in both hematologic and solid tumors including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), T cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC) and breast cancer. In CLL, Notch 1 mutations were detected in ~12% of patients at the time of diagnosis and at a higher rate during disease progression to Richter's transformation. As such, NOTCH1 may be a valuable biomarker of therapeutic efficacy, or clinical candidacy, for novel anti-cancer agents or regimens. For prospective evaluation of NOTCH1's utility as a biomarker, it is critical to have a sufficiently sensitive, specific, and comprehensive assay to detect NOTCH1 mutations in clinical specimens. Compared to Sanger sequencing, a targeted Next Generation DNA Sequencing (NGS) assay is more comprehensive, sensitive, and specific, and is therefore better suited for clinical applications with limited specimen availability.
Aims: We have developed a custom NGS assay on the Ion Torrent PGM platform for evaluating NOTCH1 mutation status. With this validation study we determined whether the Notch NGS Assay could semi-quantitatively detect NOTCH1 activating mutations at the limit of detection (LOD) of 5% allele frequency in blood and FFPE tumor tissue collected from patients with various lymphoid malignancies.
Methods: The region of interest (ROI) includes the coding exons of Notch1 (34 exons). Primers were designed using Ion AmpliSeq™ Designer in combination with MolecularMD's proprietary primer design method. The data were analyzed with Torrent Suite 3.4.2 and the MolecularMD analysis pipeline. Cell lines containing known NOTCH1 mutations and Horizon Diagnostics NOTCH1 L1600P mutation standards were used, along with clinical samples from several sources.
Results: Accuracy: The allele frequencies of the NOTCH1 activating mutations detected, including single base substitutions (SBS) and insertions/deletions (indel), correspond with those reported by droplet digital PCR (ddPCR) of NOTCH1 mutation standards, or those reported by Sanger sequencing of serially diluted fresh or contrived FFPE DNA samples. Precision: Seven NOTCH1 activating mutations with allele frequencies above 5% LOD (3 SBS, 2 insertions, 2 deletions) were all reproducibly detected with CVs of less than 30% in 3 independent sequencing runs using serial dilution of both fresh and FFPE DNA samples respectively. LOD: The LOD of the assay is 5% for SBS and indels, as determined by sequencing 20 replicates of fresh DNA samples and 20 replicates of contrived FFPE DNA samples containing NOTCH1 activating mutations with an expected 5% allele frequency. False Positive and False Negative Rates: NOTCH1 mutations detected by NGS and Sanger or Sanger with enrichment methods in 30 blood samples (4 contrived, 26 clinical) and 32 FFPE samples (4 contrived, 28 clinical) are being compared to determine analytical sensitivity and confirm specificity.
Summary and Conclusion: This validation study demonstrates that with only 30ng DNA input, the Notch NGS Assay is capable of accurately detecting NOTCH1 activating mutations, including SBS and small indels in blood and FFPE tissue specimens. Collectively, our results indicate that the custom targeted NGS assay is suitable for mutation profiling of clinical samples. The assay is designed for use in screening clinical trial patients, and the NGS platform further accommodates expansion for multiplexed evaluation of additional genes within the same clinical specimen.

P862

ITALIAN VALIDATION OF THE MDACC PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS: ANALYSIS OF 1502 CASES

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Background: The clinical course of CLL is notable for its marked variability: some patients present with highly aggressive disease requiring immediate therapy and poor survival (death within 1-2 years of diagnosis), whereas others live therapy-free for decades. In 2007, Wierda et al proposed a predictive nomogram and a simplified prognostic index built on basic clinical characteristics (age, sex, Rai stage, number of lymph node groups) and laboratory parameters [β2-microglobulin (β2M) and absolute lymphocyte count (ALC)] universally applicable to previously untreated CLL patients to predict survival.
Aims: We performed a validation of the MDACC index in an independent series of Italian patients.
Methods: Databases of 4 Italian centers including roughly 3000 CLL patients diagnosed between 1980 and 2013 were used to evaluate the validity and reproducibility of the MDACC prognostic index. Data regarding age, sex, Rai stage, ALC and number of lymph node sites involved was complete for all patients, while β2M values were available for 1502. Nomogram and prognostic index score were calculated using the method proposed by Wierda et al (Figure 1). Progression free survival (PFS) and overall survival (OS) analyses were performed using the Kaplan-Meier method. The prognostic impact for the outcome variable was investigated by univariate and multiple Cox regression analysis.
Results: The median age of the 1502 patients was 67 years (range 27-94) with 55.7% male. The majority of patients had Binet stage A (82.9%), 847 cases (56.4%) had Rai stage 0; moreover 151 cases (10.1%) satisfied the 2008

NCI/WG criteria for cMBL (<5.0x10^9 B lymphocytes/L in the peripheral blood and no apparent lymph node, spleen or liver enlargement). All 6 parameters involved in the prognostic index were found to be independently associated with survival in this analysis (age: HR 1.085, 95%CI 1.071-1.1, P<0.0001; sex: HR 1.548 95%CI 1.204-1.989, P=0.001; ALC: HR 1.006, 95%CI 1.003-1.01, P<0.0001; number of lymph node groups: HR 2.222, 95%CI 1.671-2.956, P<0.0001; b2M: HR 1.216 95%CI 1.16-1.275, P<0.0001). The median nomogram score was 87 (range 27.4-181.8). Furthermore, when the score was evaluated as continuous variable (ie, by measuring the risk of each point increase), the total point score was associated with the OS (HR, 1.058; 95% CI, 1.053-1.064; P<.0001). According to the prognostic index 38.7% of patients were classified as low, 58.3% as intermediate and 3% as high risk. The estimated median survival times were: not reached for low risk, 13.4 years for intermediate risk, and 3.4 years for high risk. Percentage of 5- and 10-year survival probabilities were 0.983 (SE 0.006) and 0.954 (SE 0.012) for low-risk cases, 0.818 (SE 0.015) and 0.65 (SE 0.023) for intermediate-risk and 0.443 (SE 0.081) and 0.101 (SE 0.064) for high-risk. The estimated median and 5- and 10-year survival by prognostic index risk category were similar to those originally reported. The prognostic index remained significantly associated with OS also when patients were sub-grouped by period of diagnosis (1980-1995, P<0.0001; 1996-2004, P<0.0001; 2005-2013, P<0.0001). The prognostic index risk category remained a predictor of survival when analysis was limited to Rai stage 0 (P<0.0001) and cMBLs (P=0.009). Finally the prognostic index also allowed prediction of PFS in all 1502 patients (P<0.0001), in Rai 0 cases (P<0.0001) and in cMBLs (P=0.035).

Table 1. Proposed CLL prognostic index and nomogram score.

Characteristics	Point contribution			
	0	1	2	3
Age, y	-	<50	50-65	>65
β2M, mg/L	<ULN	1-2 x ULN	>2 x ULN	-
ALC, 10^9/L	<20	20-50	>50	-
Sex	female	Male	-	-
Rai stage	0-II	III-IV	-	-
No. of lymph node groups	≤2	≥3	-	-

ULN indicates upper limit of normal; ALC, absolute lymphocyte count.
*Patient total score determined by adding up the scores of the 6 components. Patients with a score of 0 to 3 were considered to be at low risk, those with a score of 4 to 7 were considered to be at intermediate risk, and those with a score ≥8 were considered to be at high risk.
Adapted from: Wierda WG, O'Brien S, Wang X, et al. Prognostic nomograms and index for overall survival in previously untreated patients with chronic lymphocytic leukemia. Blood. 2007;109:4679-85.

The formula to calculate the nomogram score for a patient is: -12.5 + [1.25 x age] + [8.32 x (β2M)] + [8.62 x (ALC, 10^9/L/100)] + [7.34 x I (sex: male)] + [11.00 x I (Rai III or IV)] + [10.84 x I (nodes 3)] where I() is the indicator function, equal to 1 if the condition in the parenthesis is met and 0 if not.

Summary and Conclusion: Our results confirm the ability of the MDACC prognostic index to predict survival among patients with previously untreated CLL. The study also extended the utility of the index by demonstrating that it retains prognostic value when applied exclusively to Rai stage 0 and cMBL patients and predicts PFS. Finally, the period of diagnosis does not seem to modify the capability of this index to predict prognosis of CLL patients.

P863

FLUDARABINE, CYCLOPHOSPHAMIDE AND LENALIDOMIDE IN RELAPSED/REFRACTORY PATIENTS WITH CLL, PRELIMINARY RESULTS OF THE PHASE1-2 GIMEMA CLL0606 STUDY

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Background: The restoration of immuno-mediated functions seems the primary mechanism of action of lenalidomide in chronic lymphocytic leukemia (CLL).
Aims: The combination of lenalidomide with an effective regimen in CLL, such as fludarabine and cyclophosphamide (FC), might result in an increased activity preserving the immune function. The activity and toxicity of schedule combining lenalidomide and FC for the treatment of relapsed/refractory patients with CLL have been evaluated in a prospective phase I/II study.
Methods: Forty patients with a median age of 66 years (range: 47-77) and a median follow-up of 14.31 months (range 3-56) have been included in the study. The median number of prior treatments was 1 (range: 1-3), 82% of patients received a prior treatment with FC or FCR. The baseline median absolute lymphocyte count was >60 x 10^9/L in 49% of cases, increased B2M values were observed in 53%, Binet stage C in 26% of cases and bulky nodes (≥5 cm) in 29% of cases. Poor-risk genetic features were present in the majority of patients, 65% were IGVH unmutated,15% showed a del11q and 25% a del17p. Treatment consisted of 6 monthly courses of FC (fudarabine: 30 mg/m^2 iv; cyclophosphamide: 250 mg/m^2, days 1-3) combined with 14 consecutive days of lenalidomide administration (days1-14). Lenalidomide was given at the starting dose of 2.5 mg daily during the first course of therapy. Subsequently, the dose has been escalated to 5 mg, the dose that was defined in the prior phase 1 of the study as the maximum tolerated dose of lenalidomide given in combination with FC. As infection prophylaxis patients received bactrim and valacyclovir and as primary prophylaxis of granulocytopenia filgrastim. Tumor lysis syndrome (TLS) prophylaxis included oral hydration and allopurinol. Patients with an increased risk of thromboembolic events received low molecular weight heparin while low-dose aspirin was given to the other cases. Patients signed a written informed consent. CLL diagnosis, treatment requirement and response were assessed according to the 2008 IWCLL guidelines. In addition, in all CR patients, CT scan evaluation was included in the response assessment.
Results: Patients received a median number of 6 treatment cycles (range, 1-6). The most common treatment extra-hematologic adverse events (AEs) of any grade (≥10% of subjects) were fatigue, stipsis, pyrexia, muscle spams and skin rash. Grade ≥3 extra-hematologic toxicities were recorded in 5 patients (12.5%; fatigue, 1 case; stipsis,1; transient increases of transaminases, 1; atrial fibrillation, 1; accidental brain injury, 1). A mild, grade 1, tumor flare reaction was observed in 1 patient, while no case of TLS or thrombosis was recorded. The most frequent grade 3/4 hematologic toxicity was represented by neutropenia (65% of cases). However, only 3 cases (7.5%) of grade ≥3 infection (pneumonia) were recorded. On an intention-to treat basis, the overall response rate is 64.1% (CR, 23%; PR 41%). One of the 5 patients with del17p achieved a CR and one a PR. At 24 months the progression-free survival is 50% (95% CI, 32.9 - 76.8%) and the overall survival 80% (95% CI, 64.8 - 99%).
Summary and Conclusion: Taken together, the preliminary results of this study suggest that the combination of FC with lenalidomide given at the maximum tolerated dose of 5 mg, may be considered an active salvage regimen with acceptable toxicity, particularly in terms of infections, in relapsed and refractory CLL patients with adverse genetic features.

P864

SKIN CANCERS ARE COMMON AND CAUSE CONSIDERABLE MORBIDITY IN PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB FOR CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: The combination of fludarabine, cyclophosphamide and rituximab (FCR) is considered standard of care in chronic lymphocytic leukaemia (CLL), achieving durable remissions and long survival in most patients. However, the combination of fludarabine and cyclophosphamide (FC) impair DNA repair in experimental models, and causes prolonged T-cell lymphopenia in patients. Thus, FCR may simultaneously induce mutagenesis and compound CLL-associated immunosuppression, increasing the risk of second malignancies. Australia has high UV exposure and high incidence of sun-related skin cancers.
Aims: We sought to describe the epidemiology and clinical outcomes of skin cancers occurring in patients with CLL treated with FCR at an Australian center.
Methods: Single-center, retrospective study of patients treated with FCR for CLL between January 2001 and September 2008. All patients were assessed for the presence of second malignancies at their first presentation. We reviewed the records of consecutive patients whose cancer was managed primarily at our center, and documented the occurrence and histology of skin lesions following FCR treatment until death or last follow-up, irrespective of relapses or subsequent therapies.
Results: The median age of the 67 study patients (44M, 23F) was 58 years (range 26-84), and 48% were in Rai stages 3 or 4 at the time of FCR therapy. The median time from diagnosis of CLL to FCR chemotherapy was 46 (range 0.2 to 179) months. Due to restrictions surrounding the funding of rituximab during the study period, only 35 (52%) of patients received FCR frontline, and the rest had FCR after failing a median of 1 (range 1-3) previous therapies. Prior to first FCR exposure, 8 (12%) of patients had an antecedent diagnosis of skin cancer, including 4 squamous cell cancer (SCC), 1 basal cell cancer (BCC), and 3 malignant melanoma. Following FCR (median follow-up 19 months, range 1 – 48), 18 (27%) patients developed one or more skin cancer, with the first skin

cancer post chemotherapy being SCC in 11 (16%), BCC in 5 (8%), and melanoma in 2 (both with antecedent history of melanoma, and metastatic recurrence at 36 and 89 months post FCR). Forty-four percent of patients with skin cancer had more than one histological subtype, with the second subtype occurring a median of 18 months after the first. The clinical consequences of SCC or BCC development were (in increasing order of seriousness): local excision or cryotherapy in 47%, extensive excision under general anaesthetic and/or requiring skin flaps in 33%, and metastatic disease in 20%. All metastatic disease amongst patients with SCC or BCC was found to be SCC only.
Summary and Conclusion: Skin cancers are common following FCR chemotherapy for CLL. The clinical consequences of skin cancers in this population can be serious, and include requirement for major surgery and/or development of metastatic disease in over 50%. Clinicians should remain vigilant for the emergence of skin cancers following FCR treatment for CLL and initiate management of these at an early stage.

P865

A POPULATION BASED EXPERIENCE ON THE USE OF RCD OR RCVP IN AUTOIMMUNE HEMOLYTIC ANEMIA IN CLL

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Background: Autoimmune cytopenias occur in 5 to 10% of patients with CLL and have a tendency to recur, with each relapse being more difficult to treat. These cytopenias occur as a result of immune dysregulation and can be triggered by some chemotherapeutic agents. The diagnosis and management can be challenging, as diagnostic criteria may overlap with CLL presentation. The combination of cyclophosphamide and rituximab has been used in small series of referred patients, but its efficacy in the population-based setting is not known. CancerCare Manitoba (CCMB) is the sole agency in the province of Manitoba (population 1.2 million) providing approval and provision for rituximab therapy for cancer patients; hence its database can be used to assess the efficacy of rituximab in the population setting.
Aims: To determine the population-based experience of the rituximab containing chemotherapy regimens, RCD/R-CVP, in the management of autoimmune hemolytic anemia (AIHA) in Manitoba.
Methods: The CCMB pharmacy database was analyzed from 1st January 2003 to 31st December 2011 to identify all rituximab based regimens used in the treatment of CLL with autoimmune cytopenias. Patients with a diagnosis of AIHA, with or without immune thrombocytopenia (ITP), were chosen for analysis. These included therapy related AIHA. Isolated ITP or other cytopenias were excluded. AIHA related to CLL was defined as: hemoglobin (Hb)<110 g/L with (a) a single marker of hemolysis (unconjugated hyperbilirubinemia in the absence of liver disease, elevated LDH, reticulocytosis, increased bone marrow erythropoiesis without bleeding) and a positive DAT or cold agglutinin or (b) two markers of hemolysis without concomitant bleeding or hypersplenism. AIHA was defined as 'simple' if it was not associated with active CLL or 'complex' if associated with progressive lymphadenopathy, rapid lymphocyte doubling within 6 months, or the presence of 'B' symptoms. The regimens used were (a) RCD: rituximab 375mg/m^2 intravenously (IV) on day 1, cyclophosphamide 750 mg/m^2 IV on day 1, and dexamethasone 20mg on day 1 and 12 mg daily from day 2-7 per cycle, which was repeated every 4 weeks, until best response with 4-6 cycles planned or (b) R-CVP. Response criteria were defined as (a) complete response (CR), if Hb >120g/L and platelets >100 x10^9/L, without transfusion requirements and normalization of LDH and reticulocytes; (b) partial response (PR) if Hb rise was ≥ 20g/L and platelets within 50-100 x 10^9/L, with a decrease in transfusion needs and (c) no response (NR) if the above requirements were not met (Leuk Lymph 2011; 52: 1401-03).
Results: Twenty four patients received RCD or R-CVP in Manitoba for AIHA +/- ITP. Two patients were excluded, as data was insufficient for analysis. In the 22 patients reviewed (M:F; 1:1), the median age at diagnosis of CLL was 63 yrs (range 46-87). Median age at therapy was 75 yrs (range 50-90). AIHA was 'complex' in 77% cases. Previous treatment for CLL was observed in 91% patients and consisted of chlorambucil in 59%, and fludarabine (F) or F + cyclophosphamide (without rituximab) in 23%. RCD was used in 20 cases and R-CVP in 2 patients. Previous therapies for AIHA consisted of prednisone in 100%, cyclosporin in 27% and splenectomy in one patient. The median Hb value prior to therapy in this cohort was 93g/L (range 61-105). The median number of cycles of RCD/R-CVP given was 4 (range 1-7), with a CR in 9/22 (41%), PR in 8/22 (36%) and NR in 23%. Median time to maximum response was 7 months (range 1-24). The peak value of Hb in patients with CR/PR was 133g/L (range 109-157). Median duration of response was 15 months (range 2-48 months). Relapse occurred in 4 patients (18%). Of these, 2 were retreated with RCD and one had a response. Complications included: 2 febrile neutropenia episodes, one cellulitis and one sepsis, which recovered with antimicrobial therapy. There were no deaths attributable to treatment for AIHA.
Summary and Conclusion: Relapsing AIHA is difficult to treat in CLL. However, this population-based study confirms the effectiveness of the RCD/R-CVP regimens in these patients with an overall response rate of 77% (41% CR) and relapse-free response of 60 percent.

P866

OPPOSITE PROGNOSTIC SIGNIFICANCE OF CELLULAR AND SERUM CIRCULATING MICRORNA-150 IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: MicroRNAs (miRs) are a new class of tumor suppressors frequently deregulated in cancer. We previously reported the deregulation of microRNA-29c and 223 in Chronic Lymphocytic Leukemia (CLL) cells. CLL is characterized by a clinical heterogeneity that can be predicted by several prognostic factors. In addition, recent studies have shown that circulating microRNA in body fluids can also be used as prognostic biomarker.
Aims: Here, we would like to investigate the expression of cellular and serum circulating microRNA-150 in CLL and to correlate these expressions with prognosis.
Methods: We investigated the expression of cellular and serum circulating microRNA-150 by real-time PCR (qPCR) from CD19+ cells or from CLL serums in a cohort of 273/252 CLL patients with a median follow-up of 78 months (range, 7-380) and correlated it to other biological or clinical parameters.
Results: We showed that miR-150 was significantly overexpressed in CLL cells (3.0 fold, P=0.0009) and CLL serum (7.5 fold, P<0.0001) compared to healthy subjects. Among CLL patients, low cellular miR-150 expression levels was associated with tumor burden markers (LDT<1year, high sCD23 and high B-2M) but also disease aggressiveness: cellular miR-150 level decreased significantly with progression from Binet Stage A to C (P<0.0085). In addition, low level of cellular miR-150 is found in poor prognostic subgroups defined by IgVH mutational status (P<0.0001), ZAP70 (P=0.0004), LPL (P<0.0001), CD38 (P<0.0001) expression and cytogenetic abnormalities (P=0.0121). In contrast, a high level of serum circulating miR-150 was associated with patient requiring a treatment (P<0.0001) or patient died during the study (P<0.0001). Patients considered positive for B2M, sCD23 or with a LDT<1year also presented a significant higher level of miR-150 in the serum. Cellular and serum miR-150 were associated with treatment-free (TFS) and overall survival (OS) with an opposite manner: patients with a low cellular miR-150 expression have a median TFS of 40 months compared with high level patients who have a median TFS of 122 months (P<0.0001). Patients with a low serum miR-150 expression have a median TFS of 111 months compared with high level patients who have a median TFS of 60 months (P=0.0066). Similar results have been observed for OS. Interestingly, no correlation was found between cellular and serum circulating miR-150. In other words, patients with high level of miR-150 in the cell were not necessary patients with low level in the serum indicating that these two parameters were independent. In addition, we observed that during disease evolution, an increase of lymphocytosis is associated to a decrease of miR-150 in the cell but with an increase of miR-150 in the serum.
Summary and Conclusion: Downregulation of cellular and upregulation of serum circulating miR-150 are associated with poor clinical evolution in CLL. However these two parameters were independent. We concluded that cellular/serum miR-150 level could be used to monitor molecularly disease evolution and as a new prognostic factor in CLL.

P867

IMPROVING SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA OVER A 40-YEAR PERIOD (1971-2011): A SINGLE INSTITUTION EXPERIENCE

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Background: Whether the availability of new treatments, along with with better general care, translate into an improvement of survival of patients with chronic lymphocytic leukemia (CLL) is not completely clear.
Aims: We analysed pattern of presentation and survival in 782 patients followed from 1971 and 2011 at a single institution (Department Hematology-Oncology, Azienda Ospedaliera Pugliese-Ciaccio, Catanzaro, Italy).
Methods: For this purpose the whole cohort was split into two groups reflecting the time of diagnosis: the period 1971-1994 (n=386) and in the period 1995-2011 (n=402).
Results: Distribution by age groups showed a shift toward an older age at diagnosis in more recent cohort (median age, 69 *vs* 68 years; P=0.02), with a significant increase of patients older than 70 years (50% *vs* 40.9%; P=0.01). Moreover, the proportion of patients in low-risk clinical stage (Binet A) at diagnosis was significantly higher from 1995-2011 than in the previous period (73.1% *vs* 52.9%:P<0.0001). After a median follow-up time of 4 years (range, 0.1-23.3 years), 315 (40.3%) of the 782 assessable patients have died. Of those censored alive, 385 were still alive and 85 (10.5%) were lost to follow-up. Median survival for the whole series was 7.6 years from diagnosis (95% CI:

7.0-8.3 years) and 15% of patients were projected to survive 16 years or longer. Patients' stratification according to the Binet staging system allowed identification of different clinical outcome (P<0.0001). Furthermore, the impact of CLL on the patients' life expectancy was clear after adjustment for age, sex and year of diagnosis. Indeed survival of patients at 5,10 and 15 years from diagnosis was 85%,64% and 55% of that expected in the general population. A direct comparison of overall survival of patients diagnosed in the period 1971-1994 and 1995-2011 revealed an increase of 10-year survival from 30% to 60% (P<0.0001)(see figure below). This improvement was observed in patients younger (P<0.0001) and older (P<0.0001) than 70 years, as well as in Binet stage A (P<0.0001) and in Binet stage B or C (P<0.0001) patients.

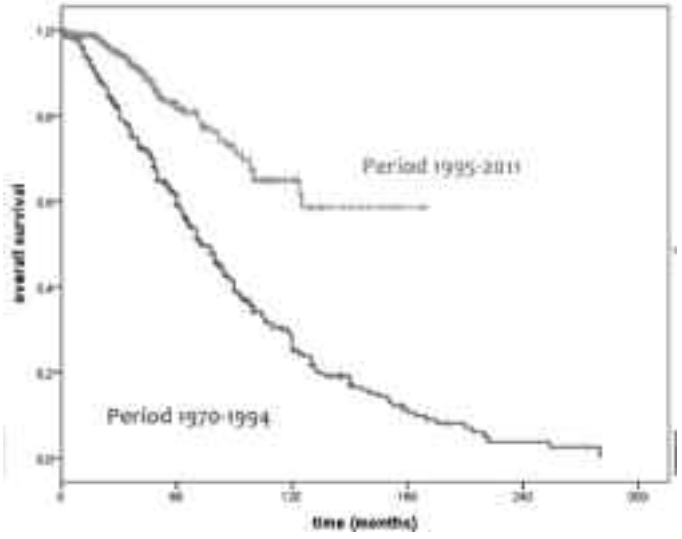

Figure 1.

Summary and Conclusion: In conclusion, this study based on a single center patient cohort demonstrates that survival in CLL is steadily improving. These results suggest also that newer treatment are changing the prognosis of CLL patients with active disease. The greater attention to regular check-ups in the last decades contributes to the survival improvements observed in Binet stage A patients who generally die because of non –CLL related causes (*i.e.*, cardiovascular diseases, second neoplasms).

P868

REDUCTION OF TUMOR LYSIS SYNDROME (TLS) RISK IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS TREATED WITH ABT-199 (GDC-0199): RESULTS OF MODIFICATIONS TO DOSING SCHEDULE AND TLS PROPHYLAXIS

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Background: CLL cells have been described as "primed for death" due to over-expression of BCL-2 and abundance of BCL-2:BIM complexes. ABT-199 is a selective, potent, orally bioavailable small molecule BCL-2 inhibitor being studied as a single agent and in combination in CLL and other B-cell malignancies. In early clinical studies in patients (pts) with relapsed/refractory CLL, events of TLS were observed, including 2 deaths and one event of acute renal failure, and adjustments to scheduling were required.
Aims: A detailed analysis was performed to identify pre-treatment risk factors for TLS, and to assess the impact of the revised ABT-199 scheduling on TLS incidence.
Methods: Data from 135 CLL pts treated with ABT-199 in 4 studies were analyzed, including all pts treated before the introduction of revised scheduling in April 2013 (n=77), and the first 58 pts treated subsequently. Pts with TLS were identified using Cairo-Bishop criteria, and medically adjudicated according to a pre-specified TLS case definition. Predictive modeling and CART analyses, PK, and clinical data were used to categorize risk and to revise dosing regimen and TLS risk mitigation measures.
Results: Among the initial 77 pts, 19 (24.7%) were identified as having had TLS: 3 clinical TLS (2 deaths and 1 acute renal failure), 16 laboratory TLS (LTLS, as designated by investigators or by medical adjudication), 9 with related adverse events and 7 with no intervention and no AE reported. TLS first manifested within 24 hrs of the 1st dose in 14 pts, 4 at 24 – 48 hrs, and 1 TLS related AE reported after a dose increase. Bulky abdominal nodes ≥ 5 cm, ALC ≥ 38k, and CrCl<80 were identified as individual risk factors for developing LTLS, but a combination of **both** lymph nodes (LN) ≥ 5 cm and ALC ≥ 38k identified all pts with clinically relevant LTLS. Using LN size and ALC (>25K to be consistent with historical cut-off) pts were classified into 3 risk groups: low risk - ALC<25k and all LN<5 cm; medium risk - ALC ≥ 25k **or** LN ≥ 5 cm but<10 cm; high risk - ALC ≥ 25k **and** LN ≥ 5 cm but<10 cm or any pt with LN ≥10 cm. Of the 19 pts with TLS, 0%, 32%, and 68% (including 3 pts with clinical TLS) were in each respective risk group. In addition, ABT-199 median C_{max} and AUC were higher among the subjects experiencing TLS. These findings led to changes to ongoing protocols. As the TLS risk was largely restricted to the first dose, changes to the dosing regimen included a reduction in the starting dose from 50 to 20 mg. Also, a change from a 3-step to a more gradual, 5-step dose ramp-up (20, 50, 100, 200, final dose) was implemented and the maximum dose was limited to 600 mg. Hospitalization to monitor and collect laboratory data was mandated at the 20 mg and 50 mg doses for all pts and at subsequent dose increases for pts in the high risk group. Detailed guidance on interventions for specific electrolyte changes and a pt management checklist were provided. As of Dec 20, 2013, 58 pts had been treated using the new monitoring and dosing schedule. No events of clinical TLS were reported and 8 pts were identified as meeting Cairo-Bishop criteria for LTLS (13.8%). Three of these pts had AEs reported; all were grade 1 and managed with rapid resolution. In general, lab changes were primarily elevations of phosphate/decreases in calcium, not potassium elevations.
Summary and Conclusion: Changes implemented in the ABT-199 dosing schedule and pt monitoring reduce the risk of clinically relevant TLS. Optimization of monitoring and prophylaxis measures is currently ongoing.

P869

OPEN LABEL MULTICENTER STUDY OF ELTROMBOPAG FOR THE TREATMENT OF IMMUNE THROMBOCYTOPENIA SECONDARY TO LYMPHOPROLIFERATIVE DISORDERS

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Background: Immune ThrombocytoPenia (ITP) secondary to Lymphoproliferative Disorders (LPDs) is a condition poorly responsive to conventional treatments [*i.e.* Intravenous immunoglobulin (IVIG), steroids] with an overall response rate (ORR) generally lower than in primary ITP, and usually less than 50%. Therefore, new treatment strategies are needed. Eltrombopag, a thrombopoietin mimetic effective in primary ITP, might represent a new treatment sparing these severely immunocompromised patients from inappropriate cytotoxic therapies or steroids, not otherwise demanded by the underlying disease.
Aims: The aim of this phase 2, single arm, open-label, prospective, multicenter, safety/efficacy study was to assess the proportion of responders to eltrombopag and its safety profile in patients with LPDs.
Methods: To be included patients had to be diagnosed with ITP following predefined criteria (Visco et al, Blood 2008), do not necessitate any cytotoxic treatment for the following 6 months, have platelet count less than 30 x 10^9/L, or between 30 and 50 x 10^9/L in case of bleeding manifestations. Subjects initiated study medication at an oral dose of 50 mg/day, with dose adjustments allowed every 2 weeks (+/- 25 mg) for toxicity, non-response or platelet count below 60 x 10^9/L or over 400 x 10^9/L. Maximum allowed dose was 150 mg/day. Response was defined according to International Working Group definitions (Rodeghiero et al, Blood 2009). A total of 18 patients are scheduled to be enrolled with the optimal two-stage design. Eltrombopag was kindly provided by GlaxoSmithKline, Italy.
Results: Between the 1st of october 2012 and the 31st of december 2013, 10 patients have been enrolled by 6 centers. Informed consent was obtained by all patients. Median age was 63 years (43-80), five were females, 9 had chronic lymphocytic leukemia (CLL), and one had Hodgkin's lymphoma in remission status. Median time from LPD diagnosis and start of eltrombopag was 57 months (2-152). Four of the 10 patients had received cytotoxic treatments directed to the LPD, at least 14 months before enrollment. At the time of study entry, all patients had received previous treatments for ITP (all had steroids, 7 had IVIG, 5 had previous rituximab, 3 had cyclosporine, 2 had azathioprine). Last treatment for ITP had been delivered at least 2 months before study entry, apart from steroids. Steroids were maintained by the treating physician at a stable dose despite platelet count below 30 x 10^9/L with tapering within 2 weeks of eltrombopag initiation. Median time from first diagnosis of ITP and start of study medication was 7 months (1-77), with 7 of the 10 patients having chronic ITP. All patients except one were refractory to steroids. Median lymphocyte count at study entry for patients with CLL was 7.2 x 10^9/L (3.4-43.4), and median platelet number was 18 x 10^9/L (7-28). Overall, eltrombopag was well tolerated. The study drug was administered for a median time of 5.8 months (1.3-9), with

6 of the 10 patients still on treatment. One patient had mild itching while assuming the drug that was well controlled with symptomatics. No ≥ grade 2 adverse events were reported. No significant increase of bone marrow reticulin was documented in patients treated for more than 24 weeks. All patients were evaluable for early response (measured after 4 weeks of eltrombopag), while 6 patients were evaluable for response after 24 weeks. The ORR was 80% at 4 weeks, with 60% achieving complete remission (CR), at a median eltrombopag dose of 50 mg. The ORR after 24 weeks was 60% (all CR) with a median dose of 50 mg. The line chart of average response in terms of median platelet number ± interquartile range (IQR) is shown in Figure 1.

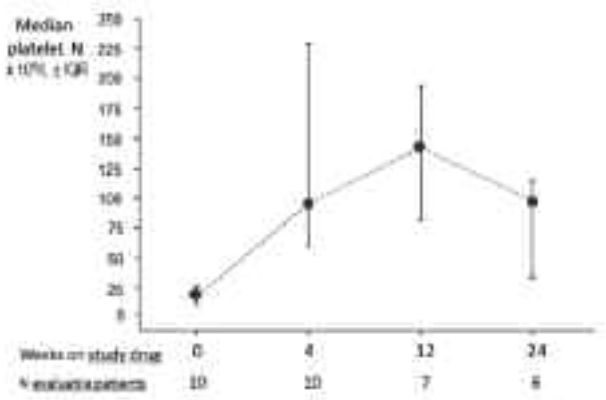

Figure 1.

Summary and Conclusion: This preliminary data show that eltrombopag is active and well tolerated in ITP secondary to LPDs. This trial was registered at ClinicalTrials.gov Identifier: NCT01610180.

P870

IS ZAP-70 STILL A KEY PROGNOSTIC FACTOR IN EARLY STAGE CHRONIC LYMPHOCYTIC LEUKEMIA? RESULTS OF THE ANALYSIS FROM A PROSPECTIVE MULTICENTER OBSERVATIONAL STUDY

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Background: The most popular markers in chronic lymphocytic leukemia (CLL) focus on the identification of aggressive disease and include CD38, *IGHV* mutational status and ZAP-70; however evaluation of the latter is often inconsistent due to lack of standardization consensus.
Aims: We investigated i) the most accurate methods of identifying ZAP-70 expression levels; ii) the predictive power of ZAP-70 for *IGHV* mutational status; iii) the ability to predict progression free survival (PFS); and iv) novel gene markers which may surrogate *IGHV* mutational status.
Methods: A cohort of 487 newly diagnosed Binet stage A patients were prospectively enrolled (clinicaltrial.gov ID: NCT00917540). ZAP-70 expression was evaluated using: 1) Western blotting, 2) flow cytometry (FC), using an isotype-matched antibody as negative control (ZAP-70 I/C) or ZAP-70 expression in normal T cells (ZAP-70 T) as an internal positive control. ZAP-70 expression was also determined as MFI ratio between T- and B-cells (ZAP-70 T/B). Highly-purified B-cells were employed for GEP analysis (GeneChip® Gene 1.0 ST Array, Affymetrix Inc., Santa Clara, CA).
Results: Cases were randomly split into CLL-Training and CLL-Validation cohorts. ZAP-70 WB strong, ZAP-70 I/C 40%, ZAP-70 T 40% and ZAP-70 T/B of 1.5 significantly predicted PFS in both the training and validation sets. In a Cox multivariate analysis, in which ZAP-70 WB strong, ZAP-70 I/C 40%, ZAP-70 T 40% and ZAP-70 T/B 1.5 were forced, the latter remained the sole variable maintaining an independent association with PFS. In Cox multivariate analysis, when all ZAP-70 procedures were forced together with *IGHV* mutational status, ZAP-70 T/B 1.5 lost its predictive power, while *IGHV* mutational status maintained an independent association with PFS. Three-year re-assessment of ZAP-70 levels showed that ZAP-70 expression differed in 26%>50% of patients. Finally, we performed an independent supervised analysis by *IGHV* mutational status in CLL-Training (102 cases) and CLL-Validation (114 cases) sets taking into consideration all those genes showing a fold change superior or equal to that of *ZAP70*. Thirty-one genes (23 up-and 8 down-regulated) and 23 genes (18 up- and 5 down-regulated) showed a fold change superior or equal to that of *ZAP70* in CLL-Training and CLL–Validation sets, respectively; notably, 20 common genes (15 up and 5 down) overall were found to be differentially regulated in the 2 cohorts analysed. Two (*SNORA70F* and *NRIP1*) of the 5 down-modulated and 6 (*SEPT10*, *ZNF667*, *TGFBR3*, *MBOAT1*, *LPL* and *CRY1*) of the 15 up-modulated genes were found to be significantly associated with a reduced risk of disease progression in both training and validation sets. Notably, when all of the above mentioned genes were forced in a Cox multivariate model together with *IGHV* mutational status, only CRY1 (H.R. 2.3, 95% C.I. 1.1-4.9, P=.027) and MBOAT1 (H.R. 2.1, 95% C.I. 1.1-3.7, P=.018) maintained their independent prognostic impact, while *IGHV* and *SNORA70F* lost their prognostic power, supporting the hypothesis that these two genes could surrogate the predictive value of *IGHV* mutational status.
Summary and Conclusion: Regardless of the detection method adopted, the independent predictive power of ZAP-70 expression for progression in early-stage CLL remained unconfirmed. Moreover, ZAP-70 expression proved relatively unstable over time. Novel gene markers, *i.e.* CRY1 and MBOAT1, may surrogate IGHV mutational status and be involved in natural progression of CLL.

P871

EVALUATION OF IMMUNE RESPONSE TO 13-VALENT PNEUMOCOCCAL CONJUGATED VACCINE (PCV13) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder associated with severe impairment of the immune system in a substantial proportion of patients. This is directly linked with an increase in susceptibility to bacterial and viral infections. About 50 to 80% of patients diagnosed with CLL die from infectious complications. Most infections in CLL patients is caused by capsular bacteria: Streptococcus pneumoniae and Haemophilus influenzae. Patients with CLL who have low levels of antipneumococcal antibody are particularly at risk for severe and recurrent pneumococcal infections. In the U.S. and in many EU countries, vaccinations against Streptococcus pneumoniae are recommended for immunocompromised patients, such as patients with CLL. For many years, 23-valent pneumococcal polysaccharide vaccine (PPV23) was used. Antibody responses to PPV23 vaccine are inadequate in most patients with CLL, that induced response only in about 20-25 % of patients. Since 2012, in the prevention of pneumoccocal infections in immunocompromised adults, 13-valent pneumococcal conjugate vaccine (PCV13) has been used that efficacy in patients with CLL has not yet been studied.
Aims: The aim of this study was to assess the efficacy of vaccination in patients with CLL using PCV13.
Methods: The study included 24 previously untreated patients with CLL in stage 0 - 2 according to Rai calssification and 15 healthy subjects as a control group. The percentage of plasma cells, defined as CD19+/++IgD/CD27, was analysed before vaccination and 7 days after the immunization, the level of specific anti-pneumococcal antibodies and the level of IgG and IgG1, IgG2, IgG3, IgG4 immunoglobulin subclasses were evaluated prior to vaccination and 4 weeks after vaccination.
Results: The positive response to vaccination was defined as at least a two-fold increase in specific anti-pneumococcal (anticapsular) antibody titers as compared to the titer prior to the vaccination. Such a criterion of response was fulfilled in 100% of healthy subjects and in 58.3% of the patients with CLL. The percentage of plasma cells after vaccination was significantly lower ($p<0.0001$) in patients with CLL comparing to the control group. Both in patients with CLL as well as healthy subjects, there was a statistically significant increase in the level of IgG2 subclass after vaccination ($p=0.0301$). The patients with adequate antibody response to PCV13 had significantly less advanced stages of CLL, higher total IgG levels and IgG2 and IgG4 subclass levels. There was no no significant vaccine-related reactions, no increase in peripheral blood lymphocyte count and no changes in laboratory markers of disease activity.
Summary and Conclusion: Protective immunization of patients with CLL using the PCV13 is safe and induces an effective immune response in a large proportion of patients. To achieve the optimal postvaccinal response it is recommended to the use the PCV13 as early as possible after the diagnosis of CLL with determination of post-vaccination antibody levels.

P872

INITIAL CHARACTERISTICS, TREATMENT AND PROGNOSIS ACCORDING TO AGE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A SINGLE-CENTER ANALYSIS BASED ON 949 PATIENTS

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Background: There is little information about the characteristics, treatment, and prognosis of large series of patients with CLL according their age at diagnosis.
Aims: To investigate clinico-biological features, outcomes, and prognosis in patients with CLL according to their age.
Methods: 949 patients diagnosed with CLL between 1990 and 2012 in a single center according to IWCLL criteria were categorized by age at diagnosis into four groups:<59 years (n=345), 60-69years (n=237), 70-79 years (n=234) and ≥ 80 years (n=133). Disease-attributable mortality was calculated using the *relsurv* package (R software environment). Several models (additive, multiplicative and transformed) were tested as appropriate and their goodness-of-fit estimated using Brownian bridge statistics.
Results: Baseline characteristics of patients are shown in the Table. Median follow-up time was 7.8 years (0.1-23.2) for the overall series. In contrast to younger patients, both sexes were equally distributed within the elderly (p<.05). While younger patients presented more often with intermediate clinical stage (Binet B, Rai I/II; p<.05) and higher absolute blood lymphocyte counts (p<.05), patients above 80 years were more likely to be diagnosed in either initial (Binet A, Rai 0; p<.05) or advanced disease (Binet C, Rai III-IV; p<.05), lower Hb levels (p<.05) and higher B2M (p<.05). No biological differences (FISH cytogenetics, *IGHV*, ZAP-70, CD38, *NOTCH1, SF3B1*) were found among different groups of age. Elderly patients were treated later than younger ones (median TTFT 3.1 *vs*. 7.0 *vs*. 8.4 years *vs*. not reached; p<.05). In addition, treatment given changed significantly with increasing age: alkylating agents (32% *vs*. 45% *vs*. 69% *vs*. 72%), purine analogs (35% *vs*. 25% *vs*. 12% *vs*. 5%), chemoimmunotherapy (27% *vs*. 20% *vs*. 8% *vs*. 0%), other (72% *vs*. 5% *vs*. 0% *vs*. 23%) (all p<.05). Consequently, response rates decreased with rising age (72% ORR with 32% CR *vs*. 63% ORR with 29% CR *vs*. 55% ORR with 16% CR *vs*. 35% ORR with 12% CR; p<.05). Finally, actuarial overall survival (OS) was shorter in older patients (15.7 *vs*. 11.9 *vs*. 7.9 *vs*. 4.3 years; p<.05), but, of note, disease-specific mortality was not significantly different across age groups (27% *vs*. 25% *vs*. 23% *vs*. 19% at 8 years; p=.26).

Table 1.

	<59 years (n=345)	60-69 years (n=237)	70-79 years (n=234)	≥80 years (n=133)	p-value
Sex, male (%)	225 (65)	140 (59)	120 (51)	70 (53)	.004
Creatinine clear (mL/min)	79 [34-136]	77 [44-123]	69 [19-120]	64 [26-120]	<.001
Binet (%) A	253/335 (75)	191/231 (84)	190/227 (84)	108/131 (82)	<.001
B	66/335 (20)	28/231 (12)	20/227 (9)	5/131 (4)	
C	16/335 (5)	10/231 (4)	17/227 (8)	18/131 (14)	
B2M mg/ml	2.34±1.53	2.51±1.35	3.24±1.99	3.78±1.94	<.001
ZAP-70 high (%)	102/256 (40)	59/178 (33)	52/165 (32)	16/62 (26)	NS
IGHV/UM (%)	118/231 (51)	56/144 (39)	51/117 (44)	17/40 (43)	NS
FISH (%)					
13q-	80/252 (32)	67/175 (38)	61/157 (39)	24/57 (42)	NS
Normal	69/252 (27)	52/175 (30)	45/157 (29)	17/57 (30)	
+12	35/252 (14)	23/175 (13)	24/157 (15)	5/57 (9)	
11q-	29/252 (12)	11/175 (6)	11/157 (7)	4/57 (7)	
17p-	39/252 (15)	17/175 (10)	16/157 (10)	7/57 (12)	

Summary and Conclusion: While younger patients tended to be diagnosed in intermediate stages (stage B and I/II) and with higher blood lymphocyte counts, older patients were diagnosed at more advanced phases of the disease. However, no significant differences in the distribution of biological risk markers according to patients' age were found and no differences in disease-specific mortality were observed.

P873

IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA A HIGH NUMBER OF CELLS WITH TRISOMY 12 IS ASSOCIATED WITH A POOR OUTCOME. ANALYSIS OF DATABASE OF SPANISH GROUPS OF CYTOGENETICS (GCECGH) AND CLL (GELLC)

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Background: Cytogenetic abnormalities in chronic lymphocytic leukemia (CLL) establish subgroups of patients with different prognosis and survival. In addition, CLL patients with a high number of losses in 11q or 13q have a worse prognosis. Trisomy 12 (+12) have been classically considered as harboring an intermediate prognosis. However, their exact clinical characteristics and outcome along with additional prognostic factors have not been ascertained in large series.
Aims: To analyze in a multicentric study whether the number of +12 lymphocytes in patients with CLL has an influence in overall survival (OS) and time to first therapy (TFT).
Methods: A total of 2,561 patients registered in DataBase of CLL of GCECGH and GELLC were included. Clinical and biological data, FISH information (11q, 12, 13q and 17p probes) and follow-up were recorded.
Results: 355 patients (13.9%) presented the +12. The final analysis was limited to 289 cases (178 males) after excluding patients with monoclonal B-cell lymphocytosis, those that had +12 as clonal evolution or cases with inadequate follow-up. Most of patients (71%) were in Binet's clinical stage A. At the time of the analysis, 22% of the patients had dead and 53 % had progressed. Median OS of patients with +12 was 129 months (CI95%, 100-148) and TFT was 66 months (CI95%, 55-76). A total of 174 patients (60.2 %) presented the +12 in<60% of cells. Interestingly, median OS was 159 months (CI95%, 119-182), while it was of 96 months (CI95%, 58-134) for those with +12 ≥60% of cells (P=0.015) (Figure). In addition, advanced Binet's clinical stage (P<0.0001), lymphadenopathy (P=0.001), splenomegaly (P=0.001), lymphocyte count >30x10^9/L (P=0.04), high serum LDH (P=0.009), high $\beta_2$microglobulin levels (P<0.0001) and expression of CD38 (P=0.04) were associated with a short OS. In the multivariate analysis, only Binet's stage (P=0.002) and high serum $\beta_2$microglobulin (P=0.02) resulted significant in predicting OS. Regarding TFT, in patients with<60% +12, the median TFT was 72 months (CI95%, 58-86) *vs* 57 months in cases with ≥60% (P=0.007) (Figure). Other significant variables for TFT were Binet's clinical stage (P<0.0001), B symptoms (P=0.02), lymphadenopathy (P=0.02), splenomegaly (P=0.001), high lymphocyte count (>30x10^9/L) (P<0.0001) and high LDH (P=0.007). In the multivariate analysis, only clinical stage (P=0.01) and a number of +12 in ≥60% of cells (P=0.013; HR: 1.58; CI95%, 1,2-2,3) resulted of significance in predicting TFT.

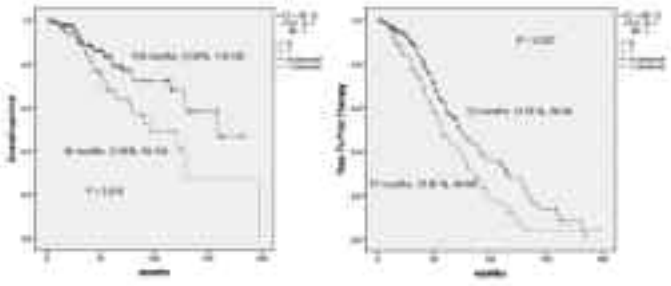

Figure 1.

Summary and Conclusion: In patients with CLL, a high percentage of cells with +12 is associated with a shorter TFT and OS. FIS: PI 12/00281, FEHH 2013-2014 and 'Junta de Castilla y León' (MH).

P874

COMPARATIVE EFFECT OF BENDAMUSTINE PLUS RITUXIMAB (R) *vs* FLUDARABINE PLUS CYCLOPHOSPHAMIDE PLUS R ON THE LEVELS OF PERIPHERAL BLOOD LEUCOCYTE POPULATIONS IN ADVANCED-STAGE CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Immunochemotherapy may improve the life expectancy of chronic lymphocytic leukemia (CLL) patients and has proven to be more efficient than chemotherapy alone in depleting malignant cells. Despite this, little is known about its precise immunomodulatory effects and immune toxicity *vs* standard regimens.

Aims: To evaluate the effects of bendamustine *plus* rituximab (BR) *vs* fludarabine *plus* cyclophosphamide *plus* rituximab (FCR) on the distribution of pathological and normal leucocyte subsets in peripheral blood (PB) from advanced-stage CLL patients.

Methods: PB neoplastic and normal immune cell subsets were analyzed in 97 CLL patients (Binet B/C) before treatment; from these, 72 patients were treated with BR and 25 with FCR. Leukocyte subsets were identified (prior therapy: M0; after 1 course: M1; and at 3 months after treatment: M2) by flow cytometry, with antibodies against CD3, CD4, CD5, CD8, TCRgd, CD19, CD20, CD27, CD38, CD45, CD56, sIgM, sIgA, sIgG, sIgl and sIgk.

Results: At M0, no clinical differences (p>0.05) were found between patients who were later treated with BR *vs* FCR, although a fewer patients treated with BR had not been previously treated *vs* the FCR group (50% *vs* 80%; p>0.05). Also, the absolute count of PB malignant cells was lower in BR *vs* FCR group at M0 (59,953±70,168 *vs* 88,500±79,297 cells/uL, p<0.05); as expected, in each group, a reduction (p<0.05) of all PB leukocyte subsets was achieved at M2, in comparison to basal levels from the corresponding group. Upon comparing the efficacy of each protocol in depleting pathological cells after treatment, the number of tumor B cells was lower in FCR *vs* BR patients (175±630 *vs* 1,541±6,740; p=0.006); actually, in comparison to M0 levels, a much higher rate of reduction of clonal cells was seen with FCR than with BR (500-fold of reduction *vs* 40-fold). This finding was consistent with the fact that patients treated with FCR had a higher rate of cases with minimal residual disease<0.01% at M2 *vs* BR-patients (61% *vs* 29%; p=0.01), although the complete remission rate was similar (40% *vs* 41%, p>0.05). Differences were also found between both groups as regards the absolute number of PB normal leukocyte subsets (Fig1), mainly after the first course. In particular, FCR-treated patients had higher counts of normal B-cells at M1 than those treated with BR, being the differences statistically significant (p≤0.05) for naïve, IgG memory B cells and total plasma cells (Fig1). In addition, dendritic and NK cells were significantly increased at M1 in patients treated with FCR. However, after finishing treatment (M2), the degree of reduction of normal PB leukocyte subsets was in general more intense than at M1, but without statistically significant differences between the two groups of patients, with the exception of circulating plasma cells and monocytes, found to be significantly lower among FCR patients (Fig1). However, the reduction rate at M2 was more profound with FCR *vs* BR, particularly for normal total B cells (>1000-fold *vs* 12-fold reduction) and CD4+ T cells (>12-fold *vs* 6-fold reduction).

Summary and Conclusion: Despite all PB leucocyte subsets are affected by BR and FCR, both pathological and normal B cells (as well as CD4+ T cells) are more efficiently depleted by FCR, although this effect is reached slower in comparison to BR. Further studies will offer more accurate insights into the biology of cell recovery and the clinical impact of neoplastic/normal B-cell depletion during and after therapy in CLL.

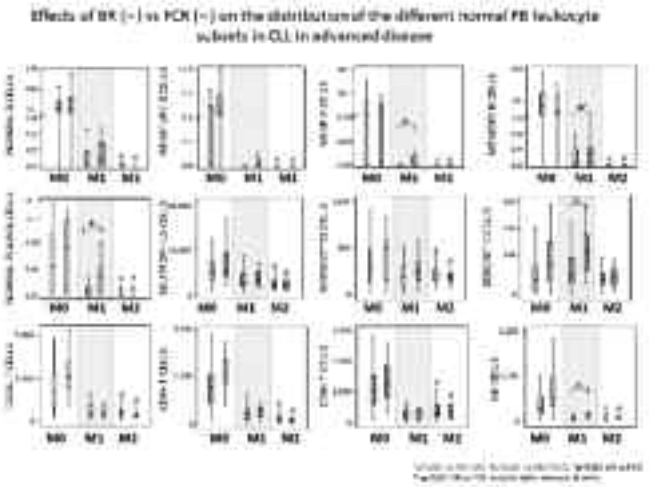

Figure 1.

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EFFICACY AND SAFETY OF BENDAMUSTINE IN COMBINATION WITH RITUXIMAB FOR ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. AN ITALIAN RETROSPECTIVE MULTICENTER STUDY

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Background: The front-line therapy for CLL young patients is chemo-immunotherapy with Fludarabine-Cyclophosphamide and Rituximab (FCR). Recently the German CLL study group reported an interim analysis of CLL10 trial, who compared FCR *vs* BR. Both treatment showed high response rate, no firm recommendation of one regimen over the other can be given at the present time regarding the first line use in CLL patients with good physical fitness. FCR regimen can result in a significant myelosuppression and a high rate of early and late infections, suggesting that it may be too toxic and therefore unsuitable for this large subpopulation of patients. However, many CLL patients are elderly and with comorbidities. Bendamustine (B) in association with Rituximab (RTX) (Benda-R) have shown to be efficacious and safe as frontline therapy in elderly and or unfit patients. Insufficient data are available in patients older than 75 years regarding the efficacy and safety, nevertheless more incidence of extra-hematological toxicity was noted in this subgroup.

Aims: We report our multicentre retrospective study focusing on responses and toxicities rate in elderly patients with CLL.

Methods: We report data of 66 patients with age ≥ 65 years previously untreated CLL observed in 12 Italian centres from November 2000 to December 2013. All patients were assigned to receive 6 courses of Bendamustine (90 mg/sm for 2 consecutive days) and RTX (375 mg/sm for the first course and 500 mg/sm for subsequent cycles) every 28 days. The primary end points were the ORR (complete response CR and partial response PR) and haematological or extrahematological toxicities. Forty-three male and twenty-three female with a median age of 72 years (range, 65-87 years) were included in the study. Six patients were unfit with a CIRS score of 7 or more. All patients had ECOG less than 2. Eleven patients were Binet A, 28 Binet B and 27 Binet C stage. The median lymphocytes count at diagnosis was 33.203/mmc (range, 2.200-140.000). Fish analysis was performed in 50/66 patients: 35 patients showed normal karyotype, del13q14 or +12, 13 pts del11qand 2 pts del17p. The analysis of the IGHV status, available in 46 patients, showed 24 patients with somatic mutation and 22 patients with germ-line sequences.

Results: A mean number of 5.46 courses of BR was given and the Bendamustine dose was reduced by more than 10% in 36 patients (54.5%). The ORR rate was 86.3%: twenty-one patients (31.8%) obtained a complete response and thirty-six patients (54.5%) obtained a partial response. Gender, age (65-69, 70-74, >75

years), Binet stage, fitness status, lymphocyte counts, del11, IGHV status did not show impact on response and time dependent variables. Only the presence of del17 had an impact in terms of response to therapy (p=0.023) and progression free survival (p<0.001). Grade III/IV hematological toxicity was recorded in 24 patients (36.4%). Extra-hematological toxicity grade I-III (skin reactions, gastrointestinal and cardiovascular symptoms, infusion related reactions, etc). was noticed in 33 patients. Nine patients were admitted to the hospital. Progression Free Survival was 79% at 2 years, Time To Retreatment was 90.3% at 2 years and Overall Survival was 89.6% at 2 years.
Summary and Conclusion: Retrospective data from this group of elderly CLL patients indicate that Benda-R front-line at standard dose provides a high response rate with a good safety profile, even if more than 50% of patients experienced a Bendamustine dose reduction until 70 mg/sm. Even in this cohort of patients we confirmed del17 as a bad prognostic parameter.

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MANAGEMENT OF RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS PREVIOUSLY TREATED AND RE-TREATED WITH RITUXIMAB IN DAILY PRACTICE: INTERIM RESULTS OF THE PERLE STUDY (ML 25664)

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Background: Despite recent therapeutic advances, chronic lymphocytic leukemia (CLL) remains an incurable disease and patients (pt) experience multiple relapses. CLL pt are generally ≥ 70 and have comorbidities. Rituximab (R) is approved in relapsed CLL in combination with chemotherapy based on the REACH study results which was conducted in young pt (median age 62 years) and combined with the standard of care FC-R (Fludarabine-Cyclophosphamide-Rituximab) only. The benefit of FC-R in old pt is still debated, and efficacy and safety of other R-based regimens in pt previously treated with R is not clearly defined.
Aims: The aim of this French non-interventional study is to describe the management in daily practice of relapsed/refractory CLL pt previously treated with rituximab in 1st or 2nd line and retreated with a R based regimen.
Methods: In this prospective, multicenter, longitudinal, national, non-interventional ongoing study, 320 pt with relapse/refractory CLL, treated again with a rituximab-based regimen are planned to be recruited.
The primary objective is to describe the different chemotherapy regimens combined with rituximab. Secondary objectives include efficacy (overall response rate, complete response rate, partial response rate, progression free survival, time to next treatment, overall survival); description of rituximab use (doses, number of cycles, etc.), chemotherapy regimens combined with R by subgroup (refractory pt, elderly pt ≥ 70 years old, 17p and 11q deletions) and safety. Pt will be followed-up for up to 2 years. In the current analysis we focused on the induction treatment data of the first 200 pt included.
Results: From April 2011 to March 2013, 200 pt have been included and 192 were evaluable in the interim analysis for the primary objective. Median age is 72 years [35-89], and 56% are ≥ 70 years old. Seventy-one percent of the pt are men. Twenty four (13%) pt have 17p deletion and 28 (15%) pt have 11q deletions. A hundred and six (55%) pt are in first relapse, 86 (45%) in second relapse and 12 (6%) pt are refractory. Subgroup analyses were not done due to the limited number of pt in each subgroup. The median time between diagnosis and 1st treatment is 2 years, and is not different whether pt are in 1st or 2nd relapse. The main treatments administered are as follows: 38 pt (21%) received R + purine based regimen, among them 29 received FCR regimen, 101 pt (56%) R-Bendamustine, and 34 pt (19%) R+alkylating agent including 13 treated with R-Chlorambucil (R-Chl). Response is based on investigators assessment. The ORR is 75%, 90% in R-FC pt 75% in R-Bendamustine pt and 69% in R-Chl pt. Twenty two pt (11%) have stable disease, and 26 (14%) have progressive disease during induction treatment. Most frequent adverse events (all grades) are hematologic (24%) including neutropenia (11%), and infectious (13%). AEs leading to treatment discontinuation occurred in 16 pt (8%), and 14 pt (7%) died during induction treatment, 8 due to disease progression and 6 due to AE (2 aspergillosis, 1 progressive multifocal leukoencephalopathy, 1 septic shock, 1 pneumonia, and 1 anuria).
Summary and Conclusion: In this non interventional study, interim results show a median age of 72 years which is consistent with the median age of CLL pt. The main induction treatment prescribed is R-Bendamustine for 56% of pt. The ORR is higher when assessed using centers usual practices. The study is still ongoing, further data will be available on induction treatments and to assess PFS, OS and safety.

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ANALYSIS OF MERKEL CELL POLYOMAVIRUS PROGNOSTIC SIGNIFICANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Merkel Cell polyomavirus (MCPyV), a ubiquitous DNA tumor virus, has been found to be associated with Merkel cell carcinoma and chronic lymphocytic leukaemia (CLL). Previous studies have reported conflicting results on the frequency and potential pathogenetic role of MCPyV in CLL. Moreover, the prognostic significance of MCPyV is unknown in CLL.
Aims: The aim of this study was to evaluate the association of MCPyV with CLL and to investigate the occurrence of MCPyV infection in relation to the course of CLL.
Methods: DNA CLL samples consecutively obtained from CLL patients (n=119) before treatment was tested for MCPyV using both quantitative real-time polymerase chain reaction analysis and next-generation sequencing. Furthermore, 21 patients were tested repeatedly after CLL therapy. Therefore, 140 samples were tested in total. Only samples being positive by both methods were considered to really be positive for MCPyV. The MCPyV have been compared with classical and biological prognostic CLL factors.
Results: We found that 13/119 CLL cases (11%) were positive for MCPyV. Between the groups of MCPyV-positive and -negative patients, there was no significant difference in the sex (5 positive females from 49; 8 positive males from 70); age (median age 60.5 years for positive and 60 years for negative patients); cytogenetics (unfavorable cytogenetics (del 17p or 11q) in 38% of positive patients and 24% of negative patients); presence of p53 defect (23% of positive patients *vs*. 20% of negative patients); *IGHV* mutational status (unmutated in 69% of positive patients and 76% of negative patients). Ad baseline, advanced Rai stage (III and IV) was found more frequently in MCPyV negative patients (15% *vs*. 41%; P=0.04). Therapy (FCR regimen or alemtuzumab) was also initiated more frequently in the negative group (46% *vs*. 68%; P=NS). There was no difference in overall response rate (86% in positive patients *vs*. 80% in negative patients), median progression-free survival (20 months in MCPyV negative *vs*. 24 months in MCPyV positive patients), and overall survival (not reached) between both groups. One CLL patient positive for MCPyV also developed Merkel cell carcinoma. Interestingly, in six initially MCPyV positive patients repeatedly tested, the virus was undetectable after treatment in four of them. We did not observe any new positivity after treatment in initially MCPyV negative patients.
Summary and Conclusion: This study provides the first analysis of the prognostic role of MCPyV in CLL. The occurrence with MCPyV seems to be a relatively rare event during the natural history of CLL. MCPyV is also unlikely to influence the outcome of CLL patients and MCPyV positivity may often disappear after CLL therapy. Supported in part by Research Grants MSMT CR CZ.1.05/1.1.00/02.0068 (CEITEC), MSMT CR CZ.1.07/2.3.00/20.0045 (SuPReMMe), IGA NT13493/2012, VaVpI CZ.1.05/2.1.00/01.0030; by the European Commission under the Health Theme of the 7th Framework Programme for Research and Technological Development GA 306242; and by the Czech Leukemia Study Group – for Life.

P878

A PATHOLOGICAL ABDOMINAL ULTRASONOGRAPHY AT DIAGNOSIS OF CLL SIGNIFICANTLY REDUCES THE TIME TO PROGRESSION IN PATIENTS WITH RAI 0 OR 1 DISEASE

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Background: The most common staging systems for CLL were developed in 1975 and 1981 and rely on standard blood cell count and the presence of enlarged lymph nodes by physical examination. While different imaging procedures are often performed in CLL patients (pts), their role has never yet validated. Recently some study have evaluated the role of computered tomography (CT) as a prognostic significance in pts with early stage disease or after first-line therapy; however CT cannot be used routinely, especially in pts with early stage CLL whose life expectancy may be very long.
Aims: Aims of the study were to investigate if ultrasonography (US) could be useful at diagnosis and in follow up and if a pathologic US (PU) showing the presence of abdominal lymphadenopathy at diagnosis could identify pts with different risk of evolution.
Methods: Between 1999 and 2011, 255 pts with Rai 0 or Rai 1 CLL had a US performed at diagnosis. Lymph nodes more than 10 mm in diameter were considered abnormal.
Results: Median age was 67 (range 41-85). 195 pts had Rai stage 0 (76.5%) disease and 60 Rai stage 1 (23.5%). Median absolute lymphocyte count was 16.7 x 10^9/L (range 5.1-199). Overall, PU were present in 53/255 (20.8%) pts, with lymph nodes up to 120 mm. PU were significantly more frequent in

pts with Rai 1 disease (24/60 – 40%) than in Rai 0 (29/195 – 14.9%) disease (p<0.01), in patients with unmutated disease (19/51 – 37.3%) than in pts with mutated disease (19/127 - 15%) (p=0.002) and in pts with absolute lymphocyte count (ALC) at diagnosis >20 x 10^9/L (20/61 – 32.8%) than in pts with ACL<20 x 10^9/L (33/194 – 17%) (p=0.03). No significant differences were present in age, hemoglobin concentration, absolute neutrophil count, platelet count or CD38 positivity. Pts with PU had a shorter time to progression (median 62 months *vs* not reached - p<0.001). (fig. 1). If the whole population is divided in three groups, pts with Rai 0 disease and no PU (group A), with Rai 0 disease and PU (group B) or pts with Rai 1 (group C), the time to progression (TTP) is significantly different, with median TTP not reached for group A, 73 months for group B and 37 months for group C (p<0.001). Among patients with Rai 1 disease, median TTP was 60 months in pts with PU and 18 months in pts without PU (p=0.056). PU had shown a prognostic significance also in pts with unmutated disease with median TTP of 63 *vs.* 19 months (p=0.04). Among patients with mutated disease, there was no significant difference in median TTP (108 months for pts with PU and not reached for pts without PU). No differences in overall survival could be shown. In 13 pts progression of lymphadenopaties or splenomegaly were shown by US as indication of an immediate treatment: in 4/13 pts this was confirmed by CT and in 10/13 pts an increase of ACL was also present but not yet indicative for treatment.

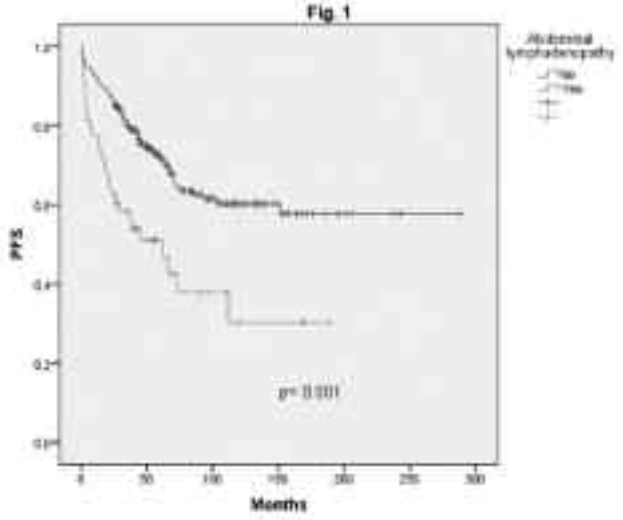

Figure 1.

Summary and Conclusion: The presence of PU at diagnosis can identify a subgroup of Rai 0 CLL with shorter TTP; this confirms results reported with CT. PU retains its importance also when unmutated pts are considered; among mutated pts, a statistically significant difference could not be shown, also due to the low number of events in this group (7/19 pts). An US performed at diagnosis appears to be useful in low-risk CLL patients: it is a non-toxic imaging technique that can identify subgroups of low-risk pts at diagnosis and it can be repeated in follow-up to evidence progressive abdominal disease.

P879

IN VITRO COMPARATIVE STUDY ON DIRECT KILLING OF CLL CELLS BY SMALL TARGETED THERAPEUTIC MOLECULES

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Background: In chronic lymphocytic leukemia (CLL), molecular studies point to the involvement of the deregulation of important cellular signaling pathways. New drugs affecting those pathways are currently developed. For drugs in early clinical testing it is important to analyze their *in vitro* killing capacity in pharma-independent head-to-head comparative experiments.

Aims: This study evaluates *in vitro* sensitivity of CLL cells to emerging targeted therapeutics and compared with conventional cytostatic agents using a high-throughput drug-sensitivity screening system based on automated fluorescence digital microscopy. The system is particularly suited for testing drug sensitivity on primary tumor cells from hematological malignancies and has been developed and validated on CLL cells.

Methods: Fresh primary CLL cells from 42 consecutive patients with CLL (22 indolent and 20 progressive or refractory), were analyzed. Informed consent was obtained from each patient. Cells were cultured for 72h on microtiter plates in a total human blood lysate-based medium. The anti-tumor effects of 30 small therapeutic molecules and of 30 cytostatic agents were measured at 4 different concentrations in triplicates in a short-term fluorescence survival assay. An equimolar concentration range was applied for the small targeted molecules in order to allow head –to-head comparison whereas for the cytostatic agents it was selected by approximating the *in vivo* achieved concentrations. Killing Efficiency (KE%), a value graded from 0 to 100, was calculated for each drug. KE% is a weighted sum of dose dependent killing-capacity where the weighting factors are the logarithms of the individual drug dilutions.

Results: Results are depicted in fig 1. The highest direct killing capacity among the small molecules tested was observed for two Bcl-2 inhibitors (ABT-199 andABT-737), one survivin inhibitor (YM-155), and one selective CDK inhibitor (dinaciclib). Small molecules, in contrast to cytostatic agents, were equally effective in cells from indolent and progressive CLL. Clinically promising drugs such as ibrutinib, lenalidomide and idelalisib showed a relatively low direct KE, in line with some previous observations and suggesting partly other mechanisms of action. The killing-capacity of cytostatic agents confirms the findings from a previous study with a notable effect for vinorelbine and daunorubicin. The sensitivity difference between cells from indolent and progressive patients was statistically significant for 10/40 tested cytostatic agents tested (p<0.05)) but no such difference was observed for the small therapeutic molecules. As expected, CLL cells from progressive, and in particular refractory CLL, had limited or no sensitivity to fludarabine, bendamustine and chlorambucil. There was no dose dependent drug sensitivity for dexamethasone or prednisone.

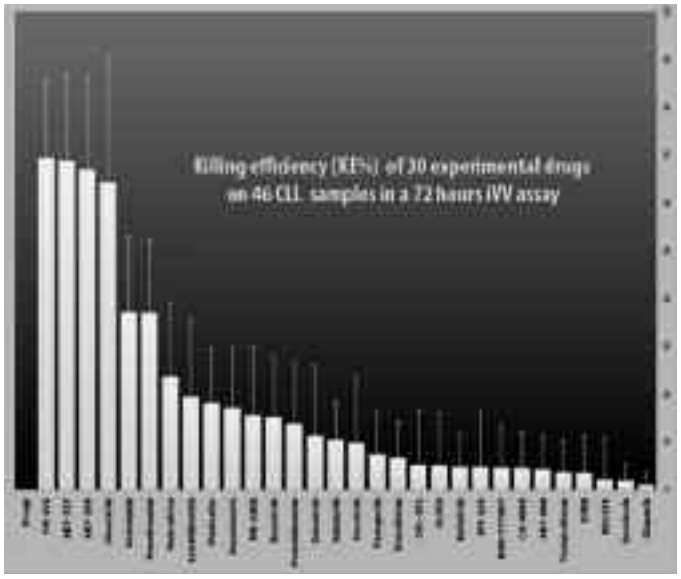

Figure 1.

Summary and Conclusion: This study provides a comparative *in vitro* testing under standardized conditions of the direct cytotoxic capacity of small therapeutic molecules in pipeline for CLL. Such analyses may help to identify drugs of particular interest to explore further, alone and in combination.

P880

UBLITUXIMAB (TG-1101), A NOVEL GLYCOENGINEERED ANTI-CD20 MAB, IN COMBINATION WITH IBRUTINIB IN PATIENTS WITH CLL AND MCL; RESULTS OF AN ONGOING PHASE II TRIAL

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Background: Ublituximab (UTX) is a novel, chimeric mAb targeting a unique epitope on the CD20 antigen, glycolengineered to enhance affinity to FcγRIIIa receptors, thereby demonstrating significantly greater ADCC than rituximab (RTX). Glycoengineered anti-CD20 mAbs have recently demonstrated greater efficacy (ORR, PFS) than RTX in CLL (NEJM, 2014). A Phase I/Ib trial with UTX monotherapy in patients with relapsed/refractory CLL reported an ORR of 45% with rapid and sustained lymphocyte depletion. Data with ibrutinib (IB) + rituximab shows high response rates however, CR and minimal residual disease (MRD) negativity rates remain low. To potentially accelerate and improve responses, IB was combined with UTX.

Aims: Herein we report data from the Phase 2 of UTX + IB in patients with relapsed/refractory CLL and MCL.
Methods: Eligible patients have relapsed/refractory CLL or MCL, with an ECOG PS<3. Informed consent was obtained in all patients. Study Design: 6 patient safety run-in followed by open enrollment. UTX (2 cohorts at 600 and 900 mg for CLL and 1 cohort at 900 mg for MCL patients) is administered weekly x 3 in Cycle 1 followed by Day 1 of Cycles 2 - 6. IB is started on Day 1 and continued daily at 420 mg for CLL and 560 mg for MCL patients. MRD assessed via central lab. Primary endpoint: Safety and Dose Limiting Toxicities (DLT). Secondary endpoint: Efficacy (ORR, CR rate, MRD).
Results: As of Feb 26, 2014, 4 CLL and 2 MCL patients have been enrolled: Median age 72 years old (range 52-76); all male. Median prior Tx=3 (range 1-4); Median ECOG PS: 1 (range 0 – 2). All patients are evaluable for safety; no DLTs or safety concerns have been observed. The most frequent AE has been Cycle 1/Day 1 infusion related reactions in CLL patients. Clinical activity has been observed in the CLL patients by physical exam (efficacy evaluation too early for MCL patients). Anticipated enrollment of 20+ patients by June 2014.
Summary and Conclusion: UTX + IB has been well tolerated to date with early clinical activity observed. Known lymphocytosis caused by IB in CLL patients has been minimized with the addition of UTX. MRD and full response analysis will be presented at the meeting. Additional studies are ongoing in patients with CLL and NHL with UTX in combination with other novel targeted agents including PI3Kδ inhibitors.

Chronic myeloid leukemia - Clinical 2

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DO CHRONIC MYELOID LEUKEMIA PATIENTS WITH LATE "WARNING" RESPONSES BENEFIT FROM SWITCHING THERAPY TO A SECOND GENERATION TYROSINE KINASE INHIBITOR?

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Background: In the latest recommendations of the European LeukemiaNet guidelines for the management of chronic-phase Chronic Myeloid Leukemia suboptimal responses have been reclassified as "warning responses". In contrast to previous recommendations current guidance advises close monitoring without changing therapy. There is little literature available regarding the potential benefit of changing treatment of patients categorized as late warning responders (patients with complete cytogenetic response (CCyR) without major molecular response (MMR)), and even more importantly, most of the published data focuses on patients classified according to previous editions of ELN recommendations (after 18 months of treatment).
Aims: The aim of this study was to describe outcomes of late warning patients, classified by the latest ELN recommendations, in order to explore the potential benefit of treatment switching in these patients.
Methods: Our registry includes a cohort of 945 CP-CML patients that are treated with first-line imatinib and are not involved in clinical trials. Registries were approved by the corresponding Ethical committees, and all the procedures were performed in accordance with international legislation. The monitoring and treatment strategies were chosen at the hematologist's discretion and thus reflect the actual treatment of CML patients outside clinical trials. All data was recorded by data managers that operated independently from the physicians taking care of the patients. From this cohort we identified 198 patients with a warning response after 12 months of treatment (patients with a complete cytogenetic response but no major molecular response [MMR]). Patients who underwent treatment change due to intolerance were identified but not categorized as late warning responders.
Results: One hundred and fourty six patients (group 1) remained on imatinib, while 52 patients (group 2) changed treatment to a Second Generation Tyrosine Kinase Inhibitor (2GTKI). The overall probabilities of obtaining MMR and $MR^{4.5}$ by 24 months on an intention-to-treat basis were 10% *vs*. 21 % respectively, whereas by 48 months the corresponding probabilities were: 21% *vs*. 44%.

Overall survival (OS) was 97% *vs.* 92% by 24 and 48 months respectively, whereas progresion free survival (PFS) was 98% *vs.* 96% for the same periods of time.Changing therapy resulted in a significant improvement in the probability of a MMR: 24% *vs.* 42% by 12 months and 43% *vs.* 64% by 24 months (p=.002); as well as the probability of achieving a deep molecular responses ($MR^{4.5}$): 1% *vs.* 19% and 7% *vs.* 23% by 12 and 24 months respectively (p=<0.001). Treatment change was also associated with an increased stability of cytogenetic responses, reflected by the observation that 17 patients (11%) in group 1 lost CCyR, compared to 2 patients (3%) in group 2 (p=.001). Similarly, treatment change significantly influenced the probability of remaining in MMR and $MR^{4.5}$ at the last follow-up: 36% *vs.* 49% and 14% *vs.* 25% implying a relative risk of 1.5 (1.2; 1.9) for MMR and a relative risk of 1.8(1.0; 3.3) for $MR^{4.5}$ in the group of patients who changed treatment *vs.* patients continuing imatinib. These improvements did not correlate with an increase in overall survival (OS) or progression-free survival (PFS).Treatment change was generally well tolerated. However, 10 patients in Group 2 (19%) discontinued treatment due to side effects with long-term side effects leading to 4 patients (2%) discontinuing imatinib.

Summary and Conclusion: To our knowledge our study represents the largest study of late warning patients treated with imatinib (including the published data regarding outcomes of late suboptimal responders under the old classification scheme). Our study shows how late warning responders had an excellent prognosis in terms of PFS and OS, and offers an empirical demonstration of the adequacy of classifying these responses as a warning, if survival outcomes are the objectives of the treatment. However, if the objectives include lowering (mitigating) the probability of treatment failure as well as obtaining deeper molecular responses, our study demonstrates that switching to a 2GTKI is the preferred option.

P882

SENSOR INTERIM DATA WITH MUTATION ANALYSIS: SWITCHING TO NILOTINIB AFTER MOLECULAR SUBOPTIMAL RESPONSE TO IMATINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background: Clinical trials have shown that nilotinib (NIL) is superior to imatinib (IM) as frontline treatment for chronic myeloid leukemia in chronic phase (CML-CP) and can improve responses in pts resistant or intolerant to IM, but the optimal treatment for patients (pts) with suboptimal response (SoR) to IM is unclear.
Aims: The Study to Evaluate Nilotinib in CML pts with SubOptimal Response (SENSOR, NCT0104387) was designed to evaluate the safety and efficacy of NIL in pts with CML-CP with SoR to frontline IM. Results of the planned 12-mo interim analysis are presented.
Methods: SENSOR is a multicenter, phase 4, open-label study in which adult pts with SoR to firstline IM switched to NIL 400 mg twice daily, with planned 24-mo follow-up. Per European LeukemiaNet 2009 criteria, SoR was defined as complete cytogenetic response, but no major molecular response (MMR; BCR-ABL ≤ 0.1% on the International Scale [IS]) after at least 18 mo. MMR rate at 12 mo was the primary endpoint. BCR-ABL^{IS} transcript levels were assessed by a central laboratory at screening, monthly for 3 mo, and every 3 mo thereafter. Mutation analyses (using direct sequencing and the polymerase chain reaction invader method), were conducted centrally at baseline (BL) and end of study in all pts; additional analyses were done every 3 mo for pts with BL mutations and in pts not in MMR with a ≥ 5-fold increase in BCR-ABL from the lowest level. The invader method, introduced to detect 25 IM-resistant point mutations with increased sensitivity, cannot detect other mutations or alternative splicing abnormalities.
Results: Between Dec 2009 and Feb 2012, 45 pts enrolled. At BL, median BCR-ABL/ABL^{IS} ratio was 0.24% (range, 0.11%>3.49%) after a median of 23.0 mo (range, 17.0-103.3) of IM. At the data cutoff, 19 pts (42%) completed the 24-mo study, 21 (47%) were ongoing, and 5 (11%) discontinued. At 12 mo, 51% of pts achieved MMR and 4.4% achieved BCR-ABL^{IS} ≤ 0.0032%. The cumulative rate of MMR by 12 mo was 67%. Most pts with mutations at any time (12/15) achieved MMR. Direct sequencing detected point mutations and alternative splicing abnormalities, such as exon 7 deletion (del) and exon 8/9 35 base pair insertion (bp ins). Twelve pts had newly detected mutations during the study; of these, 9 pts achieved MMR (6 with exon 8/9 35 bp ins, and 1 each with either E459K, Y393C, or T319A). These mutations were identified by direct sequencing, not the invader method. One pt had a newly detected T315I mutation using both methods; this pt progressed at 5.4 mo and died at 9.4 mo, never achieving MMR. This was the only pt who progressed or died. Thus, the 12-mo progression-free survival and overall survival (95% CI) were 97.8% (85.3%>99.7%) and 97.7% (84.9%>99.7%), respectively. Safety data were consistent with other studies of pts who switched to NIL after prior IM. The most common any-grade drug-related adverse events were hyperbilirubinemia (53.3%), increased ALT level (28.9%), headache (28.9%), hypophosphatemia (26.7%), rash (26.7%), and increased lipase level (24.4%).

Table 1.

Pt No.	BL Mutation Analysis: Direct Sequencing	BL Mutation Analysis: Invader Method*	Post-BL Mutation Analysis: Direct Sequencing	Post-BL Mutation Analysis: Invader Method*	MMR Achieved
1	E459K + exon 8/9 35 bp ins	E459K	E459K	E459K	Yes
2	Exon 7 del	–	–	–	Yes
3	–	Q252H	–	–	Yes
4	E255K	E255K	E255K + exon 8/9 35 bp insertion†	E255K	Yes
5	M244V	M244V	E459K†	–	Yes
6	–	–	Y393C†	–	Yes
7	–	–	T319A†	–	Yes
8	–	–	Exon 8/9 35 bp ins†	–	Yes
9	–	–	Exon 8/9 35 bp ins†	–	Yes
10	–	–	Exon 8/9 35 bp ins†	–	Yes
11	–	–	Exon 8/9 35 bp ins†	–	Yes
12	–	–	Exon 8/9 35 bp ins†	–	Yes
13	–	–	Exon 8/9 35 bp ins†	–	No
14	–	–	Exon 8/9 35 bp ins†	–	No
15‡	–	–	T315I†	T315I†	No

Gray highlight indicates pts with BL mutations using either method. Bold text indicates mutations able to be detected by the invader method.
* The invader method was used to detect the following IM-resistant point mutations: M244V, L248V/R, G250E, Q252H/R, Y253F/H, E255K/V, E274K, F311L, T315A/I, F317L, M351T, F359V, V379I, L387M, H396P/R, S417Y, E459K, and F486S.
† Newly detected mutation during study.
‡ This pt progressed to blast crisis at 5.4 mo and died at 9.4 mo.

Summary and Conclusion: After switch to NIL for 12 mo, 51% of pts with SoR to frontline IM achieved MMR. Additional follow-up is required to evaluate long-term outcomes. These results support data from other international studies (eg, ENESTcmr) showing that switch to NIL improves responses in pts with detectable disease on IM. Direct sequencing and the invader method complemented each other in detection of certain point mutations, but direct sequencing detected additional mutations and alternative splicing abnormalities in pts with SoR to frontline IM. Most pts in this study with point mutations detected at any time (with any method) achieved MMR with NIL.

P883

TYROSINE KINASE INHIBITOR (TKI) SWITCHING: EXPERIENCE FROM SIMPLICITY, A PROSPECTIVE OBSERVATIONAL STUDY OF CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) PATIENTS IN CLINICAL PRACTICE

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Background: Few data from clinical practice describe treatment switching patterns in patients (pts) with CP-CML treated with TKIs.
Aims: To investigate patterns of, and reasons for, TKI switching in pts with CP-CML who discontinued first-line treatment.
Methods: SIMPLICITY is an ongoing observational cohort study of adult pts with newly diagnosed CP-CML receiving first-line treatment with imatinib (IM), dasatinib (DAS) or nilotinib (NIL) in Europe (Eu) and the United States (US) and outside of clinical trials (NCT01244750). The primary study objective is to understand TKI management patterns in clinical practice. The study includes three prospective cohorts of pts treated with IM, DAS or NIL as initial therapy since 2010 (the study opened after first-line approval of all three TKIs) and a historical cohort treated with IM since 2008. Data on treatment discontinuation and treatment switching in all pts with ≥12 months of follow-up are presented for prospective cohorts.

Results: 949 pts (Eu: 34%, US: 66%) were enrolled through 3 October 2013, initially treated with IM (N=415), DAS (N=275) or NIL (N=259). Median follow-up was 1.6 years. Demographics were consistent across all cohorts (median age: 56 years of age at first-line TKI, 56% male). Across all regions, 733 of 949 pts were followed for ≥12 months after initiation of first-line TKI (IM: n=375, DAS: n=178 or NIL: n=180). Of these, 30.1%, 17.4% and 22.8% of patients discontinued IM, DAS and NIL, respectively, within a year of initiation. The main reason for TKI discontinuation was physician-reported intolerance (IM: 62.8%, DAS: 80.6%, NIL: 87.8%). Physician-reported primary resistance, leading to discontinuation, was only noted in IM-treated pts (8.8%). Median time to first discontinuation varied by TKI (IM: 127 days, DAS: 129 days, NIL: 56 days). A proportion of pts who discontinued first-line TKI within 12 months switched to a second-line TKI (IM: 70.8%, DAS: 54.8%, NIL: 58.5%), while no further TKI treatment information was available for the remaining pts at the time of data lock (IM: 29.2%, DAS: 45.2%, NIL: 41.5%). Of pts who switched to a second-line TKI within 12 months, 55.0% and 45.0% of IM-treated pts switched to DAS and NIL, respectively; most DAS-treated pts switched to IM (70.6% *vs.* 29.4% to NIL) and 54.2% and 45.8% of NIL-treated pts switched to DAS and IM, respectively. In Eu, the proportion of pts discontinuing was highest in the IM cohort (IM: 27.7%, DAS: 6.1%, NIL: 11.4%); in the US, the proportion of pts discontinuing was more evenly distributed (IM: 31.6%, DAS: 20.0%, NIL: 26.5%). Few pts, all in the US, discontinued therapy due to financial reasons (IM: 6.8%, DAS: 3.4%, NIL: 2.8%). Median time to first discontinuation was consistently higher in the US, with the exception of median time to discontinuation of IM, which was longer in Eu (130.0 *vs.* 122.5 days in the USA). Among pts in Eu who received a second-generation TKI as second-line therapy, NIL predominated (16/26 NIL *vs.* 10/26 DAS), whereas, in the US, DAS predominated (47/78 DAS *vs.* 25/71 NIL).

Summary and Conclusion: The proportion of pts discontinuing first-line treatment for CP-CML, and reasons for discontinuation, vary by TKI. While intolerance was the primary reason for treatment discontinuation in all TKI cohorts during the first 12 months, primary resistance was reported in IM-treated pts only. Additional analyses will be presented that identify further differences between TKI cohorts, as well as types of intolerances leading to first-line treatment discontinuation.

P884

SAFETY AND TOLERABILITY OF DASATINIB IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AND PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ ALL): POOLED ANALYSIS OF OVER 2400 PATIENTS

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Background: CML has become a manageable chronic disease for the majority of patients (pts). However, several rare, severe, and irreversible adverse events (AEs) have been reported in tyrosine kinase inhibitor (TKI)-treated populations. To better understand the incidence of such events previously reported in the context of individual trials, a large pooled dataset from Ph+ leukemic pts treated with dasatinib was interrogated for the incidence of reversible or irreversible nonhematologic AEs.

Aims: The objective of this analysis is to report clinical safety data from a large adult population of dasatinib-treated pts (n=2440) derived from 9 single-arm or comparative clinical trials of CML or Ph+ ALL resistant or intolerant to imatinib (IM; n=2182) or newly diagnosed CML in chronic phase (CML-CP; n=258).

Methods: All pts who received ≥1 dose of dasatinib 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily are included. Duration of treatment was defined as the interval from the first to the last dose of study drug and may include intermittent dose interruptions. AEs were graded using NCI CTCAE Version 3.0, and investigator AE terms were coded and grouped by System Organ Class using MedDRA Version 12.1. AEs that were determined by the investigator to have a certain, probable, or possible relationship to the study drug were defined as drug-related. Very common AEs (frequency ≥10%) and AEs of interest (eg, AEs with known association with dasatinib or other TKIs, clinical significance warranting evaluation, or nonclinical data suggesting an association) were analyzed.

Results: The median duration of therapy was 37 months (range<1 to 50 months) in pts with newly diagnosed CML-CP (n=258) and 15 months (range 0 to 65.6 months) in pts with IM-resistant or -intolerant CML or Ph+ ALL (n=2182). In this pooled analysis, minimum follow-up was 3 years and 5 years in the first-line DASISION (n=258) and second-line CA180-034 (n=662) CML-CP trials, respectively. On-study drug-related AEs with frequency ≥10% (any grade; n=2440) were diarrhea, pleural effusion, headache, dyspnea, rash, fatigue, nausea, peripheral edema, musculoskeletal pain, hemorrhage, pyrexia, vomiting, abdominal pain, infection, and cough. Drug-related AEs, such as pleural effusion, thrombocytopenia, or neutropenia, led to discontinuation in 16% of the 2440 pts. Fluid-related AEs (drug-related, any grade) were reported in 44% of pts and included: pleural effusion (30%), superficial edema (22%), pericardial effusion (5%), generalized edema (4%), congestive heart failure/cardiac dysfunction (3%), pulmonary edema (2%), pulmonary hypertension (1%), and ascites (0.5%). The single pulmonary arterial hypertension AE reported was not considered drug-related. Cardiac AEs, including ischemic AEs, rhythm abnormalities, and congestion were infrequent (Table), as were other AEs that may be related to vascular thrombosis. All-cause cerebrovascular accidents and transient ischemic attacks (TIAs) were reported in 0.6% and 0.4% of pts, respectively, and were rarely drug-related. Peripheral arterial occlusive disease (PAOD) and PAOD-related AEs have been reported (le Coutre *Blood* 2013;122:1489) in 0.3% of pts in a larger, 11-study dataset (n=2705).

Table 1. Drug-related cardiac AEs

	First-line CML-CP n=258			CML or Ph+ ALL post-IM n=2182		
	Any grade	Grade 3-4	Grade 5	Any grade	Grade 3-4	Grade 5
Drug-related cardiac AEs, n (%)	18 (7.0)	3 (1.2)	1 (0.4)	282 (13.4)	89 (4.1)	8 (0.3)
Pericardial effusion	7 (2.7)	2 (0.8)	0	112 (5.1)	33 (1.5)	1 (<0.1)
Congestive heart failure/cardiac dysfunction	4 (1.6)	1 (0.4)	0	67 (3.1)	37 (1.7)	4 (0.2)
Palpitations	4 (1.6)	0	0	45 (2.1)	0	0
Arrhythmia	3 (1.2)	1 (0.4)	0	74 (3.4)	9 (0.4)	1 (<0.1)
Myocardial infarction	1 (0.4)	0	1 (0.4)	4 (0.2)	4 (0.2)	0
Cardiomegaly	1 (0.4)	0	0	11 (0.5)	0	0
Angina pectoris	0	0	0	13 (0.6)	8 (0.3)	0
Pericarditis	0	0	0	12 (0.5)	2 (<0.1)	0
Ventricular arrhythmia	0	0	0	5 (0.2)	0	0
Acute coronary syndrome	0	0	0	2 (<0.1)	2 (<0.1)	0
Cor pulmonale	0	0	0	2 (<0.1)	2 (<0.1)	0
Myocarditis	0	0	0	2 (<0.1)	2 (<0.1)	0
Drug-related QT prolongation, n (%)	1 (0.4)	1 (0.4)	0	15 (0.7)	8 (0.4)	0

Summary and Conclusion: Analysis of a large pooled dataset from dasatinib clinical trials did not identify new safety signals. Although pleural effusions are considered common (frequency >10%), they are generally reversible. AEs that are potentially irreversible, including vascular occlusive AEs, were rarely reported in dasatinib-treated pts.

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DASATINIB PHARMACOKINETICS AND ITS CORRELATION WITH CLINICAL RESPONSE IN CHRONIC MYELOID LEUKEMIA: A SUBANALYSIS OF THE DARIA-01 STUDY

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Background: In the International Randomized Study of IFN *versus* STI571 study, imatinib plasma exposure, measured following the first month of treatment with the standard dose, correlated with therapeutic efficiency. However, there have been a few reports regarding the relationship between dasatinib plasma concentration and clinical results.

Aims: To characterize the relationship between dasatinib pharmacokinetic exposure, efficacy, and tolerability with data from the DARIA-01 study.

Methods: We conducted a multicenter, open label, phase 2 study of mid-term continuation and effectiveness of dasatinib therapy in patients with chronic myeloid leukemia in chronic phase (CML-CP). Plasma trough levels were obtained on day 28 (steady state).

Results: Thirty-two CML-CP patients were included in the study. The median

age was 52.5 years (range 20–86). Twenty-four (75.0%) previously untreated CML-CP patients and the remaining eight (25.0%) were switched from other tyrosine-kinase inhibitors (imatinib, five patients; nilotinib, three patients) because of intolerance or resistance to these drugs. Dasatinib treatment was initiated at a dose of 100 mg/day. During the initial 28 days, 28 patients continued with the 100 mg/day treatment and four received doses of 50 mg/day. Age significantly correlated with C_{min} on day 28 (p=0.0138), and was more significant after an adjustment in the dasatinib dose according to the weight of the patients on day 28 (C/D ratio; p=0.0088; Fig.1). The C/D ratio on day 28 (>1.6) significantly correlated with a higher incidence of pleural effusion during the first month (p=0.049) and/or dose reduction or interruption during the initial 3 months (p=0.009). On the other hand, the higher C/D ratio on day28 was not statistically-significant increase of major molecular response achievement at 3 months.

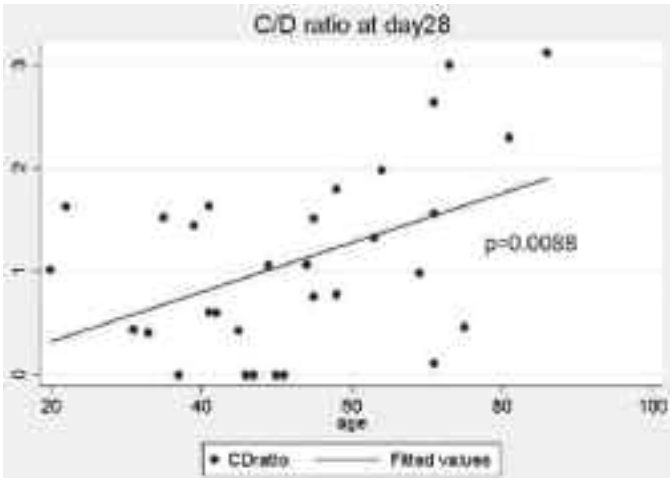

Figure 1.

Summary and Conclusion: Our finding reveals that the C/D ratio on day 28 appeared to have a predictive value for pleural effusion and dose reduction or interruption during the initial 3 months. Thus, pharmacokinetic monitoring could be beneficial in determining optimal therapy conditions for CML treatment, particularly in elderly patients.

P886

LONG-TERM FOLLOW-UP OF PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN COMPLETE MOLECULAR RESPONSE WITH ALPHA-INTERFERON AFTER TREATMENT DISCONTINUATION

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Background: Treatment with alpha-interferon (IFN) has been widely employed before the advent of Tyrosine-kinase inhibitors in patients (pts) with Chronic Myelogenous Leukemia (CML), leading to the achievement in few cases of a prolonged Complete Molecular Response (CMolR) and to IFN discontinuation.
Aims: To evaluate the long-term follow-up after IFN discontinuation, 23 pts (M/F 11/12) who achieved CMolR at RT- nested PCR with IFN at our Institution have been revised.
Methods: Median age at diagnosis was 43,3 years [Interquartile Range (IR) 35.8 – 51.3], Sokal score was low in 18 pts, Intermediate in 3 and not evaluable in 2, median WBC at diagnosis was 39.9 x 10^9/l (IR 24.4 – 67.6). IFN was given alone in 16 pts or in association with autologous bone marrow transplantation (ABMT) in 7 pts (ABMT at onset followed by IFN in 4 pts, IFN followed by ABMT in 3 pts). Median time to Complete Cytogenetic Response was 21.4 months (IR 14.4 – 37.4), median time to CMolR was 63.7 months (IR 30.3 – 106.0).
Results: After a median period of IFN treatment of 105.8 months (IR 56.1 – 127.3), all pts discontinued IFN due to medical decision for prolonged CMolR (12 pts), intolerance (8 pts) or planned ABMT (3 pts). After 12.5 months from IFN discontinuation, 1 patient developed a sudden extramedullar lymphoid blast crisis and died from disease progression. Four pts needed to start a new treatment with imatinib [2 for cytogenetic relapse after 24,8 and 44,0 months, 1 for molecular relapse after 39,8 months and 1 for progressive rise in molecular transcript but still in Major Molecular Response (MMolR) after 39,7 months], all achieving a new molecular response which was complete in 3 out 4. The remaining 18 pts are still off-therapy after a median time from IFN discontinuation of 125.5 months (IR 86.9 – 205.3); among them, 5 pts resulted always negative at the molecular follow-up, 6 pts presented a sporadic positivity but always resulted in MMolR with bcr-abl ratio<0.1 and 7 showed a mild rise of transcript levels with a long-lasting stable positivity (bcr-abl ratio<0,5 without further increments). At the last molecular evaluation, 11/18 pts were in CMolR, 4/18 in MMolR and 3 had bcr-abl ratio between 0.5 and 0.1. The 5-year and 10-year cumulative overall survival was 95.5%. The cumulative 5-year event-free survival was 77.4%, with a stable plateau in the subsequent follow-up (last event recorded 39.8 months after IFN discontinuation).
Summary and Conclusion: Our data show that CML pts who achieved a prolonged CMolR with IFN and discontinued the treatment had a very low risk of disease recurrence; it is worth of note that in many cases the reappearance of a bcr-abl positivity<0.5 did not precede a disease relapse but was sporadic or stable over long time, suggesting a possible role for immunological mechanisms induced by IFN.

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FIRST LINE TREATMENT OF CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WITH THE GENERIC FORMULATIONS OF IMATINIB MESYLATE

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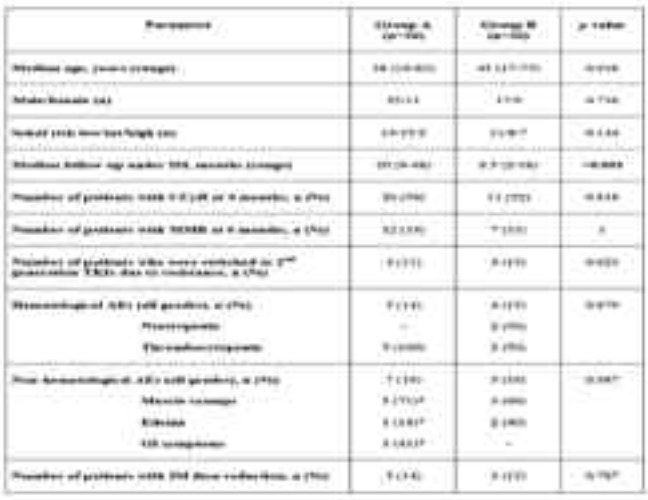
Background: The high cost of tyrosine kinase inhibitors (TKIs) developed for chronic myeloid leukemia (CML) is a major concern for the health care payers, especially in countries with restricted resources. Reimbursement policies encourage generic drug use to lower the prices. It is true that generics lead to considerable cost savings but they also give rise to questions associated with their efficacy, safety and quality. In Turkey, there are three commercially available generics of imatinib mesylate (IM) in the market, which are approved for the treatment of CML. There is a price difference between the original IM (Glivec) and generics, and due to the reimbursement policy in Turkey, patients who prefer to receive the original molecule, should pay the price difference.
Aims: The aim of this multicenter study was to evaluate the efficacy and tolerability of generics of IM, and to compare these with Glivec when used among patients with chronic phase (CP) CML in the upfront setting.
Methods: There were two study groups, thirty-six patients who started TKI treatment between January 2010 and June 2013 with Glivec (Group A), and 26 patients who were diagnosed between August 2012 (when generics became available in Turkey) and December 2013, in which a generic of IM was initiated upfront (Group B). Patients' demographics, imatinib dose, Sokal risk scores, adverse events (AEs), and follow-up periods were noted from the patients' files retrospectively. Imatinib response was evaluated according to the criteria recommended by the European LeukemiaNet. Molecular response (MR) was classified based on *BCR-ABL1* to control gene transcript ratios, expressed on the International Scale. Major molecular response (MMR) was defined as ratios ≤0.1%, and the MMR and complete cytogenetic response (CCyR) rates of both arms were calculated.
Results: The two groups were balanced regarding age, gender, and Sokal risk scores (Table 1). Patients in group A had a longer median follow-up under IM than patients in group B (20 months *vs*. 8.5 months). There were five patients in group B with a follow-up duration less than 6 months. They all have complete hematological response (CHR), but while calculating the CCyR and MMR rates, these patients were excluded. The CCyR rates at 6 months for groups A and B were 56% and 52%, respectively. There was no significant difference between the groups regarding MMR rates at 6 months of IM treatment (33% *vs*. 33%). During the follow-up, four patients in group A, and 4 in group B were switched to 2nd generation TKIs due to resistance (p=0.623). The rates of hematological and non-hematological AEs were similar in both groups (Table 1), and the percent of patients who needed a lower dose of IM (300 mg daily) due to AEs were 14% and 12% for groups A and B, respectively. There was no switch to 2nd generation TKIs in the both study groups due to intolerance. After a median follow-up of 29 months under the original molecule, eight patients were switched to a generic due to the reimbursement policy. They were all in MMR at the time of the switch, and they maintained their responses during the follow-up.
Summary and Conclusion: TKIs are now the mainstay of treatment of CML, and patients with CML live close to normal life spans. The current prices of TKIs are high, and the launch of generics might reduce health care costs. Among our patient cohort, the generics were at least non-inferior to the original molecule regarding efficacy and tolerability when used in the upfront setting. Prospective randomized trials with larger number of patients are needed to address the efficacy of generics of IM in patients with CML.

Table 1. The characteristic of patients in both groups (AE, adverse event; CCyR, complete cytogenetic response; GI, gastrointestinal; IM, imatinib mesylate; MMR, major molecular responser) *There were 3 patients with more than one AE (one with both GI symptoms and muscle cramps, and the other had edema and muscle cramps), so the rates of non-hematological AEs in this patient group were added up to >100%.



P888

LONG-TERM RESULTS OF A PROSPECTIVE RANDOMIZED TRIAL OF HIGH-DOSE IMATINIB WITH OR WITHOUT INTERFERON-A IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background: Before tyrosine kinase inhibitors (TKIs) became the standard frontline therapy, interferon-α (IFN-α) achieved durable cytogenetic responses in occasional patients with chronic myeloid leukemia in chronic phase (CML-CP). Trials of the combination of imatinib and IFN- α have yielded conflicting results.
Aims: We report the long-term outcome of a randomized trial of imatinib ± IFN-α, with a minimum follow-up of 104 months (last patient, first visit 06/2005).
Methods: We conducted a prospective, randomized clinical trial on newly diagnosed CML-CP randomized to receive 800 mg of imatinib daily with (IM800+IFN; n=43) or without (IM800; n=48) weekly subcutaneous injections of 0.5 μg/kg polyethylene glycol (PEG)–IFN-α-2b. Administration of IFN-α-2b started 6 months after the start of imatinib for patient randomized to that arm. Patients aged ≥18 years with CML-CP were eligible if performance status ≤2 by the Eastern Cooperative Oncology Group, serum creatinine and total bilirubin<1.5 x the upper limit of normal. The Kaplan-Meier method was used to calculate overall survival (OS; dated from the start of imatinib until death from any cause), event-free survival (EFS; from the start of imatinib to loss of complete hematologic response, loss of major cytogenetic response, transformation to accelerated [AP] or blast phase [BP], or death from any cause), transformation-free survival (TFS; from the start of imatinib to transformation to AP or BP or death), and failure-free survival (FFS; from the start of imatinib to any event defined above, imatinib discontinuation for any reason, or death.) The chi-square test was used to compare response rates between groups and a log-rank test was used for univariate comparisons.
Results: The median follow-up duration was 107 months (range, 6-121 months). Cumulative response rates and rates of OS, EFS, TFS, and FFS are given in Table 1. The median duration of PEG–IFN-α-2b therapy was 335 days (range, 7-1191 days), and all patients discontinued PEG–IFN-α-2b. The complete cytogenetic response rate (CCyR), major molecular response (MMR), major response ≥4.5-log reduction (MR4.5), and complete molecular response rate(CMR) at 6 months after high-dose imatinib treatment before PEG–IFN-α-2b therapy in IM800 + IFN group were 74%, 60%, 21% and 2% compared to comparable rates of 83%, 67%, 21% and 6% in IM800 group, respectively(*P*=0.877). The CCyR, MMR, MR4.5 and CMR at 12 months from start of all therapy in IM800 + IFN group were 77%, 65%, 26% and 7% compared to comparable rates of 83%, 73%, 21% and 10% in IM800 group, respectively (*P*=0.888). The groups' best response rates and rates of 10-year FFS, TFS, EFS, and OS did not differ significantly.

Table 1. Patient Characteristics and Outcomes

	IM800 + IFN [n=43]	IM800 [n=48]	P value
Cumulative Response, %			
CMR	58	60	0.825
MR4.5	67	75	0.426
MMR	81	88	0.420
CCyR	86	88	0.838
10-y Outcome, %			
FFS	60	67	0.436
TFS	97	95	0.639
EFS	61	88	0.094
OS	88	91	0.873

Summary and Conclusion: The addition of PEG–IFN-α-2b to high-dose imatinib as a frontline therapy does not confer a clinical benefit in patients with CML-CP.

P889

FOUR-YEAR FOLLOW-UP OF PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA RECEIVING DASATINIB: EFFICACY AND SAFETY

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Background: The Chinese dasatinib registration study, initiated in 2007, enrolled 119 chronic myeloid leukemia (CML) patients (pts) intolerant of, or resistant to, imatinib, including 59 pts with chronic-phase (CP) CML. After 18 months of follow-up, 50.8% and 91.5% of pts with CML-CP achieved a major cytogenetic response (MCyR) or complete hematologic response (CHR), respectively. Furthermore, none of the CML-CP pts who achieved a MCyR died or experienced disease progression at 18 months. The safety findings supported prior Phase 1/2 trials, with common adverse events (AEs) including neutropenia and thrombocytopenia. Here we present the efficacy and safety results from the 4-year follow-up of this pivotal Chinese study.
Aims: To evaluate the long-term efficacy and safety of dasatinib for the treatment of Chinese pts with CML-CP who were resistant or intolerant to imatinib, and to investigate the association between pre/on-treatment factors and treatment efficacy after 4 years of follow-up.
Methods: This was a single-arm, Phase 2 study conducted at 10 centers in China. Adult, Chinese pts with imatinib-resistant or -intolerant CML-CP received oral dasatinib 100 mg/d until disease progression, unacceptable toxicity, or at the investigator/patient's discretion. The primary endpoint was the rate of MCyR and secondary endpoints included time to MCyR, rate of progression-free survival (PFS), and incidence of AEs. Pts who discontinued treatment were not routinely followed up, therefore disease progression and survival data are not available for these pts post study departure. The association between pre- and on-treatment factors and complete cytogenetic response (CCyR) was investigated by univariate regression. Written, informed consent was obtained from all pts and the study was registered at clinicaltrials.gov (NCT00529763).
Results: After 4 years of follow-up, 72.9% (43/59) of pts remained on treatment. The cumulative rate of the primary endpoint, MCyR, was 66.1% (39/59; 95% CI, 52.6–77.9); an increase of 30% from that observed at 18 months (50.8% [30/59]). Median time to MCyR was 12.7 weeks (range, 4.3–206.1). Interestingly, all pts who achieved MCyR also met the criteria for CCyR.
The 4-year PFS was 85.7% (95% CI, 77.0–95.4), and rates at 12, 24 and 36 months were 94.7%, 91.2% and 85.7%, respectively; no pts experienced disease progression between 36 and 48 months. After 4 years of follow-up there had been 5 deaths on study, none of which were considered to be treatment-related. None of the 20 pts who achieved an early response (MCyR within 90 days of treatment initiation) had experienced disease progression after 4 years (Figure), and these pts had a greater likelihood of achieving a CCyR than those without an early response (odds ratio 42.1, 95% CI 2.37–746.84; *P*=0.01). Dasatinib was generally well tolerated; 11.9% (7/59) of pts discontinued treatment due to AEs. Drug-related AEs of any grade were experienced by 69.5% (41/59) of pts, and Grade 3–4 by 18.6% (11/59). Reported AEs included pleural effusion (23.7% [14/59]; Grade 3–4: 3.4% [2/59]), thrombocytopenia (11.9% [7/59]; Grade 3–4: 11.9% [7/59]), and neutropenia (6.8% [4/59]; Grade 3–4: 6.8% [4/59]). Pulmonary hypertension was reported in 6.8% (4/59) of pts (Grade 3–4: 1.7% [1/59]).

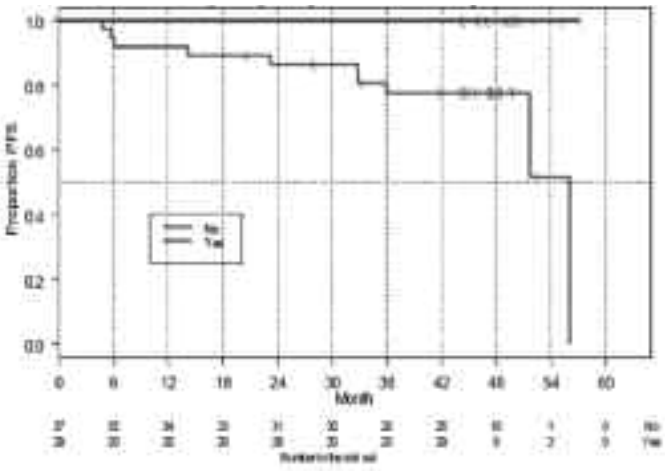

Figure: Kaplan–Meier PFS curve by early response (MCyR within 90 days of treatment initiation; green line) or no early response (red line)

Figure 1. Kaplan-Meier PFS curve by early response (MCyR within 90 days of treatment initiation; green line) or no early response (red line).

Summary and Conclusion: This 4-year follow-up study supports dasatinib treatment in Chinese pts with imatinib-resistant or -intolerant CML-CP: 72.9% of pts remained on treatment, 66.1% achieved a MCyR, and high rates of PFS were sustained from 36 months through 4 years. Dasatinib was well tolerated, with a low rate of discontinuations and a safety profile that supports previous clinical trial data.

P890

ABNORMALITY OF GLUCOSE AND LIPID METABOLISM DURING NILOTINIB THERAPY – RESULTS OF ENIGMA 2 STUDY

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Background: Recently we have published results of pilot study on CML patients demonstrating fast development of hyperinsulinaemia, peripheral insulin resistance, hypoadiponectinaemia and hypercholesterolemia during nilotinib therapy.
Aims: To analyze preliminary results from follow up study ENIGMA 2 to confirm or exclude result from the pilot study, as well as to analyze whether these abnormalities are detected in control group of patients treated with imatinib.
Methods: Patients received intensive laboratory workup before the start of TKI and after 3 month of therapy. This included fasting insulin, glucose, adiponectin and lipid serum concentration, HbA1c and oral glucose tolerance test.
Results: Between 2/2011-11/2013 twenty two CML patients initiated therapy with nilotinib and 4 with imatinib. Patients treated with nilotinib developed significant hypersinulinaemia and hyperglycaemia. We also proved significant development of insulin resistance using HOMA-2 index, that significantly increased after 3 month of nilotinib therapy (medians - 1,8 *vs*. 2,5; p=0,0098). More over, we have proved significant decrease of adiponectin concentration as well as significant increase in total cholesterol concentration. Details are presented in Table. Contrary – none of these abnormalities was detected in the control group of patients treated with imatinib, including any change in insulin resistance measured by HOMA-2 index (medians - 0,5 *vs*. 0,8; p=0,3750).

Table 1.

NILOTINIB THERAPY (n=22)			
	Start median (range)	Month 3 median (range)	p
Fasting glucose [mmol/l]	5.25 (4.8-6.1)	5.7 (4.8-6.1)	0.0003
Fasting insulin [mIU/l]	23.55 (2.4-89.0)	29.05 (3.50-84.90)	0.2482
Fasting C-peptide [pmol/ml]	0.85 (0.50-2.40)	0.85 (0.60-1.30)	0.0064
Fasting HbA1c [mmol/mol]	38 (34-50)	37 (30-51)	0.0221
Fasting adiponectin [mg/l]	3.86 (0.66-43.14)	6.34 (1.01-22.35)	0.0094
Total cholesterol [mmol/l]	5.00 (2.60-6.60)	5.65 (4.50-7.70)	0.0004
Triglycerides [mmol/l]	1.68 (0.58-5.27)	2.46 (0.62-4.38)	0.1840
HDL cholesterol [mmol/l]	1.10 (0.50-2.30)	1.50 (0.80-2.30)	0.0002
LDL cholesterol [mmol/l]	2.65 (1.20-4.40)	3.20 (1.10-5.70)	0.0007
Non-HDL cholesterol [mmol/l]	3.50 (1.50-5.30)	4.05 (2.50-6.20)	0.0025
IMATINIB THERAPY (n=4)			
	Start median (range)	Month 3 median (range)	p
Fasting glucose [mmol/l]	5.0 (4.70-5.10)	5.35 (4.90-5.70)	0.1250
Fasting insulin [mIU/l]	4.05 (2.60-5.90)	8.10 (2.50-37.90)	0.2500
Fasting C-peptide [pmol/ml]	0.60 (0.50-0.70)	0.50 (0.40-0.60)	0.2500
Fasting HbA1c [mmol/mol]	38.5 (35.0-42.0)	35.5 (33.0-37.0)	0.0938
Fasting adiponectin [mg/l]	6.27 (2.21-13.30)	13.35 (5.00-35.30)	0.1250
Total cholesterol [mmol/l]	5.50 (5.30-6.10)	4.80 (4.20-5.60)	0.1250
Triglycerides [mmol/l]	2.135 (0.940-2.680)	0.900 (0.560-1.230)	0.1250
HDL cholesterol [mmol/l]	1.30 (1.00-1.50)	1.70 (1.30-1.90)	0.0938
LDL cholesterol [mmol/l]	3.70 (3.40-4.30)	2.55 (2.10-2.80)	0.1250
Non-HDL cholesterol [mmol/l]	4.30 (4.00-4.70)	3.25 (2.70-4.00)	0.0938

Summary and Conclusion: Our preliminary results from presented study proved fast development of peripheral insulin resistance already during the first 3 month of nilotinib therapy as underlying cause of glucose and secondary also lipid metabolism impairment during nilotinib treatment. Moreover, this was not proved for patients treated with imatinib. Final data from target of 40 patients on nilotinib and 10 patients on imatinib will be presented during the meeting.

P891

HIGHER RATE OF MAJOR MOLECULAR RESPONSE (MMR) WITH NILOTINIB *vs* IMATINIB IN CHINESE PATIENTS (PTS) WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTCHINA 24-MO UPDATE

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Background: In the global ENESTnd study, nilotinib resulted in superior efficacy *vs* imatinib in pts with newly diagnosed CML-CP. ENESTchina is a local, randomized, multicenter phase 3 study of nilotinib *vs* imatinib in Chinese pts with newly diagnosed CML-CP. ENESTchina met its primary endpoint with a significantly higher rate of MMR at 12 mo in the nilotinib arm (52.2%) *vs* the imatinib arm (27.8%; *P*<0.0001).
Aims: This analysis aims to evaluate the safety and efficacy of nilotinib *vs* imatinib in ENESTchina after all pts completed the mo 24 visit or discontinued early.
Methods: Adult Chinese pts (N=267) with newly diagnosed (≤ 6 mo) CML-CP were randomized to nilotinib 300 mg twice daily (n=134) or imatinib 400 mg once daily (n=133; dose escalation to 600 mg/day was allowed). Randomization was stratified by Sokal risk score and prior interferon therapy. Response rates were compared between arms using a stratified Cochran-Mantel-Haenszel test. Freedom from progression to accelerated phase/blast crisis (AP/BC) and overall survival (OS) were estimated by Kaplan-Meier analysis and compared between arms using a stratified log-rank test. *P* values for secondary endpoints were not adjusted for multiple comparisons and are provided for descriptive purposes only.
Results: In the nilotinib and imatinib arms, respectively, the median age was 41 y and 39 y, and 67.9% and 60.9% of pts were male. Median dose intensities were nilotinib 579.4 mg/day (range, 167-600) and imatinib 399.4 mg/day (range, 247-574). By the data cutoff, 18 pts (13.4%) in the nilotinib arm and 15 pts (11.3%) in the imatinib arm discontinued treatment, most commonly due to suboptimal response/treatment failure (nilotinib, n=6; imatinib, n=4) or disease progression (per investigator's judgment; nilotinib, n=4; imatinib, n=6). Compared with imatinib, nilotinib resulted in higher rates of MMR by 24 mo (Table; *P*=0.0096), MMR at 24 mo (*P*=0.0395), and durable MMR at 24 mo (defined in table footnote d; *P*=0.0001). Rates of MMR by 24 mo were higher on nilotinib *vs* imatinib across all Sokal risk groups. Median time to first MMR

among pts who achieved MMR was shorter on nilotinib *vs* imatinib. Rates of complete cytogenetic response (CCyR) were high and comparable in both arms. Four pts in the nilotinib arm and 5 pts in the imatinib arm progressed to AP/BC (calculated) on treatment; estimated 2-y freedom from progression to AP/BC was 96.8% on nilotinib and 96.1% on imatinib (*P*=0.7481). Two pts in each arm died on study (due to cerebral hemorrhage and disease progression [1 each] in the nilotinib arm and non-Hodgkin's lymphoma and disease progression [1 each] in the imatinib arm), all after discontinuing treatment. Estimated 2-y OS was 98.5% in each arm. Both drugs were well tolerated, and no new safety signals were identified. No cases of peripheral artery disease were reported in either arm; 1 pt (nilotinib arm) with a history of cerebral infarction had an ischemic cerebrovascular event, and 1 pt (imatinib arm) had an asymptomatic creatinine kinase-MB elevation with no clinical or electrocardiographic findings. New or worsening biochemical abnormalities were typically grade 1/2, including all cholesterol elevations and all glucose elevations except 2 (both in the nilotinib arm). New or worsening grade 3/4 biochemical abnormalities occurring in ≥ 5% of pts in either arm included elevated lipase (14.3% and 6.8% of pts on nilotinib and imatinib, respectively), elevated magnesium (6.0% and 5.3%, respectively), and decreased phosphate (4.5% and 5.3%, respectively).

Table 1.

	Nilotinib n = 134	Imatinib n = 132
Response rates, n (%)		
MMR by 24 mo	91 (67.9)	70 (53.0)
Low Sokal risk score[a]	52 (75.4)	41 (59.4)
Intermediate Sokal risk score[a]	29 (65.9)	24 (55.8)
High Sokal risk score[a]	10 (47.6)	5 (23.8)
MMR at 24 mo	82 (61.2)	65 (49.2)
Durable MMR at 24 mo[b]	66 (56.7)	57 (37.9)
CCyR by 24 mo	112 (83.6)	103 (78.0)
Median time to MMR,[c] mo (25th-75th percentiles)	6.00 (5.39-8.31)	10.86 (5.45-14.53)
Nonhematologic adverse events of any grade in ≥ 10% of pts in either arm regardless of relationship to study drug, n (%)[d]		
Rash	47 (35.3)	17 (12.9)
Myalgia	16 (12.0)	9 (6.1)
Nasopharyngitis	14 (10.5)	22 (16.7)
Eyelid edema	4 (3.0)	23 (17.4)
Diarrhea	4 (3.0)	15 (11.4)
New or worsening biochemical abnormalities of any grade in ≥ 30% of pts in either arm, n (%)[e]		
Bilirubin (total)	116 (87.2)	25 (18.9)
Alanine aminotransferase	77 (57.9)	47 (35.6)
Phosphate (inorganic phosphorus)	66 (49.6)	95 (72.0)
Cholesterol (total)	66 (49.6)	11 (8.3)
Lipase (blood)	55 (41.4)	59 (44.7)
Glucose – high	47 (35.3)	36 (27.3)
Calcium – low	46 (34.6)	67 (50.8)
Aspartate aminotransferase	43 (32.3)	27 (20.5)
Potassium – low	28 (21.1)	66 (50.0)
New or worsening grade 3/4 hematologic abnormalities in ≥ 5% of pts in either arm, n (%)[f]		
Thrombocytopenia	34 (25.6)	40 (30.3)
Neutropenia	28 (21.1)	29 (22.0)
Leukopenia	13 (9.8)	23 (17.4)
Lymphopenia	6 (4.5)	16 (13.6)
Anemia	5 (3.8)	8 (6.1)

[a] Pts with low Sokal risk scores: nilotinib, n = 69; imatinib, n = 69. [b] Pts with intermediate Sokal risk scores: nilotinib, n = 44; imatinib, n = 43. [c] Pts with high Sokal risk scores: nilotinib, n = 21; imatinib, n = 21. [d] Defined as MMR at both 12 and 24 mo with no confirmed loss of MMR in between. [e] Among pts who achieved MMR. [f] Among pts who received ≥ 1 dose of study drug (nilotinib, n = 133; imatinib, n = 132).

Summary and Conclusion: Through 24 mo of follow-up, MMR rates were consistently higher on nilotinib *vs* imatinib. This study confirms the superiority of nilotinib over imatinib for the treatment of pts with newly diagnosed CML-CP, as initially demonstrated in ENESTnd.

P892

FOUR-YEAR OUTCOME OF 215 NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH NILOTINIB FRONTLINE: A GIMEMA CML WORKING PARTY ANALYSIS

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Background: Nilotinib (NIL) is a potent and selective BCR-ABL inhibitor approved for the frontline treatment of chronic myeloid leukemia (CML) based on the results of the ENESTnd study. The sustained superiority of NIL *vs*. imatinib (IM) was confirmed after 5 years of follow-up (Saglio et al, abs. 92, ASH 2013). However, few data are available on pts treated frontline with NIL outside of company-initiated trials.

Aims: To analyze the response rates and outcome in a large, independent cohort of newly diagnosed CML pts treated frontline with NIL-based regimens.

Methods: We analyzed 215 early chronic phase pts, enrolled in 2 multicenter phase II studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or treated at the Bologna University Hospital, with NIL 300 mg or 400 mg BID as initial treatment; 123 pts received a sequential treatment with NIL and IM, with a 3-months rotation period. The median age was 53 years (range 18–86). Ten out of 215 pts (5%) had a high EUTOS score. The median follow-up was 43 months (range 18–69 months). We assessed: the rates of Complete Cytogenetic Response (CCyR) and Major Molecular Response (MMR); the rates of optimal responders at each milestone according to ELN 2013 recommendations; the overall survival (OS; any death included), progression-free survival (PFS; progression to accelerated/blast phase [AP/BP] and deaths for any cause), failure-free survival (FFS; failures according to ELN 2013 recommendations and deaths for any cause), and event-free survival (EFS; events: failures, permanent discontinuation of NIL for any cause, including deaths). All analysis was made according to the intention-to-treat principle.

Results: The cumulative rates of CCyR and MMR were 93% and 88%, respectively. At 3 months, 82% of the pts were in Partial Cytogenetic Response and 90% had a BCR-ABL/ABL (IS)<10%; at 6 months, 86% were in CCyR and 83% had a BCR-ABL/ABL (IS)<1%; at 12 months, 72% were in MMR; all these pts were optimal responders according to ELN 2013 recommendations. Overall, 64 (30%) pts permanently discontinued NIL: 31 (14%) for adverse events or intolerance; 22 (10%) for failures (figure); 11 (5%) for other reasons. In detail, 10 (4.6%) pts discontinued NIL for cardiovascular events, including 3 for peripheral arterial occlusive disease and 2 for acute myocardial infarction. Eight (3.7%) pts progressed to AP/BP, all during the 1st year of therapy, and all pts subsequently died (after a median of 13 months, range 1- 34 months). NIL-resistant mutations were identified in 5 of these pts (4 T315I; 1 Y253H). No difference in the rate of progression to AP/BP was observed between pts receiving NIL alone or NIL and IM in sequential schedule. During the follow-up, 8 patients developed a secondary resistance (3 loss of CHR, 3 loss of CCyR, and 2 confirmed loss of MMR). Overall, 15 (7%) pts died, in 7 cases for reasons unrelated to CML progression. The estimated 4-year OS, PFS, FFS, and EFS were 93%, 93%, 86%, and 69%, respectively.

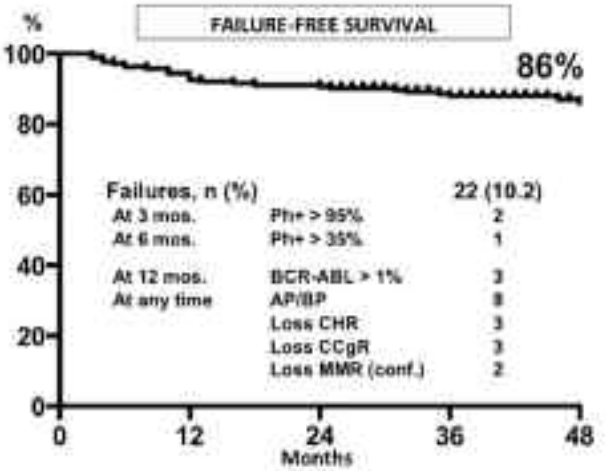

Figure 1.

Summary and Conclusion: Our National experience confirmed that pts treated frontline with NIL-based regimens obtained fast and high rates of complete cytogenetic and major molecular response, and most pts were optimal responders according to ELN recommendations. Of note, all progressions to AP/BP occurred early (in the first year). Overall, 93% of the pts were estimated to be alive and progression-free at 4-years, with 69% of the pts still on NIL.

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P893

PONATINIB IN PATIENTS (PTS) WITH PHILADELPHIA CHROMOSOME–POSITIVE (PH+) LEUKEMIAS RESISTANT OR INTOLERANT TO DASATINIB OR NILOTINIB, OR WITH THE T315I MUTATION: LONGER-TERM FOLLOW-UP OF THE PACE TRIAL

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Background: Ponatinib is a potent oral pan–BCR-ABL inhibitor with activity against native and mutant forms of BCR-ABL, including the resistant T315I mutant.
Aims: The efficacy and safety of ponatinib (45 mg QD) were evaluated in the phase 2 PACE trial.
Methods: 449 pts resistant or intolerant (R/I) to dasatinib or nilotinib or with the T315I mutation were enrolled and gave informed consent. Data are as of 3 Sept 2013; median (range) follow-up was 24 (0.1-35) months. NCT01207440.
Results: Pts were heavily pretreated: 58% received ≥3 prior tyrosine kinase inhibitors. At analysis, 42% remained on study (55% chronic-phase chronic myeloid leukemia [CP-CML]). The most common reasons for discontinuation were progressive disease (21%) and adverse events (AEs) (14%; most common was thrombocytopenia, 4%). The table shows response rates at any time. In CP-CML, 89% of pts maintained major cytogenetic response (MCyR) for at least 2 yrs; progression-free survival (PFS) and overall survival (OS) at 2 yrs were 67% and 86%, respectively. For accelerated-phase (AP) CML, blast-phase (BP) CML, and Ph+ acute lymphoblastic leukemia (ALL), OS at 2 yrs was 72%, 18%, and 21%, respectively. The most common treatment-emergent AEs (≥30%) were thrombocytopenia (43%), rash (40%), abdominal pain (40%), headache (36%), constipation (36%), and dry skin (36%). Pancreatitis and pneumonia were the most common serious AEs (SAEs; both 6%). Vascular occlusive AEs [SAEs] were reported as follows: overall 20% [14%], including cardiovascular 9% [6%], cerebrovascular 6% [4%], peripheral vascular 6% [4%] (collectively, arterial thrombotic events [ATEs]), and venous thromboembolic 5% [3%]. In a multivariate analysis, higher dose intensity, older age, and cardiovascular risk factors were associated with a higher likelihood of an ATE; pts with and without cardiovascular risk factors experienced these events. OS at 2 yrs was not reduced in pts with an ATE (73%) *vs* without an ATE (69%); MCyR in CP-CML pts with *vs* without an ATE was 70% *vs* 51%.
Summary and Conclusion: Ponatinib has substantial clinical activity in heavily pretreated pts with Ph+ leukemias. Vascular occlusive events were observed. Ponatinib is an important treatment option for pts in whom the need and benefit outweigh the risk.

Table 1.

	R/I, n (%)	T315I, n (%)	Total, n (%)
CP-CML	N=203	N=64	N=267
MCyR	113 (56)	46 (72)	159 (60)
CCyR	98 (48)	45 (70)	143 (54)
MMR	63 (31)	37 (58)	100 (38)
AP-CML	N=65	N=18	N=83
MaHR	40 (62)	11 (61)	51 (61)
BP-CML	N=38	N=24	N=62
MaHR	12 (32)	7 (29)	19 (31)
Ph+ ALL	N=10	N=22	N=32
MaHR	5 (50)	8 (36)	13 (41)

Responses at any time after first dose

P894

CLINICAL IMPACT OF DOSE MODIFICATION AND DOSE INTENSITY ON RESPONSE TO PONATINIB IN PATIENTS (PTS) WITH PHILADELPHIA CHROMOSOME–POSITIVE (PH+) LEUKEMIAS

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Background: Ponatinib is a potent oral pan–BCR-ABL tyrosine kinase inhibitor with clinical activity in pretreated pts with Ph+ leukemias. Dose modification of ponatinib was used to manage adverse events (AEs).
Aims: This post hoc analysis assessed the clinical impact of dose modification and dose intensity on outcomes of pts in the phase 2 PACE trial.
Methods: All pts provided informed consent. Ponatinib starting dose was 45 mg QD. Dose reduction was defined as any reduction below 45 mg/day; dose interruption was defined as treatment withheld for ≥3 consecutive days. Efficacy analyses were performed on chronic-phase chronic myeloid leukemia (CP-CML) pts (N=267). Analysis of arterial thrombotic events (ATEs) included all pts (CP-CML, blast-phase CML, accelerated-phase CML, Ph+ acute lymphoblastic leukemia; N=449). Data are as of 3 Sept 2013; median (range) follow-up was 24 (0.1-35) months for all pts. NCT01207440.
Results: 78% of CP-CML pts had dose modification within the first 12 months (82% at any time). Responses in pts with and pts without modification were comparable (Table). Of 149 responders, 87 (58%) achieved major cytogenetic response (MCyR) at 45 mg/day, 46 (31%) at 30 mg/day, and 16 (11%) at 15 mg/day. Most pts who had a dose reduction after achieving a response maintained that response: 97% maintained MCyR; 96% maintained complete cytogenetic response (CCyR); and 92% maintained major molecular response (MMR). Among pts with a dose reduction lasting ≥6 months after achieving a response at a higher dose, 100% (33/33) maintained MCyR (96% [26/27] CCyR and 93% [13/14] MMR). While dose intensity was the most significant predictor of MCyR by 12 months (multivariate analysis), substantial responses occurred at lower doses; estimated response rates were ~75% at 45 mg/day, ~60% at 30 mg/day, and ~30% at 15 mg/day. ATEs occurred in 17% of pts; each 15 mg/day reduction in average daily dose is predicted, by multivariate analysis, to lead to ~40% reduction in risk of ATE. 2-yr overall survival was similar for CP-CML pts who had dose modifications (86%) and those who did not (86%), and for pts who had ATEs (85%) and those who did not (87%). Of pts with ATEs, 46% had dose modifications.
Summary and Conclusion: Most of the CP-CML pts in the PACE trial had dose modifications. Pts who underwent dose modification still responded to treatment. Dose modification was an effective management tool for CP-CML patients. Careful consideration of the potential benefits and risks of ponatinib should guide treatment decisions.

Table 1.

CP-CML	n	MCyR, %b	CCyR, %b	MMR, %a
Dose modificationa	218	58	47	38
No dose modification	49	47	45	35

[a]At any time; [b]By 12 months

P895

LONGER-TERM FOLLOW-UP OF A PHASE 1 STUDY OF PONATINIB IN PATIENTS (PTS) WITH PHILADELPHIA CHROMOSOME-POSITIVE (PH+) LEUKEMIAS

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Background: Ponatinib is a potent oral pan–BCR-ABL tyrosine kinase inhibitor (TKI) that is active against native and mutated forms of BCR-ABL.
Aims: The safety and anti-leukemic activity of ponatinib in pts with chronic myeloid leukemia (CML) or Ph+ acute lymphoblastic leukemia were evaluated in a phase 1 clinical trial.
Methods: Pts (N=81) with resistant/refractory hematologic malignancies were enrolled and gave informed consent in this ongoing, open-label, dose-escalation, phase 1 study. Ponatinib was dosed once daily (2–60 mg). 65 pts had Ph+ leukemia and are included in this analysis (data as of 15 Oct 2013). Median (range) follow-up for 25 pts still on treatment was 45 (38–57) months. NCT00660920.
Results: The median age of pts was 55 yrs; median time since diagnosis was 6.5 yrs. Pts were heavily pretreated (94% had received ≥2 prior TKIs, 62% ≥3). 65% had baseline BCR-ABL mutations (29% with T315I). 38% (58% chronic phase [CP] CML) of pts remained on study at the time of analysis. Adverse events (AEs) and progression were the most common reasons for discontinuation (20% and 17%, respectively). The most common treatment-emergent AEs were rash (52%), fatigue (52%), abdominal pain (51%), headache (48%), and arthralgia (46%). Treatment-emergent vascular occlusive events were observed in 23% (serious events) and 37% (all events) of pts, including cardiovascular (15% serious, 23% all) peripheral vascular (5%, 9%), cerebrovascular (5%, 8%), and venous thrombotic (0%, 5%) events. Significant anti-leukemic activity was observed: among CP-CML pts, major cytogenetic response (MCyR), complete cytogenetic response (CCyR), and major molecular response (MMR) rates were 72%, 65%, and 51%, respectively; 75% of pts with MCyR, 69% with CCyR, and 53% with MMR are estimated (Kaplan-Meier [KM]) to maintain response for at least 3 yrs (4-yr KM estimates: 75% MCyR, 53% MMR). Of 28 CP-CML pts with CCyR, 23 remained on study at analysis (17 with continuous CCyR); of 22 pts with MMR, 20 remained on study at analysis (12 with continuous MMR).
Summary and Conclusion: Substantial and durable responses were observed with ponatinib in heavily pretreated CP-CML pts. Vascular occlusive events were observed. Risk and benefit considerations should be evaluated when utilizing ponatinib in this patient population.

P896

A NATIONAL EXPERIENCE OF THE USE OF PONATINIB IN PATIENTS FAILING MULTIPLE TYROSINE KINASE INHIBITORS CONFIRMS EFFICACY IN A HEAVILY PRE-TREATED COHORT OF PATIENTS WITH PH+ LEUKAEMIAS

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Background: Ponatinib is a highly potent third generation tyrosine kinase inhibitor(TKI) licensed for use in patients with Ph positive leukaemias who have failed two or more prior TKI and/or have a documented T315I kinase domain mutation. Ponatinib first became available in the United Kingdom in 2011 through the phase II PACE study and subsequently through an expanded access scheme.
Aims: To examine efficacy of Ponatinib in Patients failing Multiple Tyrosine Kinase Inhibitors.
Methods: From May 2011 to September 2012, 66 patients received ponatinib for chronic myeloid leukaemia (CML) in chronic phase (CP) (n=57), accelerated phase (n=3), second or subsequent CP (n=3), blast crisis (n=1) or Ph positive ALL (n=2). Of the patients treated in CP, 10/57 had the T315I mutation. Six patients received ponatinib for relapse after allogeneic stem cell transplantation and in 4 of these the T315I mutation had been identified before and/or after transplant. In the 51 patients treated without transplant, a T315I mutation was identified in 6 and all had received two prior TKI. However the majority of these patients (45/51) received ponatinib for poor responses and/or intolerance of other TKI without evidence of kinase domain mutations, 4 after failing a single second generation TKI, 14 after failing two TKI and 27 after 3-4 prior TKI.
Results: Focussing on the 51 patients who received ponatinib in CP and without a previous transplant, the median age was 62 years (range 20-78 yrs). The median duration of ponatinib treatment was 9 months (range 0.25-31 mths). 21/51 (41%) and 20/51 (39%) patients remained on ponatinib at 12 and 24 months respectively. The reasons for discontinuation were resistance only in 8, intolerance only in 5 and resistance and intolerance in 15 patients. Patients were considered evaluable for response if they received at least 3 months of therapy (n=48). Six patients commenced treatment in complete cytogenetic remission (CCyR) (4 of whom were also in major molecular response - MMR) and all 6 maintained these levels of response. Achievement of CCyR was seen in 15 of the remaining 42 patients including 3 of the 6 patients with a T315I mutation at the start of treatment. CCyR and MMR were seen in 12/36 (33%) and 3/36 (8%) of patients without and 3/6 (50%) and 3/6 (50%) with the T315I mutation.
Summary and Conclusion: More precise data collection for the type and grade of toxicities is on-going. Our national experience reflects the data emerging from the phase II PACE study confirming useful efficacy of ponatinib in a previously heavily treated patient cohort in whom the need and benefit outweigh the risk.

P897

OUTCOMES OF THIRD-LINE BCR-ABL1 TYROSINE KINASE INHIBITORS IN THE TREATMENT FAILED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS WHO HAVE RECEIVED TWO PRIOR TKIS

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Background: With the availability of several TKIs, in patients who have failed to 2 prior TKIs, anyone of the remaining TKIs can be considered for salvage therapy. However, further evaluation on the use of a novel TKI as third-line therapy is needed.
Aims: The aim of this study was to evaluate the response rates and outcomes of third-line TKI therapy in the treatment failed patients with chronic phase chronic myeloid leukemia who have received 2 prior TKIs.
Methods: We evaluated 100 CP CML patients who failed to 2 prior TKIs. Main study objectives were to evaluate major cytogenetic response (MCyR) by 12 months, cumulative incidence (CI) of major molecular response (MMR), progression-free survival, and overall survival. Molecular responses were monitored using qRT-PCR assay in3-month intervals, and then 6-month intervals after achieving major molecular response (MMR). MMR was defined as a BCR-ABL1 transcript level of 0.1% or lower on the international scale (IS).
Results: A total of 100 patients were received third-line therapy with dasatinib (n=48), radotinib (n=28), nilotinib (n=14), and bosutinib (n=10). 65 men and 35 women were included and their median age was 41 years (range, 18-73). The percentages of patients with low, intermediate and high Sokal risk scores were 19%, 30% and 17%, respectively with unknown risk score in 34%. 93 patients received IM as first-line therapy and 7 patients received 2G-TKI as first-line therapy. At the start of first-line therapy, 92 patients were in CP, 7 in AP, and 1 in BP. As second-line therapy, IM (n=7), dasatinib (n=43), nilotinib (n=24), radotinib (n=22), and bosutinib (n=4) were used. At the start of second-line therapy, 84 patients were in CP, 14 in AP, and 2 in BP. With a median follow-up of 20.1 months (range, 0.2-81.6 months) since the start of the third-line TKI, 55 patients continue on third-line TKI therapy. Forty-five patients were permanently discontinued from third-line TKI treatment due to intolerance (n=18), failure (n=6), progression (n=14), death (n=3) and others (n=4).Overall, 18 patients died. MCyR rate at 12 months after third-line TKI, in 31 patients with cytogenetic assessments at 12 months was 74.2% (23/31 patients). The3-year OS and PFS after third-line TKI were 81.1%and 76.0%, respectively. 50 patients with MCyR at the time of third-line TKI had a better 3-year OS (100% *vs*. 69%, P<0.001) and PFS (95.5% *vs*. 51.3%, P<0.001) than those of 50 patients without MCyR.
Summary and Conclusion: Our data showed the favorable outcomes of CP CML patients treated with a third-line TKI. The patients with MCyR at the time of third-line TKI showed a better outcome. It implies that the use of anyone of remaining TKI, as third-line therapy may induce feasible outcomes in CP patients. Further study focusing on selecting the patients who may benefit from a third-line TKI is needed.

P898

PREDICTIVE FACTORS FOR OUTCOME TO NOVEL BCR-ABL1 TYROSINE KINASE INHIBITORS IN IMATINIB FAILED CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Background: The first BCR-ABL1tyrosine kinase inhibitor (TKI), imatinib mesylate (IM), has become a first-line therapy for chronic phase (CP) chronic myeloid leukemia (CML).However, approximately one third of IM-treated patients discontinue therapy due to an inadequate response or adverse event. More potent novel TKIs such as dasatinib, nilotinib, radotinib, bosutinib and ponatinib have developed and these agents have shown high rates of hematologic and cytogenetic responses after failure of IM therapy. However, factors associated with long-term benefit from 2nd line therapy are not confirmed.
Aims: The aim of this study was to evaluate the predictive factors for outcome to novel TKIs in IM failed CP CML.
Methods: We evaluated 328 imatinib failed CP CML patients who had been treated with novel TKIs. Predictive factors for failure-free survival (FFS), progression-free survival (PFS), and overall survival (OS) were evaluated. FFS was measured from the day of 2ndline therapy initiation to death, progression to AP or BC, treatment failure according to 2013 ELN recommendations or contact, whichever came first. OS included any death regardless of causes, and PFS included progression to AP or BC as well as death resulting from any reason. OS and PFS were also collected on patients who were treated with other TKIs after 2nd-line TKI discontinuation.
Results: A total of 328 patients were treated with 2nd line TKI, dasatinib (n=131), nilotinib (n=103), radotinib (n=76), bosutinib (n=16), and ponatinib (n=2). 205 men and 123 women were included and their median age was 43 years (range, 15-77). The percentages of patients with low, intermediate and high Sokal risk scores were 23%, 31% and 24%, respectively with unknown Sokal risk scores in 22%. At diagnosis, 315 patients were in CP, 9 in AP, and 4 in BP. With a median follow-up of 28.4 months (range, 0.2-108.6 months) since the start of 2nd line TKI, 194 patients continue on therapy. 134 patients were permanently discontinued due to intolerance (n=74), failure (n=31), warning (n=1), progression (n=18), death (n=3) and others (n=7).The3-year FFS, PFS, and OS were 79.7%, 85.1%, and 89.1%, respectively. After adjusting for factors affecting FFS on univariate analyses, multivariate analyses showed that AP/BP at diagnosis and presence of BCR-ABL1 kinase domain abnormalities (KDA) at baseline were associated with a lower FFS. Less than PCyR on IM had a lower FFS, compared with those with CCyR on IM (RR of 3.25, P=0.016). AP/BP at diagnosis, presence of KDA at baseline and less than PCyR on IM were also associated with a lower PFS and OS. Increasing age was a predictive factor for lower OS.
Summary and Conclusion: Our data showed the predictive factors for outcome to secondline TKIs in imatinib failed CP CML. Disease phase at diagnosis, previously cytogenetic response on IM, and presence of KDA at baseline had the value of predicting the outcomes. It implies that the patients with these factors may require more precise monitoring on secondline therapy. Further study focusing on selecting the patients who may benefit from a third-line TKI is needed.

Chronic myeloid leukemia - Clinical 3

P899

OUTCOMES OF PREGNANCY AND THERAPEUTIC APPROACHES IN CHRONIC MYELOID LEUKEMIA DURING PREGNANCY

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Background: Therapy approaches during pregnancy in females with chronic myeloid leukemia (CML) are still discussable, practical experience is limited. Preventing of CML progression and fetus safety are main issues in management of CML at pregnancy.
Aims: To analyze pregnancy outcomes and management of CML during pregnancy.
Methods: A retrospective and prospective information about adult patients with Ph+ positive CML and pregnancy was gathered. Cases for CML females have been collected since 2006 till February 2014 from database of Hematology Research Center (Moscow) and clinics of Russian Federation. Data structure regarded CML treatment and monitoring, pregnancy data and outcomes, newborns characteristics and follow-up.
Results: An information about 54 completed cases has been recorded in 46 female patients (8 patients had 2 subsequent pregnancies). Chronic phase (CP) CML at diagnosis was in 44 women, accelerated phase (AP) was in 2 women. In 38 (70,4%) cases healthy children were born, 5 (9,3%) pregnancies resulted in spontaneous abortion, 2(3,7%) ended in premature delivery with death of children; in 9 (16,6%) cases an elective abortion was performed. In 28(74%) of 38 cases of successful delivery patients conceived during tyrosine kinase inhibitors (TKI) uptake: 23 on imatinib, 3 on dasatinib, 2 on nilotinib; 10 (26%) of 38 conceived without TKI therapy (5 of 10 with newly diagnosed CML). In 26 of 28 cases TKI treatment was discontinued immediately after pregnancy confirmation within the 1st trimester. Generally in 14 of 38 cases females were observed without therapy after the 1st trimester and for whole pregnancy period. In 13 of 14 cases deep remission at pregnancy diagnostics was achieved: molecular response 4 logs (MR4), major molecular response (MMR). In 1 of 14 cases therapy was postponed till delivery for woman with newly diagnosed CML in 3rd trimester. In 24 of 38 cases treatment interventions for CML were performed in 2nd – 3rd trimester due to lack of remission or for newly diagnosed CML. Females got the following therapy: interferon alpha in 9 cases, hydroxycarbamide in 2 and TKI in 12 cases (10 –imatinib, 2-nilotinib). In 2 of 12 cases TKI were used for whole pregnancy period. The infants born under TKI exposure had no birth abnormalities. However, a low weight (≤2500 g) was observed in 6 of 12 newborns exposed to TKI after 2nd trimester and/or for whole pregnancy. In 5 of 6 those cases there was a premature delivery. Further weight recovery and development of children were normal. Two cases of premature delivery and death of children were in females non-compliant to TKI with no treatment at pregnancy and hematologic relapse. Among 5 cases of spontaneous abortions 3 patients conceived on imatinib, for 2 data were not obtained. In 9 cases of elective abortions 6 were performed in women on imatinib, 2 on dasatinib, 1 case in newly diagnosed CML.
Summary and Conclusion: Management of CML at pregnancy is based on careful assessment of risks for female and fetus. Treatment approaches highly depend on grade of remission and pregnancy terms Choice of therapeutic interventions in patients with significant leukemic burden is a problem without standard decision. Although successful outcomes are possible in cases of using TKI on late stages of pregnancy, risks of TKI exposure at human fetus remain unknown. A safe way for risks minimization for women with childbearing potential with CML is to avoid pregnancy until achieving stable deep molecular remission.

P900

LONG-TERM EVALUATION OF VASCULAR TOXICITY IN PATIENTS WITH PH+ LEUKEMIAS TREATED WITH BOSUTINIB

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Background: Vascular toxicities (eg, peripheral arterial occlusive disease [PAOD]) have been associated with BCR-ABL tyrosine kinase inhibitor (TKI) treatment (tx).
Aims: We evaluated vascular (peripheral, cardiovascular, and cerebrovascular) toxicities based on tx-emergent adverse events (TEAEs) with bosutinib (BOS) in 2 ongoing studies.
Methods: This analysis included data from a phase 1/2 study of BOS 2nd/3rd/4th-line tx (2L/3L/4L, up to 3 y) in Ph+ leukemia patients (pts) resistant/intolerant to prior TKIs and a phase 3 study in chronic phase CML pts of BOS *vs* imatinib (IM) 1st-line tx (1L; up to 2 y).
Results: 12.7% of BOS pts had vascular TEAEs (**Table**), with no significant differences for BOS *vs* IM in the randomized frontline BELA study (1L: $P \geq 0.122$). Individual cardiovascular TEAE incidences were low; only angina pectoris (1.2%) and coronary artery disease (CAD 1.2%) occurred in >1% of BOS pts. No individual cerebrovascular TEAE occurred in >3 BOS pts. Individual peripheral vascular TEAEs were uncommon; only hypertension (6.4%) occurred in >2 BOS pts (1L: 6.0% [BOS] *vs* 4.4% [IM], $P=0.427$). Only 1 pt had PAOD (BOS 3L; y 1). Newly occurring vascular TEAE rates decreased with longer BOS tx (2L/3L/4L: y1, 40/570 [7.0%]; y 2, 21/273 [7.7%]; y 3, 8/208 [3.8%]; 1L [BOS *vs* IM]: y 1, 16/248 [6.5%] *vs* 9/251 [3.6%]; y 2, 5/183 [2.7%] *vs* 5/209 [2.4%]). Risk factors for vascular TEAEs ($P \leq 0.036$) were age ≥65 y (both studies) and history of vascular disorders (2L/3L/4L). Vascular TEAEs were managed mostly by concomitant medication (61.5% of affected pts); few pts required dose delays (n=15) or reductions (n=1). Discontinuation due to vascular TEAEs occurred in 6 (0.7%) BOS pts (2L/3L/4L: CAD n=2, myocardial infarction n=2, cerebrovascular accident n=1; 1L: cerebral haemorrhage n=1 [*vs* 0 IM, $P=0.497$]).

Table 1.

	Pooled	2L/3L/4L	1L	
	BOS* (n=813)	BOS* (n=570)	BOS* (n=248)	IM† (n=251)
Median (range) tx duration, mo	–	11.1 (0.03–83.4)	13.1 (0.03–49.6)	13.9 (0.3–46.9)
Vascular TEAEs, %	12.7	13.5	10.9	7.6
Cardiovascular	3.4	4.2	1.6	1.2
Cerebrovascular	1.8	2.3	0.8	0.4
Peripheral vascular	8.7	8.7	8.1	4.8

*200 or †400 mg/d starting dose

Summary and Conclusion: Vascular TEAE incidences with BOS in Ph+ leukemia pts were low individually and overall and not significantly different *vs* IM (1L). Peripheral vascular TEAEs, except hypertension (occurring in 6% of patients), are uncommon with BOS.

P901

CHROMOSOMAL ABNORMALITIES IN PHILADELPHIA CHROMOSOME (PH)-NEGATIVE METAPHASES IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE TREATED FRONTLINE WITH NILOTINIB OR DASATINIB

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Background: Patients(pts) with CML develop chromosomal abnormalities in Ph-negative metaphases while on treatment with tyrosine kinase inhibitors(TKI). Previous reports have focused on these events in pts treated with imatinib, but there is limited information on this phenomena among pts treated with nilotinib or dasatinib.
Aims: To determine the frequency of appearance of chromosomal abnormalities(CA) in Philadelphia chromosome-negative metaphases in newly diagnosed CML patients treated with nilotinib or dasatinib and to describe the significance of such events.
Methods: We conducted a retrospective analysis of all newly diagnosed CML pts treated with nilotinib or dasatinib as initial therapy in prospective trials at our institution between from July 2005 to August 2012. A review of all cytogenetic analyses was performed and pts who developed chromosomal abnormalities in Philadelphia chromosome-negative metaphases at any point in therapy were identified. Characteristics of such abnormalities such as time of appearance, number of metaphases in which they were seen and duration of persistence were noted.
Results: 245 pts were treated with nilotinib (n=124) or dasatinib (n=121). Out of these, 52 (21%) pts developed chromosomal abnormalities in Ph-negative metaphases. 35 (67%) were male. Median follow-up was 51 months. Median time from initiation of TKI to appearance of CA was 19.5 months. Frontline therapy was nilotinib in 33 (63%; 27% of all pts treated with nilotinib) and dasatinib in 19 (37%; 16% of all pts treated with dasatinib) patients. Nine (17%) pts had more than one CA either simultaneously or consecutively, for a total of 68 abnormalities. The most common CA was alterations of chromosome Y (deletion or addition) in 9 (17%) pts; the most common autosomal abnormality was deletion 10 in 3 pts. 44 (65%) events were seen in one metaphase only out of which 39 (89%) never reappeared. The remaining five the persisted were all sex chromosome abnormalities. 24 (35%) events were clonal, most commonly +8 and -Y, (3 pts each). 16 (67%) pts with clonal CA in Ph-negative metaphases continued to maintain chronic phase of disease during entire follow-up period. The rates of MMR and CMR (transcript ratio less than $10^{-4.5}$) in these patients were 90% and 58%, respectively, for pts with clonal CA; 97% and 55% for those with non-clonal CA; and 92% and 54% in those with no CA. Progression to accelerated or blast phase was seen in three (15%), five (16%) and 25 (13%) pts, respectively. A total of three pts with clonal CA in this analysis died: one in complete cytogenetic remission and two in major molecular remission at time of death. Causes of death were complication of esophageal cancer, myocardial infarction and sepsis, respectively. Only one pt received stem cell transplant for transformation to a lymphoid blast phase and continued to be in remission at last follow-up. None of the pts with CA has developed a second leukemia.

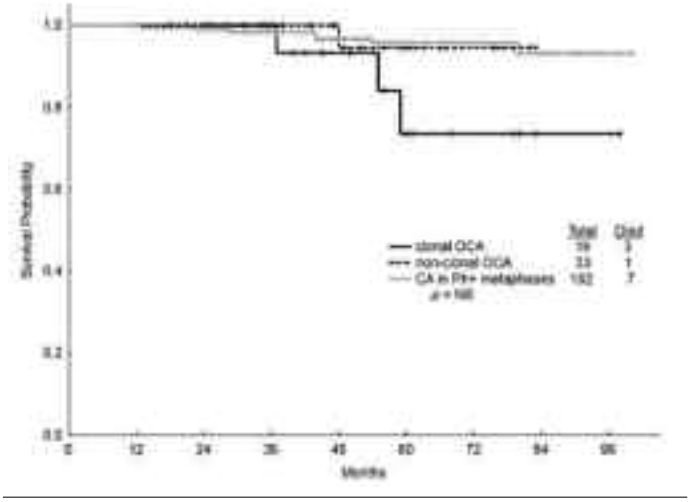

Figure 1.

Summary and Conclusion: Chromosomal abnormalities in Ph-negative metaphases in pts with newly diagnosed CML-CP treated with 2nd generation TKI are relatively common but bear no adverse consequences. In particular, no occurrence of second leukemias has been detected. Continued monitoring is required to better define significance of these events.

P902

CLINICAL EXPERIENCE OF BOSUTINIB UNDER COMPASIONATE USE PROGRAM IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO IMATINIB, DASATINIB AND NILOTINIB

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Background: Bosutinib (BOS) is a dual Src/Abl tyrosine kinase inhibitor that has confirmed a potent activity in patients (pts) resistant or intolerant to previous tyrosine kinase (TKI) inhibitors with a good safety profile. However, the European Medical Agency has approved BOS only for patients (pts) resistant or intolerant to imatinib (IM) and for whom nilotinib (NI) and dasatinib (DA) are not considered appropriate treatment options. Efficacy and safety data have been published of 115 pts who had been pretreated with IM and DA or NI, however only data of 3 pts have been collected in fourth-line setting.
Aims: The aim of this study is to present the first data reported of pts treated in Spain with BOS in previous heavily treated patients.
Methods: We have collected data of 29 pts among the 35 pts (85%) that have been treated with BOS since 2012 under a Spanish Compassionate Use Program in 16 centers. The study was approved by regulatory authorities and all pts signed informed consent document. Four patients (13%) received BOS in third line (after failure or intolerance to IM and NI or DA) while 25 pts were treated with BOS after receiving IM, DA and NI (one also received ponatinib). The most common indication was pts intolerant to three previous TKI (38%), while 24% were resistant to the 3 TKIs. Patients disposition and main baseline characteristics are shown in table 1.
Results: Considering those pts in fourth line (25), the median follow up was 7.23 months RI=[3,67_9,17]. Time to best response was 5.13 months RI=[2.10-8.32]. BOS was discontinued in 3 pts (12%): 1 death (after progression to advanced phase) (4%), 1 resistance (4%) and 1 intolerance (4%). Event free survival (Guilhot J, *et al.* Blood 119(25): 5963-5971) was 88%. BOS was usually well tolerated. Grade 3-4 anemia, neutropenia and thrombocytopenia occurred in 6%, 8% and 8% respectively. The most common nonhematological toxicities were diarrhea (48%), nausea (20%), rash (8%), increased ALT/AST (12%) and abdominal pain (12%). Grade 3-4 nonhematological toxicities were observed in 4 pts (1 nausea, 1 gastrointestinal bleeding, 1 ALT/AST elevation and 1 diarrhea). BOS has been proved to be a useful treatment option regardless the indication of use. Patients were classified according to the status when BOS was started. Group 1 consisted of 12 patients (46%) who started treatment as failures (less than CCyR); while 13 patients in group 2 (54%) started treatment with at least CCyR. Responses in group 1 were: CCyR 25% (3/12), MMR 16% (2/12), 75% (9/12) maintained baseline response and 8% (1 patient) progressed. For patients in group 2, probabilities of improve molecular response, sustained baseline response and progression were: 50% (4/8), 77% (10/13) and 15% (2/13) respectively.

Table 1.

Pts, N(%)		3 (13)	7 (24)	11 (38)	4 (14)	4 (14)	29 (100)
Age of diagnosis, med yr		43	47	55	61	54	54
Age of BOS initiation, med yr		47	61	67	72	61	61
Sokal index at diagnosis, N	high	0	1	0	1	0	2
	intermediate	1	2	5	1	1	10
	low	1	4	6	1	3	14
Time from first TKI to BOS, med yr		6	10	10	11	11	10
Duration of IM treatment, med, mo		16	16	23	62	87	60
Duration of DA treatment, med, mo		17	16	26	27	27	23
Duration of NI treatment, med, mo		10	14	10	29	24	16

Summary and Conclusion: We are presenting (to our knowledge) the largest series of patients treated with BOS forth line. Our study shows the results in 25 patients treated with bosutinib after failure or intolerance to 3 previous TKIs. In resistant patients, BOS has shown probabilities of CCyR and MMR (25% and 16%, respectively), similar to those described in third line. Bosutinib has also shown a very good safety profile in patients intolerant to 3 previous TKI, and one-half of them improved the response after switching to Bosutinib. Our results show Bosutinib is a safe and effective option for those patients in 4rd line of TKIs, Ongoing analysis of a larger series will be presented at the meeting.

P903

LONG-TERM ASSESSMENT OF CARDIAC TOXICITY IN PATIENTS WITH PH+ LEUKEMIAS TREATED WITH BOSUTINIB

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Background: Cardiac toxicities are a potential concern for long-term treatment (tx) with BCR-ABL tyrosine kinase inhibitors (TKI).
Aims: We evaluated cardiac toxicities based on reported tx-emergent adverse events (TEAEs) in 2 ongoing studies of bosutinib (BOS).
Methods: This analysis included data from a phase 1/2 study of Ph+ leukemia patients (pts) resistant/intolerant to prior TKIs (all with prior imatinib [IM]) on BOS 2nd/3rd/4th-line tx (>1L; up to 3 y) and a phase 3 study in chronic phase CML pts on BOS or IM 1st-line tx (1L; up to 2 y). A logistic regression analysis was performed.
Results: Factors associated with cardiac TEAEs were history (hx) of hyperlipidemia/increased cholesterol (both studies); age ≥65 y, hx of cardiac disorder, ECOG >0 (>1L); and hx hypertension (1L). A numerically higher incidence of cardiac TEAEs **(Table)** was seen in BOS- *vs* IM-treated pts (1L; not statistically significant [NS; Fisher's exact]). Individual cardiac TEAE incidences were low with BOS and NS *vs* IM (1L). In the 1L study, ECG QT prolonged was the only individual TEAE reported in >2% of BOS or IM pts (2.8% *vs* 3.2%). Newly occurring cardiac TEAE rates decreased over time on BOS (>1L: 11.2% [y 1], 7.0% [y 2], 6.3% [y 3]; 1L [BOS *vs* IM]: 8.5% *vs* 6.0% [y 1], 3.8% *vs* 2.4% [y 2]) for pts on tx during that year. Cardiac TEAEs were managed mainly by concomitant medication (40.3% of affected pts) and dose delays (25.8% of affected pts). Cardiac TEAEs leading to BOS discontinuation were low (2.0%; 12 pts >1L; 4 pts 1L): cardiac arrhythmias n=1, coronary artery disorders n=4, heart failures n=5, pericardial disorders n=5, ECG QT prolonged n=1; NS *vs* IM (1L: 4 *vs* 0; *P*=0.06).

Table 1.

	Pooled	>1L	1L	
	BOS* (n=418)	BOS* (n=370)	BOS* (n=248)	IM† (n=251)
Median (range) tx duration, mo	–	11.1 (0.03-83.4)	11.1 (0.03-49.6)	13.3 (0.1-48.9)
Cardiac TEAEs (any grade, HLGT), %	15.2	16.5	12.1	9.6
Cardiac arrhythmia	5.7	6.5	4.8	2.8
Coronary artery disorder	3.4	4.2	1.6	1.2
Heart failure	2.9	3.9	0.8	0.8
Myocardial disorder	2.1	2.8	0.4	0
Pericardial disorder	2.9	3.5	1.6	0
ECG QT prolonged, %	1.1	0.7	1.6	3.2

*500 or †400 mg/d starting dose

Summary and Conclusion: Cardiac TEAE incidences were generally low in BOS-treated Ph+ leukemia pts. Overall, high level and individual cardiac TEAE incidences were NS *vs* IM (1L). Heart failure incidence was low with BOS and similar to IM.

P904

DESIGN OF RAPID FLUORESCENT MOLECULAR BEACON BASED PCR ASSAY FOR DETECTION AND QUANTIFICATION OF BCR –ABL TRANSCRIPTS IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Chronic myeloid leukemia(CML) is clonal myeloproliferative neoplasm of hematopoietic stem cells which is consistently associated with balanced reciprocal translocatiion between chromosome 9 and 22. This creates a minute 22 chromosome called Philadelphia chromosome having the fusion gene BCR-ABL. In 95% of patients BCR-ABL fusion gene codes for oncogenic fusion protein with constitutive tyrosine kinase activity which is causal of morphological and clinical manifestations of the CML. Apart from 5% of atypical cases, a clear detection of BCR-ABL fusion gene/transcript qualifies a person to be a patient of CML. The treatment mainly involves Tyrosine Kinase Inhibitors(TKIs) like imatinib that results in complete remission in 60-70% of patients and also increases the overall survival. A peripheral blood smear with cytogenetics of bone marrow is the gold standard for initial detection worldwide. In developing countries like India except peripheral smear,cytogenetics diagnostic is not always tenable. Lack of proper laboratory facilities and cost of undergoing cytogenetics initially and at regular intervals is unaffordable by substantial population of patients. Moreover the sensitivity of the technique is not more than 1-5%, and disadvantages also include high cost,dependence on metaphases and the fact that it is an invasive procedure. Another technique which is recommended by WHO/ELN is real time RT-PCR for dose response monitoring and MRD (minimal residual disease) detection. Realtime RT-PCR is very expensive and time consuming given the prolong nature of disease

which warrants regular detection at defined intervals.Therefore once again it is not the method of choice in developing countries like India

Aims: To design a rapid, inexpensive, easy visualization method for detection and quantification of BCR-ABL transcripts in chronic myeloid leukemia patients.

Methods: Peripheral blood samples of untreated patients were collected after informed consent. RNA was isolated by trizol method and cDNA prepared using commercial kit.The quality of the cDNA was tested with five housekeeping genes. In house primers were designed for BCR–ABL amplification. Specific molecular beacons were used to detect and quantify transcripts by measuring fluorescence in an ELISA reader. The assay was evaluated against qPCR using published primers. The study was approved by the institutional committee sofAIIMS, Safdurjung Hospital No. 14-11-EC (19/26) and ACBR (No. F.50-/Eth.com/ACBR/11/2106).

Results: An molecular beacon based PCR assay was developed for easy and quick visualization of BCR-ABL transcripts. The specificity of the assay was confirmed by competitive experiments. The assay was found to be sensitive enough to detect 500 fg of plasmid containing the chimeric cDNA. A linear increase in fluorescence was observed with increase in template concentration in the PCR reaction (Figure 1:Semi log plot of Plasmid DNA (clone containing the BCR-ABL insert) concentration *vs* fluorescent intensity obtained by hybridization of 65 nM of molecular beacon with amplicon b2a2 obtained by PCR of BCR-ABL plasmid). Clinical evaluation of assay was done using 50 patient samples.

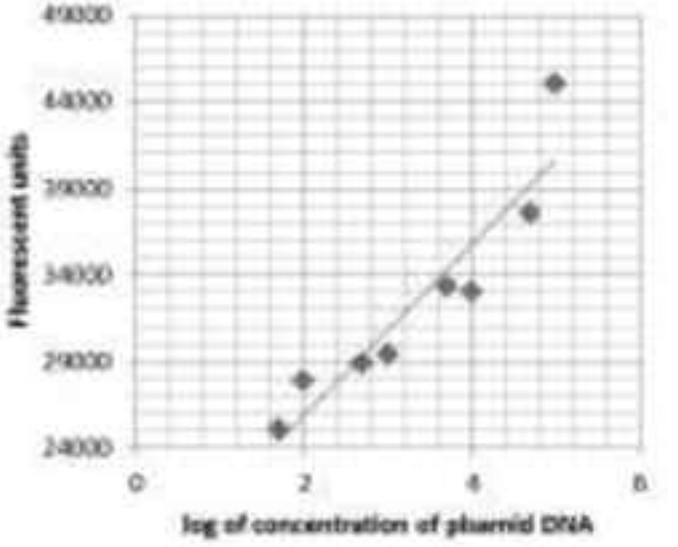

Figure 1.

Summary and Conclusion: We propose that beacon based method can simultaneously detect and quantify the BCR-ABL transcripts in rapid and easy manner when the patient approaches for diagnosis thus eliminating the need for cytogenetics and qPCR. The current method is as sensitive and specific as commercially available qPCR. Since the method is cost effective, and does not require expertise, therefore we envisage that we can use it in resource poor settings.

P905

HYPOPHOSPHATEMIA AS A PREDICTOR OF RESPONSE IN CHRONIC MYELOID LEUKEMIA (CML) TREATED WITH IMATINIB

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Background: Response to treatment with Imatinib is uneven among patients with CML. Hypophosphatemia has been described has an adverse effect and, although its pathogenesis is still unclear (Imatinib-induced renal tubulopathy, dysregulation of bone remodelling, secondary hyperparathyroidism) it may also play a role in monitoring adherence, efficacy, and even predicting response.

Aims: To look for clinical/laboratorial factors associated with hematologic (CHR), complete cytogenetic (CCyR), major (MMR) and complete molecular remission (CMR).

Methods: We assessed clinical records of 135 patients with chronic phase CML diagnosed in a single centre between 2001 and 2012. Median follow-up from start of Imatinib was 69months [8;168].

Results: The median age at diagnosis was 51years [16;89], and 73(54.1%) patients were male. 28 patients showed resistance to Imatinib, less frequently in those with hypophosphatemia (<2.7mg/dL) during the first year of treatment (p=.008). 103(73.7%) patients achieved complete hematologic remission (CHR) within 3months; 64(47.4%%) achieved CCyR within 6 months; 40(29.6%) reached MMR within 18months; and 32(23.7%) achieved CMR. Among these 32 patients, 59.4% were female (p=.048); 62.5% reached CCyR within 6 months (p=.026); and 50% had MMR within 18months (p=.006); additionally, there was an inferior median LDH at diagnosis for this group (626 *vs*. 743U/L; p=.025). The presence of hypophosphatemia at 6 and 12months of treatment was correlated with a higher probability of achieving CCyR within 6months (p=.001) and MMR within 18months (p=.04), respectively. 81.8% of the patients achieving CMR had hypophosphatemia during the first year of treatment (p=.08). On the other hand, age, Sokal/Hasford risk stratification, and other laboratory parameters at diagnosis were not correlated with response. Seventeen (12.5%) patients progressed to accelerated disease, 14 had blast crisis; and 21(15.6%) died (11 due to CML). The 10-year progression-free survival (PFS)/ overall survival (OS) were superior in those patients with CCyR (PFS: 88.6% *vs*. 0%; p<.0001/ OS: 83.1% *vs*. 29.9%; p<.0001); MMR (PFS: 96.5% *vs*. 23.5%; p<.0001/ OS: 86.6% *vs*. 41.8%; p<.0001); CMR (PFS 100% *vs*. 56.2%; p<.0001/ OS: 100% *vs*. 72.5%; p=.005); as well as those with hypophosphatemia during the first year of treatment (PFS: 82.9% *vs*. 58.3%; p=.012/ OS: 86.8% *vs*. 53.9%; p=.021).

Summary and Conclusion: In our group of patients, hyphosphatemia seems to correlate with early, deeper responses and improved survival, so it may establish itself as an important biomarker while monitoring patients treated with Imatinib.

P906

RISK AND IMPACT OF TUBERCULOSIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A NATIONWIDE POPULATION-BASED STUDY IN TAIWAN

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Background: BCR-ABL tyrosine kinase inhibitors (BCR-ABL TKIs) have greatly improved treatment responses, overall survival, and disease-free survival in patients with chronic myeloid leukemia (CML). Some recent studies have reported an increased risk of tuberculosis (TB) infection during BCR-ABL TKI treatment, but none of these studies contained adequate comparison groups. Moreover, inferring increased TB risk during BCR-ABL TKI therapy is questionable because BCR-ABL TKIs improve hematopoietic function and patient immunity. It is impossible to conduct randomized studies that compare TB risk during BCR-ABL TKI treatment with a placebo group due to ethical issues of withholding TKI therapy. Therefore, a well-designed large-scale study is the best way to address this issue.

Aims: We aim to evaluate the relationship between CML and TB, and to determine the risk and the impact of TB development.

Methods: A national survey included 1,082 CML patients retrieved from the Taiwan National Health Insurance database between 1998 and 2011. A total of 10,820 subjects without CML that were matched for age, sex, and comorbidities comprised the matched non-exposed cohort. TB development was the main outcome; its impact was measured by the overall mortality, and the risk factors were identified by a multivariate Cox proportional hazards model.

Results: We found that the risk of TB was higher in the CML cohort (adjusted HR 3.76, 95% CI 2.24–6.31, p=0.001), for both pulmonary (adjusted HR 3.23, 95% CI 1.82–5.73, p<0.001) and extrapulmonary (adjusted HR 9.77, 95% CI 2.61–36.59, p=0.001) TB. Specific risk factors were age 60 or older (HR 3.24, 95% CI 1.19–8.83, p=0.022),being male (HR 13.49, 95% CI 1.79–101.54, p=0.012), receiving hematopoietic stem cell transplantation (HR 10.50, 95% CI 2.77–39.80, p=0.001), and interferon-α therapy (HR 3.34, 95% CI 1.32–8.47, p=0.011). BCR-ABL TKIs did not increase the risk of TB in patients with CML (HR 0.51, 95% CI 0.19–1.38, p=0.184). CML patients with TB had a higher mortality rate than those without (adjusted HR 2.04, 95% CI 1.02–4.08, p=0.043).

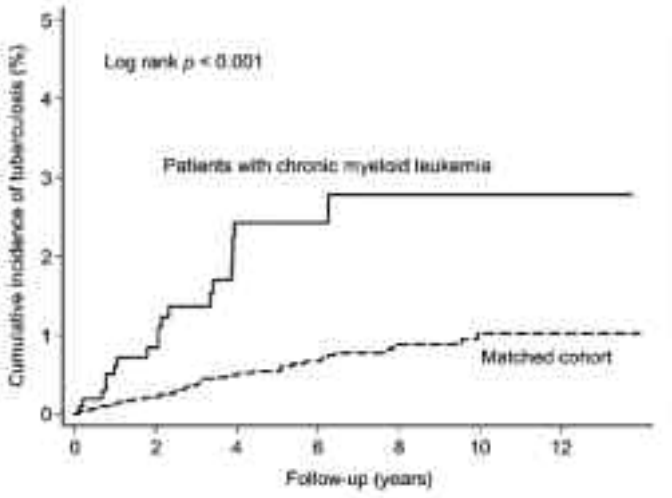

Figure 1.

Summary and Conclusion: The incidence of TB is significantly higher in CML patients, especially for patients that are male, aged 60 or older, or those who received either hematopoietic stem cell transplantation or interferon-α treatment. Careful screening strategies for TB should be considered for high-risk CML patients.

P907

MULTICENTER CLINICAL STUDY TO EVALUATE A UTILITY OF FRET-BASED DRUG SENRITIVITY TEST THAT PREDICTS EARLY MOLECULAR RESPONSE IN NEWLY DIAGNOSED CML PATIENTS TREATED WITH NILOTINIB

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Background: The tyrosine kinase inhibitors (TKIs), imatinib, nilotinib and dasatinib are used as the first-line therapy for newly diagnosed chronic myeloid leukemia (CML). To predict a clinical outcome, Sokal, Hasford and EUTOS scores are widely used. But it is controversial that these methods are useful in 2nd generation TKI era. We have developed a FRET (fluorescence resonance image transfer)-based method to evaluate sensitivity of CML cells to TKIs (Mizutani *et al.* Clin Cancer Res. 2010).
Aims: In this study, we evaluate a clinical utility of FRET-based drug sensitivity test in newly-diagnosed CML-CP patients treated with nilotinib.
Methods: We conducted Eso-FANTA study (Estimation of outcome by FRET Analysis for Newly diagnosed CML-CP patients treated with Tasigna). In this multicenter phase II clinical trial, nilotinib (300 mg BID) was administered and the molecular responses were monitored. FRET analysis was performed to analyze the drug-sensitivity at the diagnosis. Bone marrow mononuclear cells were isolated and transfected with FRET probe. After 18 to 24 hours of transfection, the cells were treated with 2 microM nilotinib and then subjected to microscopic analysis to determine FRET efficiency. RQ-PCR evaluation of BCR-ABL mRNA was performed to monitor the clinical efficacy of nilotinib.
Results: From October 2010 to January 2014, 31 patients were registered in the study, and the median follow-up period was 9 months (range: 1-24). Nilotinib sensitivity was evaluated in 30 patients. By FRET analysis, 22 patients were determined nilotinib-sensitive, and 8 patients were nilotinib-insensitive. There was no significant difference of Sokal Low/Intermediate and High Risk distribution in two groups. Compared nilotinib-sensitive patients with nilotinib-insensitive patients, the rates of MMR were significantly higher; 91% *vs* 43% at 6 months (p=0.031) and 100% *vs* 43% at 9 months (p=0.023).

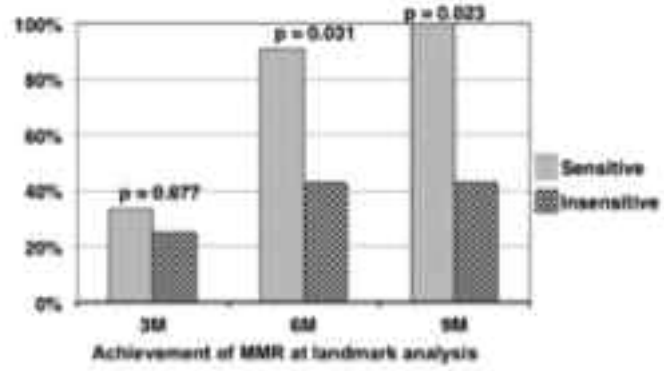

Figure 1.

Summary and Conclusion: The FRET analysis could predict the clinical efficacy of treatment with nilotinib before starting treatment. Our method will add the new tool to stratify the patients with newly-diagnosed CML-CP.

P908

FIRST PRELIMINARY REPORT OF ARAB LEUKEMIA NET (ALN) REGISTRY FOR CHRONIC MYELOID LEUKAEMIA (CML) IN THE MIDDLE EAST &NORTH AFRICA REGION.PART I EPIDEMIOLOGY OF CML IN EGYPT

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Background: Little is known about burden of CML in Middle East/ North Africa region. unpublished observations sugested a much younger incidence and the presence of diseae clusters in certain localities.Reliable epidemiological information on BCR-ABL-positive CML, is rare.Geographic and/or ethnic variations contribute to the variability of incidences among registries. Prevalence rate has increased by use of TKIs. In practice,reliable data concerning response rates to therapy in Arab nations is lacking. some CML management areas are not in line with the current recommendations. Other problematic areas are sub-optimal timing of treatment decisions under monitoring, unawareness and/or lack of new molecular monitoring techniques and of beneficial newTKIs. Median age differs between cancer registries and clinical trials by 10-20 years. Reports of clinical studies underestimate the true age of the CML population depending on the ease of access to medical servises that show great diversity in Middle East/ North Africa region.
Aims: Objective To understand and compare studies results of the previous and future and to translate their conclusion into clinical practice, there is a need for reliable basic epidemioloic data collected and analyzed through standardised CML studies designed according to recognized recommendations.
Methods: Method We analyzed data of 350 CML Egyptian patients (171 male and 179 female) Data were collected according to ELN and EUTOS recommendations by using a multicenter web based data registery portal, the ALN. (**www.aln-afme.com**).
Results: Patients Mean age at presentation was 41y (40y for males, 41y for females), The age specific rates were highest for the age group of 30-35 years. At diagnosis 84% patients were in chronic phase CML, 9.1% in accelerated, and 6.9% in blastic phase. Results of Sokal score were: Low risk 55.8% Intermediate risk 24.5%High risk 17.7% and Unknown in 2%. EURO (Hasford) score (56.3% Low risk, 18.4%Intermediate risk and13.6%High risk while 2.7% Unknown).Female patients presented with lower hemoglobin, higher platelet counts and smaller spleen size (P<0.0001).BCR-ABL transcript level was performed to 96.6%of cases Cytogenetics by FISH to 77.6%. All patients recived TKI therapy,(61% imatinib,24 nilotinib and 15 dasatinib).96%of patients achived hematologic respone,87% achived PCYR,64% achived. CCYR, and 60%MMR within a peroid of 4 years follow-up. The transplantation rate was 14% for female and 22% for male patients. Median survival /progression-free survival were equal in female and male patients.
Summary and Conclusion: We examined the demographic and clinical features of CML patients presenting at Medical Centers in Middle East/ North Africa region by analyzing data reported to ALN web portal. The study demonstrates that age-specific rates for CML are highest in age group of 30-34 years,that is much lower compared to western populations.No gender differance was found. PFS were equal in female and male patients.

P909

UPDATED REPORT OF THE AUSTRIAN CML REGISTRY

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Background: Recommendations for treatment of chronic myeloid leukemia (CML) have been constantly revised since tyrosine kinase inhibitors (TKIs) have become standard of care. While treatment goals have become more stringent and the achievement of early and deep molecular responses as well as TKI treatment discontinuation strategies are being evaluated in clinical trials, population based data on treatment, response and survival of patients treated throughout that period reflect the real life situation.
Aims: The Austrian CML registry aims to monitor and analyse outcome, disease course and management as well as related toxicities in a nationwide unselected patient cohort to provide data reflecting general pratice and contrast these results with clinical trial data.
Methods: The Austrian CML registry is an ethics committee approved online database with a web-based tool for data entry. Participating centres can contribute patient data after having obtained written informed consent. The collected data comprise diagnostic parameters, CML phase, concomitant diseases, CML specific treatment including stem cell transplantation and adverse effects.
Results: Here we summarize the CML registry data as of January 2014 with a focus on treatment, response and survival. The CML registry cohort comprises a total of 410 patients (evaluable n=400) with a total of 3300 follow-up visits. At diagnosis most patients (n=374) were in chronic phase (early, late, secondary), whereas only 9 were in accelerated phase, 3 in blast crisis. A total of 354 patients were treated at least once with imatinib and 73 and 50 patients received at least once nilotinib or dasatinib, respectively. Other treatment modalities included chemotherapy, interferons and stem cell transplantation (SCT). Calculcated overall survival at 108 months was 84.2% (CI: 78.3.2-90.5). We will present an updated response analysis according to ELN recommendations 2013 and TKI usage.
Summary and Conclusion: The majority of patients is treated with imatinib and overall survival in this population based cohort is comparable to what has been observed in clinical trials. Second generation TKIs are being used predominantly in second line treatment, their usage in front line therapy is still limited.

P910

10 YEARS EXPERIENCE OF TYROSINE KINASE INHIBITOR THERAPY FOR CML IN A SINGLE UK CENTRE: COMPARISON WITH PRE-TKI ERA AND EVALUATION OF 2013 ELN GUIDELINES

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Background: Routine use of tyrosine kinase inhibitor (TKI) therapy for chronic myeloid leukaemia (CML) was approved in the UK in 2002, transforming the treatment of this disorder. European LeukaemiaNet (ELN) 2013 guidelines include a new emphasis on early markers of response to guide switch to second generation TKIs, with important clinical and financial implications given the imminent loss of patent exclusivity on imatinib.
Aims: To assess the impact of TKIs on overall survival (OS) of CML at our institution & benchmark the outcome of TKI treated patients with 2013 ELN guidelines in a "real world" cohort.
Methods: We retrospectively reviewed outcome of all chronic phase CML patients treated in our centre in 2 cohorts; a 1990-1995 (pre-TKI) cohort and a 2000-2014 (post-TKI) cohort.
Results: Outcome of 102 CML patients was examined. There were 26 patients in the pre-TKI cohort and 76 patients in the post-TKI cohort, of whom 66 received first line TKI therapy with imatinib (n=56), nilotinib (n=6), dasatinib (n=3) and ponatinib (n=1). 10 had allogeneic bone marrow transplants as first line therapy and were excluded from analysis of TKI response. The median age was 52 (18-86) and 50 years (24-81) in the pre- and post-TKI groups respectively. Comparison of pre- and post-TKI cohorts demonstrated a dramatic improvement in survival post approval of imatinib in the UK with an 8 year survival of 14.8% and 91.1% respectively and median survival of 2.3 years and 'unreached' respectively (P<0.0001; Figure 1). Indeed, with a median follow-up of 4.6 years, only 4 deaths occurred in the post-TKI cohort, none related to CML. Accelerated phase (AP) and blast crisis (BC) occurred in 2 and 1 patient respectively. Allogeneic bone marrow transplant was carried out in two and the other patient in AP continued on first line TKI to complete cytogenetic remission (CCyR). Sufficient data to allow assessment of ELN response criteria was available in 57 patients. Optimal response throughout treatment was observed in 18 patients, with only 2 requiring switch to second line therapy due to intolerance. Major molecular remission (MMR3) was achieved in 16 (89%) with 10 (56%) complete molecular responses (CMR). ELN defined "warning" (without subsequently meeting criteria for failure) was observed in 17 patients. No progression to AP or BC was observed. Only 3 patients switched therapy for inadequate response (n=2) and intolerance (n=1). CCyR was achieved in 12 (71%), with 11 (65%) MMR3 and 1 (6%) CMR. ELN defined treatment failure at any time was observed in 22 patients, of whom 16 had a preceding ELN defined "warning". AP or BC subsequently occurred in 3 (14%; P<0.01 *vs* non-failure patients). 3 patients developed kinase domain mutations. 14 patients were switched to second line therapy, only 2 due to intolerance. CCyR was achieved in 7 (32%), with 2 (9%) MMR3 and 0 CMR. 5 patients proceeded to allogeneic bone marrow transplant. Non-relapse mortality occurred in one patient and 4 are alive and in CMR. Molecular response data was available on 31 patients at 3 months. A warning (>10% BCR-ABL), without meeting failure criteria, occurred in 14 patients. Of these early warnings, 11 patients out of 14 have achieved CCyR (79%), of whom 3 (21%) achieved MMR; 6 of these CCyR were achieved without switching therapy.

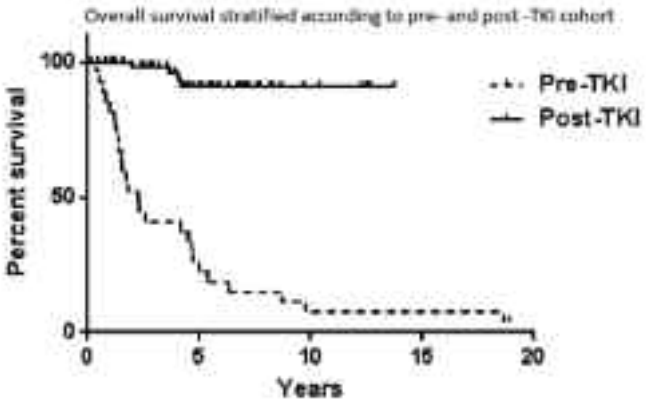

Figure 1.

Summary and Conclusion: In our single-institutional experience, first-line TKI therapy has dramatically improved OS in CML. Despite optimal response in only 32% of patients, disease progression events were rare. Most patients with "warning" at 3 months achieved CCyR, including patients who did not switch therapy, supporting ELN guidance that a "warning" should prompt close monitoring but only "failure" should prompt switch in therapy. This retrospective analysis is being extended to a further 100 patients across the Thames Valley and these results will be presented at the congress.

P911

CHALLENGES OF COMPOSITE ENDPOINTS FOR CML TRIAL EXECUTION, STATISTICAL ANALYSIS AND INTERPRETATION

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Background: Recent years have seen an exceptional improvement of overall survival (OS) in patients with chronic myeloid leukemia. Thus clinical trials using classical endpoints like OS would need either huge sample sizes or very long observation times. Composite endpoints (CE), combining survival with events like progression, not achieving complete cytogenetic or molecular remission within a certain time period (typically 12 or 18 months) or loss of remission, called progression-free survival (PFS) or failure-free survival (FFS) were introduced to allow for a faster evaluation of new treatments.
Aims: To elucidate and discuss the challenges of CE for CML trial execution, statistical analysis and interpretation.
Methods: Challenging issues of CEs in particular for the comparative interpretation of CML trials will be exemplarily discussed on the basis of two major CML trials published in 2010 (Saglio G *et al.* NEJM 2010;362:2251-59, and Kantarjian HM *et al.* NEJM 2010;362;2260-70).
Results: FFS subsumes ~ 10 singleton events ranging from death to loss of response. FFS is thus difficult to interpret and provides limited value only for informing clinical decisions as the relevance of the singleton events is very heterogeneous. Hence drug authorities like the FDA ask that CE do not mix disease outcomes with clinical management decisions. An often neglected issue is that the intention to treat analysis of CEs requires that all singleton events have been properly monitored and assessed at the time points specified in advance in the trial protocol. Thus in a large CML trial out of 1466 evaluable patients only 571 were available for the analysis of FFS (Lauseker M *et al.* Frontiers Meeting 2012). Including in the statistical analysis patients with missing information on singleton events may seriously bias results. Yet another

problem is the common lack of widely accepted uniform definitions of CEs precluding a comparative assessment accross trials, *e.g.* the definition of PFS differs between the two CML trials mentioned above.
Summary and Conclusion: There is an urgent need for consented CEs and for the development of more suitable alternatives to CEs.

P912

GENOMIC BCR-ABL1 FUSION CHARACTERISATION AND SNPS IDENTIFICATION UPSTREAM AND DOWNSTREAM OF THE BREAKPOINTS IN BCR AND ABL1 GENES OF CML PATIENTS USING NEXT GENERATION SEQUENCING

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Background: BCR-ABL1 genomic fusion sequences represent clone-specific markers for each patient with chronic myeloid leukemia (CML). Their identification allows more sensitive monitoring of minimal residual disease at DNA level. As BCR-ABL1 is a large fusion gene the characterization of DNA breakpoints is challenging. In this study we used multiplex long-range PCR (mLR-PCR) with primers enabling to amplify large PCR products (>10 kb) carrying BCR-ABL1 breakpoints. LR-PCR products were directly sequenced using next generation sequencing (NGS). Amplification of large regions upstream and downstream of the breakpoints allows identification of SNPs and other sequence features.
Aims: We aimed to characterize genomic BCR-ABL1 breakpoints and to identify SNPs in CML patients.
Methods: Two BCR-ABL1 positive cell lines (K562; JURL-MK1) and 48 CML patients who achieved a deep molecular response were included in this study. DNA was isolated from leukocytes of peripheral blood at diagnosis. We performed 2 rounds of mLR-PCR. In the 1st round, 1 forward primer located in BCR exon 13 and 10 primers located in intron 1 of ABL1 (Lange et al 1999) were used. LR-PCR products were obtained in 28/49 cases. The 2nd round of mLR-PCR was performed for the rest of samples using 1 BCR and 20 ABL1 primers (Krumbholz et al 2012, Ross et al 2010, Score et al 2010). LR-PCR products were fragmented and rapid library was prepared for 454 NGS technology (Roche Applied Science). NextGENe software (Softgenetics) was used for sequence analysis, breakpoints characterisation and SNP calling. NCBI database and reference sequences for BCR and ABL1 genes were used for breakpoints localization and SNP calling.
Results: Median length of LR-PCR products was 6 kb (range 1.5-10kb). NGS was performed in 41 samples. Characterized breakpoints were confirmed by Sanger sequencing. BCR breakpoints were located in intron 14 in 32/39 patients and in both cell lines, all carrying e14a2 mRNA, and in intron 13 in 7/39 patients with e13a2 mRNA. All ABL1 breakpoints were dispersed across intron 1. In BCR region upstream of the breakpoints, 17 annotated SNPs (1 SNP located in exon 13, 4 in intron 13 and 12 in intron 14) were detected among patients and cell lines with e14a2 mRNA (median 3 SNPs/patient or cell line, range 1-12). The frequency of minor alleles of detected SNPs varies from 0.5% to 30.7% in the 1000Genome phase 1 population (NCBI SNP database). Novel SNPs were identified in 3 patients with e14a2 mRNA in exon 13, intron 13 and exon 14. Interestingly, we did not detect any SNPs upstream of the BCR breakpoints among patients with e13a2 mRNA, except one with 3 SNPs detected. Based on SNP profiles in the BCR region we performed hierarchical clustering analysis showing 3 main distant clusters of patients; the cluster consisted of patients with e13a2 mRNA was distant to other 2 clusters which consisted of patients with e14a2 mRNA. In the downstream region of the breakpoints in intron 1 of ABL1, 51 different SNPs were identified in 12/39 patients and JURL-MK1.
Summary and Conclusion: Sanger sequencing is not suitable for sequencing of long PCR products, thus an alternative approach had to be sought for BCR-ABL1 fusion identification. The mLR-PCR followed by NGS accelerated the whole process. Moreover, this approach enabled to analyse long sequences downstream and upstream of the BCR-ABL1 fusion and to identify other sequence features. SNPs in introns may alter gene splicing and coding SNPs may alter protein conformation. Interestingly, we identified multiple SNPs upstream of the genomic breakpoints in BCR gene in all CML patients with e14a2 mRNA. No SNPs were observed in all but one patient with e13a2 mRNA. Based on the SNP profile in the BCR region upstream of the breakpoints, 3 main distinct patient clusters were formed. Further studies are warranted to find the potential association between the SNP profile, patient characteristics and outcome. Supported by IGA/NT11555 and the project for conceptual development of research organization (00023736) from the Ministry of Health of the Czech Republic.

P913

THE EFFECT OF DELAYED TKI TREATMENT ON CYTOGENETIC AND MOLECULAR RESPONSES: 8 YEAR FOLLOW-UP STUDY IN BOSNIA

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Background: Due to the inadequate health care system in Bosnia, CML patients do not get TKI therapy immediately after diagnosis. Only 16% of patients received immediate TKI treatment (within 3 months of diagnosis) from 07/2005-12/2013. About 66% of patients received therapy after 14-month median wait period.
Aims: We systematically studied the effect of delayed TKI (imatinib and nilotinib) treatment on cytogenetic and molecular responses.
Methods: This is a multicenter retrospective cohort study of all CML patients (n=135) in Federation of Bosnia and Herzegovina from 07/2005-12/2013. Patients received imatinib (once daily 400 mg or 600 mg), nilotinib (twice daily 300 mg or 400 mg) or no TKI therapy. The cutoff date was 31.12.2013, on the basis of 18 month median time on nilotinib therapy. Patients were grouped based on the length of treatment delay: immediate (0-3 months), 4-6 months, 7-12 months, and >13 month delay. Patient data was collected and analyzed in SPSS v.18.
Results: We stratified patients (n=135) into following groups: immediate therapy (16%), 4-6 month wait (13%), 7-12 month wait (15%), >13 month wait (37%), and never received TKI therapy (18%). Basic cohort characteristics and distribution of EUTOS risk score in the studied groups were well balanced. The median duration of treatment for imatinib was 36 months and nilotinib 18 months. At 60 months, the estimated rate of survival was 0% for patients who never received TKI, 91% for immediate TKI treatment (0-3 months), 80% for patients who waited 4-6 months, 86% for patients who waited 7-12 months, and 64% for patients who waited >13 months ($p<0.001$). Cytogenetic responses had highly significant associations with delayed TKI treatment. At 12 months, complete cytogenetic response (CCyR) was achieved by 70% of patients with immediate TKI treatment (nilotinib or imatinib), compared to 25% of patients with >13 month wait ($p<0.001$). At 36 months, 92% of patients with immediate treatment achieved CCyR compared to 47% of patients with >13 month wait. Molecular responses also showed highly significant associations with delayed treatment. At 24 months, 56% of patients with immediate treatment achieved MMR, compared to 26% of patients who waited >13 months ($p<0.01$). Since patients were treated with two different TKIs, imatinib and nilotinib, we further categorized patients according to the treatment drug. CCyR of patients treated with imatinib alone showed significant differences. At 12 months, 67% of patients in the immediate imatinib treatment group achieved CCyR compared to 15% of patients in >13 month wait group ($p<0.005$). The achievement of MMR showed even more drastic changes. MMR at 12 months occurred in 53% of patients with immediate treatment compared to 0% of patients in >13 month wait group ($p<0.02$). The achievement of CCyR and MMR in patients treated with nilotinib as the first line showed no association with length of treatment delay. Patients who waited >13 months had same response rates as those who received immediate treatment (86% v. 75% at 6 months or 86% v. 80% at 12 months, respectively). Similarly, MMR rates in patients treated with nilotinib in different wait groups were indistinguishable. At 12 months, 43% of patients with immediate treatment achieved MMR compared to 50% of patients who waited >13 months.
Summary and Conclusion: Patients achieved much better CCyR and MMR on nilotinib than imatinib, regardless of the length of delay to therapy. Patients on imatinib had worse response rates if they waited for treatment. Our recommendation is that if treatment is delayed for more than 3 months, the preferred treatment drug is nilotinib.

P914

THE TYROSINE KINASE INHIBITOR AXITINIB TARGETS T315I-MUTANT BCR-ABL DRIVEN LEUKEMIAS IN VITRO AND IN VIVO

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Background: The use of ABL1 kinase inhibitors has dramatically improved the outcome for chronic myeloid leukemia (CML) and Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL) patients. However, resistance after treatment still poses a major clinical challenge. The most common resistance mechanism following treatment with first or second line therapy is the occurrence of a T315I mutation in the kinase domain of BCR-ABL1. Only one

clinically available ABL1 inhibitor, ponatinib, has been shown to target this gatekeeper mutation, but has recently been associated with significant adverse effects. Hence, there is an unmet need for new and improved therapies for patients with T315I BCR-ABL1 leukemias.
Aims: In this study we set out to functionally and molecularly profile BCR-ABL1 T315I-driven CML/Ph+ALL patient samples to understand the disease pathogenesis and identify novel therapies with a drug sensitivity and resistance testing (DSRT) platform covering 306 approved and investigational oncology compounds.
Methods: Mononuclear cells isolated from patient bone marrow were plated with drugs on 384-well plates. Each compound was tested for its effect on cell growth and survival in a 10.000-fold concentration range enabling the generation of dose response curves and by comparing to healthy donor mononuclear cells, selective drug sensitivity scores (sDSS).
Results: *Ex vivo* DSRT results of one CML and two Ph+ALL patient samples with the T315I mutation revealed a marked and specific sensitivity (IC50 30-40nM) to the tyrosine kinase inhibitor axitinib, originally developed as a VEGFR inhibitor. Strikingly, sensitivity to axitinib was higher in these T315I positive patient samples than in T315I negative CML or ALL patient samples or any other leukemic samples. Supporting the notion that axitinib is a direct T315I BCR-ABL1 inhibitor we observed that Ba/F3 cells transformed with T315I BCR-ABL1 were sensitive to axitinib while the same cells transformed with wild type BCR-ABL1 were not. Finally, we discovered that axitinib has been described to have selective binding towards T315I ABL1 compared to the wild type kinase (Kd 1.5 nM *vs*. 36 nM, respectively, Davis *et al.* 2011, Nat. Biotechnol. 29:1046-1051). Based on this information, the T315I CML patient mentioned above was compassionately treated with axitinib for 2 weeks resulting in a rapid 4-fold reduction of the mutated transcript levels in blood suggesting targeted *in vivo* activity of the drug.
Summary and Conclusion: In summary, we demonstrated that axitinib is a potent BCR-ABL1 T315I inhibitor both *in vitro* and *in vivo*. In light to the fact that axitinib is currently approved as a second line therapy for renal cell carcinoma and is well tolerated in patients, there is an opportunity to repurpose axitinib for Ph+ leukemia patients with T315I mutations with significantly shorter clinical development time.

P915

BCR-ABL1 KINASE DOMAIN MUTATION ANALYSIS USING AMPLICON DEEP SEQUENCING ON THE ION TORRENT PLATFORM

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Background: *BCR-ABL1* mutation analysis is an important factor in CML therapy intervention. The majority of the mutations identified are associated to some degree with resistance to imatinib, which is the main therapy available upfront for CML patients. 2nd and 3rd generation tyrosine kinase inhibitors, which have been widely used as 2nd line following imatinib failure and recently have been approved as a first line therapy, have been shown to having selective inhibitory action on some mutations while being ineffective against others. T315I, F359V/C, F311L mutations for instance are associated with resistance to dasatinib whereas T315I, V299L, T317L/I, T315A, M351T show resistance to nilotinib. The most potent TKI inhibitor, ponatinib has shown an inhibitory effect on all mutations, including the pan resistant T315I mutation. However, its inhibitory effect has been shown to be compromised in the presence of compound mutations. The current gold standard method for mutations analysis is Sanger sequencing, which has a limit of detection of 10-20%. Although it is capable of detecting compound mutations present at levels below its limit of detection, it is incapable of discriminating whether these mutations are in-cis or in-trans –information important to understand the underlying complexity of these mutations. Additionally, Sanger seq is only semi-quantitative and relatively low throughput.
Aims: in the era of high throughput sequencing our aim is to validate the Ion Torrent PGM platform for feasibility, sensitivity and compatibility with the gold standard and implement the workflow in routine diagnostic service.
Methods: 32 clinical samples were included in the study. Samples were chosen on the basis of having either compound mutations or increasing mutational load in progressive samples identified by Sanger or pyro sequencing (n=23/32). Of these, 13/23 had compound mutations identified by Sanger and/or Pyrosequencing, 7/32 had mutations identified only by Sanger sequencing, 3/32 had mutations identified only by pyrosequencing. 6/32 had more than two compound mutations and 9/32 had no mutations identified by either method. We used 6 pairs of primers previously optimized for TKD mutation detection on the Roche GS-Junior platform as part of the IRON-II study. We used the Ion Plus Fragment Library kit and the Ion Xpress Barcode Adapters 1-16 Kit for library prep, Ion PGM Template OT2 400 Kit for templating and the Ion PGM Sequencing 400 Kit for sequencing. Data were analysed using the NextSeq software from sequence Pilot, JSI medical systems with predefined settings as a collaborative effort within the IRON-II study and a sensitivity of 1%, in addition to the Ion Reporter data analysis tool hosted by Life Technologies.
Results: There was a 100% concordance amongst results obtained by NGS *vs* Sanger and/or pyrosequencing. However, NGS detected low level mutations ranging from 2-4% in two patients, rendering them from single to compound mutation holders. The coverage was at least 2000 reads per mutation hotspot and the mutant alleles were at least 20 reads (1%) in either direction. Compound mutational status varied between cis and trans. There was no significant difference in the results obtained using both software, however the Ion Reporter software occasionally missed the F299L mutation, despite being at relatively high levels (~20%).
Summary and Conclusion: We demonstrate the feasibility and comparability of the assay we have developed on the Ion Torrent PGM next generation sequencing platform to be deployed in routine diagnostic labs providing this test. In addition, we emphasise the need for extensive standardization of settings depending on the software to be used for data analysis using appropriate quality controls.

Myelodysplastic syndromes - Clinical 2

P916

THE INFLUENCE OF DISEASE AND COMORBIDITY RISK ASSESSMENTS ON THE SURVIVAL OF MDS PATIENTS TREATED WITH 5-AZACITIDINE

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Background: The use of 5-azacitidine (5-aza) can prolong survival in patients (pts) affected by high-risk myelodysplastic syndrome (MDS), not candidate to allogeneic stem cell transplantation. The influence of the MDS specific comorbidity index on survival of these pts has been documented (Breccia, Haematologica 2012).
Aims: To evaluate if the current prognostic assessments of disease or comorbidity have an impact on the survival in this setting.
Methods: A retrospective analysis of MDS patients treated with 5-aza in 10 centers in Lombardia region was carried out. The disease risk indexes (IPSS, WPSS and IPSS-R) and the comorbidity MDS specific index (MDS-CI) were calculated at the moment of the beginning of 5-aza therapy. The Kaplan Meier method, followed by the logrank test was applied to evaluate the survival, starting from the beginning of treatment, in relationship to IPSS, WPSS, IPSS-R risk and MDS-CI risk.
Results: Data about 198 pts were collected. Median age was 70 (range 23-88). The median number of 5-aza courses administered was 6 (range 1-35). A response to the treatment (including the cases of "stable disease" maintenance) was achieved in 111 cases on 173 evaluable. The analysis showed that pts with an IPSS "high" risk at starting treatment time (evaluable in 43 pts) in comparison to pts with "intermediate-2" IPSS risk (104 pts), did not have different survival (p=0.164). On the contrary, a "very high" WPSS risk (evaluable in 42 pts) in relation to a WPSS "high" risk (85 pts) was associated to a shorter survival (p=0.049). In fact, median survival was 13.7 months for "very high" risk pts and 23.2 months for "high risk" pts. Furthermore, an IPSS-R "very high" risk at baseline (evaluable in 73 pts), in comparison to an IPSS-R "high" risk (41 pts), was associated to a strongly significant reduction in survival (p=0.0007, fig1). In this case median survival was 12.9 months for "very high" risk patients and 33.8 months for "high" risk patients. According to MDS-CI, the pts were stratified in 134 "low" risk cases, 42 "intermediate" risk cases and 11 "high" risk cases. The analysis showed that the MDS-CI category of membership did not influence the survival of these patients (p=0.672, fig 2).

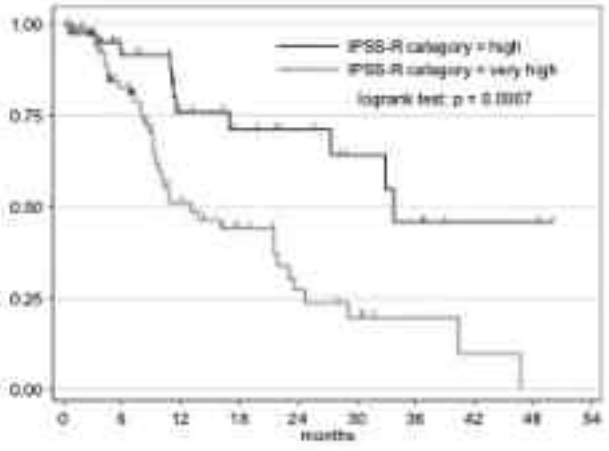

Figure 1.

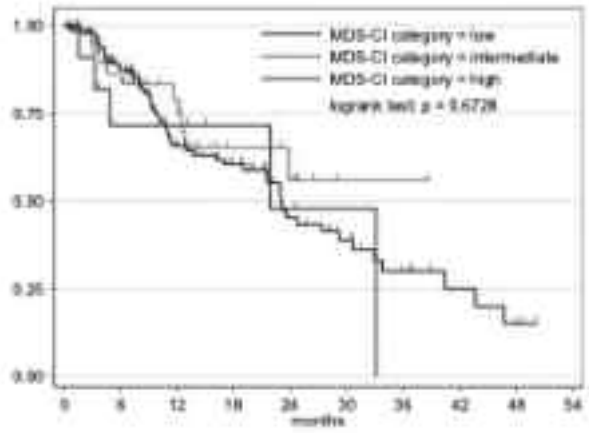

Figure 2.

Summary and Conclusion: Consistent with these data, identification of WPSS and, above all, IPSS-R risk category is useful to identify MDS pts who can have better outcome with 5-aza. The comorbidity assessment with MDS-CI did not influence the survival in the studied pts. This last result, in contrast to the other already quoted series, may be influenced by the preponderance of pts with a "low" MDS-CI risk in the described population.

P917

IRON-CHELATING THERAPY WITH DEFERASIROX IN HIGHER RISK MYELODYSPLASTIC SYNDROMES: A RETROSPECTIVE, MULTICENTER, ITALIAN STUDY

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Background: Iron-chelating therapy (ICT) is usually employed in transfusion-dependent myelodysplastic syndromes (MDS) with iron overload and lower risk disease. Indeed, with the exception of some studies focused on patients who undergo allogeneic stem cell transplantation, few specific data are currently available on the use of ICT, and in particular of the oral chelator Deferarisox (DFX), in higher risk MDS.
Aims: We aimed to evaluate practical use, efficacy, tolerability and possible effects on hematopoiesis of DFX in higher risk MDS treated in the real-life setting.
Methods: We retrospectively collected clinical data from 14 hematologic centers, all belonging to the two major Italian MDS registries (FISM and GROM) and to GIMEMA MDS Working Party. Overall, 58 patients (41 males, 17 females; median age 67 years, range 35-83) were evaluated. A well defined WHO diagnosis was obtained in 53 patients: RA (1.7%), RCMD (5.1%), RAEB-1 (19%), RAEB-2 (62%) and CMML (3.4%). R-IPSS at diagnosis was intermediate in 12.3%, high in 52.6%, and very high in 26.3% of patients, respectively. R-IPSS was not available in 5 patients. Median time from diagnosis to ICT was 10.5 months (range 0-159.5). The median number of red cell transfusions received before starting DFX was 24.5 (range 2-63), while the median levels of serum ferritin and Hb were 1688 ng/ml (range 460-7423) and 8.2 g/dl (range 6.5-10.8), respectively. Forty-one patients had previously received recombinant erythropoietin (13.4%), azacitidine (31%), or both (25.9%).
Results: DFX was administered orally at the median dose of 1000 mg per day (range 375-2500 mg), for a median time of 11 months (range 0.4-75). The initial

daily dose/kg was 5 mg in 8.8%, 10 mg in 42.1%, 20 mg in 47.4% and 30 mg in 1.7% of patients (mean dose: 14.7 mg/kg +/- 5.9). The dose was reduced in 10 patients and increased in 4 patients, due to intolerance or inefficacy, respectively. Eight patients (13.8%) showed grade 2 (5 renal, 3 gastrointestinal) and one patient (1.7%) developed grade 3 (gastrointestinal) toxicities. DFX was definitively interrupted in 7 patients (three renal and one gastrointestinal toxicities, three because of progressive disease). One patient stopped DFX after reaching normal ferritin values. In evaluable patients, median ferritin levels decreased from 1688 ng/ml at baseline (n. 55, range 460-7423), to 1396 ng/ml after one month of DFX treatment (n.47, 443-8513), to 1298 ng/ml at 6 months (n.36, 439-10112) and to 1198 ng/ml at 12 months (n.23, 198-4282) (Figure). Eight of 17 patients (47%) improved or normalized baseline increased ALT/AST levels under ICT. Thirty-one patients continued or started concomitant therapies with azacitidine, recombinant erythropoietin or lenalidomide during ICT and 4 patients received allogeneic stem cell transplantation after DFX. One patient evidenced a durable and complete trilineage response according to IWG criteria, without receiving any other active treatment in addition to DFX when hematological improvement occurred. With a median follow up of 27 months from diagnosis and 14 months after the start of ICT, median overall survival was 38 months and 24.1 months, respectively.

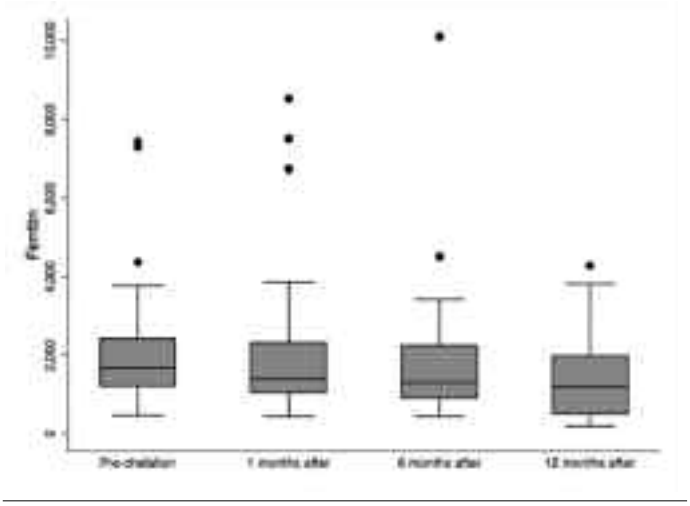

Figure 1.

Summary and Conclusion: This study specifically assessed the role of DFX in higher risk MDS patients. Though the number of patients is quite limited, in this real-world evaluation we showed that such a treatment is feasible, may be effective in lowering serum ferritin levels and, probably, in improving hepatic function. DFX may be safely administered also in patients who are receiving or are planned to receive other active therapies, including allogeneic stem cell transplantation. The effects on survival and the possibility that, as seen in lower risk MDS, DFX may induce hematological responses in higher risk cases as well, remains to be better investigated in a larger, prospective study.

P918

MYELODYSPLASTIC SYNDROMES: AN INTEGRATED MOLECULAR/ CYTOGENETIC WORKUP FOR A CORRECT PATIENTS' RISK STRATIFICATION

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Background: In MDS, cytogenetics is fundamental for the risk stratification, but in this setting non-informative karyotypes represent up to 15-20% of cases. The aCGH is able to detect new abnormalities in up to 80% of cases already tested by conventional karyotype, but it is not enclosed in the routinary diagnostic workup. More recently, the whole genome sequencing methods detected in MDS relevant mutations involving TET2, ASXL1, EZH2, CBL, IDH1/IDH2, DNMT3A. TET2 mutations have been related to a better survival in patients receiving 5-azacitidine, whereas ASXL1, TP53, and EZH2 mutations have been associated with worse outcome. Moreover, down-regulation of RPS14, associated with a good response to lenalidomide, characterizes also an elevated percentage of no 5q- MDS, with different prognostic significance in different risk categories. Finally, high WT1 expression levels have been related to shorter OS.
Aims: The aim of the study was to determine the adjuctive value offered by FISH, aCGH, and somatic mutation assays in respect of the conventional cytogenetics and to determine if the proposed new diagnostic workup will reach good sensitivity and specificity, necessary for a routinary application in the "real-life".
Methods: In this study, we assessed 50 new MDS cases by different techniques: a) conventional cytogenetics; b) FISH for chromosome 5, 7, PDGFRA, and PDGFRB rearrangements; c) aCGH; d) specific real-time PCR assay for ASXL1, EZH2, TP53, and TET2 gene mutations.
Results: After Giemsa banding, one third of our samples showed chromosomal aberrations, including +8, del(7), del(5), -Y, +6, del(13), +14, del(20), and complex karyotypes. After the FISH analysis, 17% of patients showed chromosomal abnormalities, including 5q- and del(13) that were not detected by the Giemsa banding. The aGCH allowed to detect quantitative chromosomal aberrations in 46% of cases (del(13), -7, del(12), del(16), del(17), del(11), del(8), dupl(14), 5q-). After the RT-PCR assessment, 22% of patients resulted mutated for TP53 gene; the involved nucleotides were 844 (C>T), 733 (G>A), 742 (C>T), and 853 (G>A). Four of these TP53 mutated patients showed normal karyotype, and resulted unmutated also by FISH and aCGH. The WT1 gene was over-expressed (in comparison to healthy subjects) in the 25% of the assessed cases; 50% of these patients presented with RAEB and IPSS intermediate-2/high and had a worse outcome. The half of these patients had a normal karyotype. The RPS14 gene was under-expressed in 77% of cases, analogously to the percentage already reported by our group. Clinical correlations and evaluation of the outcome of these patients are being performed at this time, but we already reported that lower RPS14 and higher WT1 levels did negatively impact on the outcome.
Summary and Conclusion: In conclusion, these data sustain the fundamental role of the integrated diagnostic work-up for MDS: indeed, 2 cases, correctly classified as affected by the 5q- syndrome after the FISH analysis, received lenalidomide. In one third of the remaining cases, the identification of TP53 and ASXL1 mutations, in addition to the abnormalities of chromosome 17, 8, 11, and 16 detected after aCGH, allowed us to scoring patients as at higher risk. These patients are now candidate to receive azacitidine.

P919

PROGNOSTIC IMPLICATION OF IPSS AND IPSS-R FOR PATIENTS WITH LOWER-RISK MDS TREATED WITH HYPOMETHYLATING AGENTS

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Background: Prognosis of patients with myelodysplastic syndrome (MDS) can be calculated using a number of scoring systems. The most commonly used system is the International Prognostic Scoring System (IPSS), and the treatment decision using hypomethylating agents (HMA) has been based on IPSS risk groups. The hypomethylating agents (HMA) are usually recommended for patients with lower-risk MDS who is not responsive to other therapies. However, some of the patients showed the deteriorating course even though they were categorized to lower risk IPSS, and the role of HMA for these patients are still on debate.
Aims: This study was conducted to know whether it is rational to treat HMA for patients with IPSS lower-risk (low and intermediate-1), and IPSS-R further discriminates the prognosis of lower-risk patients treated with HMA.
Methods: From 2006 Jan to 2012 Dec, the data of 334 patients who were diagnosed with IPSS lower risk (39 low and 295 intermediate-1) were retrospectively reviewed. The prognosis of lower-risk by IPSS and IPSS-R were analyzed in terms of overall survival (OS) and prognostic power was calculated with time-dependent Cox-hazard models. To define the role of HMA for patients with lower-risk IPSS, we categorized the lower risk patients into No-HMA group, early HMA group (HMA within 2 mo.), and late HMA group (HMA after 2 mo).
Results: Among 334 lower-risk patients (39 low and 295 intermediate-1), 195 patients (58.4%) were treated with HMA (HMA group) and 139 (41.6%) best supportive care (non-HMA group). Median time to HMA treatment was 43 days (range 0-1778 days). 3yr-OS rate was not significantly different between HMA group (43.1±4.2%) and non-HMA group (62.3±5.9%) (p=0.099). Early HMA treatment (3yr-OS 36.4±5.4%) didn't show survival benefits compared to late HMA group (54.6±6.3%) or no-HMA group (62.3±5.9%) (p=0.003). Furthermore, HMA response (CR/PR/HI) didn't guarantee OS benefits: 3yr-OS of 45.2±7.4% in responders (CR/PR/HI, n=74) and 43.4±5.0 in non-responders (SD/PD, n=121) (p=0.239). Among 39 patients with IPSS low, 11 patients (28.2%) were revised into IPSS-R very low, 24 (61.5%) into low, 3 (7.7%) into intermediate, and 1 (2.6%) into high. Among 295 patients with IPSS intermediate-1, 4 patients (1.4%) were revised into IPSS-R very low, 84 (28.5%) into low, 133 (45.1%) into intermediate, 71 (24.1%) into high and 3 (1.0%) into very high. IPSS-R could further discriminate the IPSS lower-risk patients with regard to OS: 3yr-OS rates of 100% in very low, 64.5±5.9% in low, 46.1±5.5% in intermediate, 26.1±6.6% in high, and 0% in very high (p<0.001). The survival benefits of HMA for lower risk groups (very low and low) defined by IPSS-R (n=123) was

not defined in the current study, where 3yr-OS was 83.8±6.4% in no-HMA group (n=60) *vs.* 60.5±7.1% in HMA group (n=63) (p=0.198).
Summary and Conclusion: The use of IPSS as a therapeutic decision making for HMA among lower-risk (low and intermediate-1) patients needs further investigation. The prognosis of the patients with lower-risk IPSS was successfully refined by IPSS-R, where the IPSS intermediate-1 risk was heterogeneous group, which subdivided into very low to very high by IPSS-R. However, the beneficial effect of HMA for patients with lower-risk MDS defined by IPSS-R (very low and low) also needs to be elucidated in the future studies.

P920

HIGHER BONE MARROW LGALS3 EXPRESSION PREDICTS FAVORABLE PROGNOSIS IN ADULT PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Galectin-3, a member of the *β*-galactoside-binding gene family, is a multifunctional protein implicated in cell adhesion, proliferation, and apoptosis. Alterations of galectin-3 expression are often seen in cancers and may contribute to angiogenesis, tumorigenesis and cancer progression. However, the clinical implication of Galectin-3 expression in myelodysplastic syndrome (MDS) remains unclear.
Aims: We aimed to investigate the clinical relevance of Galectin-3 expression in the patients with myelodysplastic syndrome.
Methods: In this study, we sought to investigate the prognostic relevance of *LGALS3*, the gene encoding Galectin-3, in 266 adult patients with newly diagnosed primary MDS, including 196 in original cohort and 70 in validation cohort. Thirty healthy transplantation donors were used as normal controls. *LGALS3* expression in bone marrow (BM) mononuclear cells was analyzed by quantitative real-time polymerase chain reaction. The results were correlated with FAB/WHO subtypes, clinical features, cytogenetics, other genetic alterations, and clinical outcome.
Results: *LGALS3* expression was higher in MDS patients than that of normal controls (P=0.0343). The median value of BM *LGALS3* expression in MDS patients was used as the cut-off point to define lower- and higher-expression groups. Patients with higher *LGALS3* expression had lower platelet count than those with lower expression (P=0.039). There was no difference in other clinical parameters, including age, sex, hemoglobin level, white blood cell count, and lactate dehydrogenase level between the two groups. Higher *LGALS3* expression occurred more frequently in patients with refractory anemia (RA) and RA with ring sideroblasts (RARS) than in those with RA with excess blasts (RAEB) and RAEB in transformation (RAEBT) (P<0.0001) based on the FAB classification. Regarding international prognosis scoring system (IPSS) classification MDS patients harboring higher *LGALS3* expression had higher incidence of lower-risk characteristics. However, there was no statistical difference of *LGALS3* expression in different cytogenetic groups or mutational profiles. With a median follow-up time of 57.7 months (range 0.1 to 217.3), the patients bearing higher *LGALS3* expression had lower probability of disease transformation to acute leukemia compared to those bearing lower *LGALS3* expression (P<0.001). Moreover, the patients with higher *LGALS3* expression had better overall survival (OS) than those with lower expression (median 63.1 months *vs.* 21 months, P=0.003). Similar result could be demonstrated in the 161 patients diagnosed according to WHO classification (median 72.2 months *vs.* 23.5 months, P=0.022). We distinctly identified higher *LGALS3* expression as an independent favorable prognostic factor for OS (relative risk 0.639; 95% CI, 0.421-0.970, P=0.036) irrespective of age, sex, cytogenetics and IPSS classification. This result was also validated in an independent cohort (n=70).
Summary and Conclusion: MDS patients with higher *LGALS3* expression had distinct clinic-biologic features and favorable outcome. BM *LGALS3* expression may serve as a new biomarker to risk stratify the MDS patients.

P921

VALUE OF THE MULTIPARAMETRIC FLOW CYTOMETRY IN MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA UNDER HYPOMETHYLATING AGENT THERAPY

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Background: Clinical response criteria published by the International Working Group (IWG) are mainly based on cytomorphological changes, but in real life, it is very common to see inter-observer variability. Multiparametric Flow Cytometry (MFC) should allow physicians to systematize and standardize this assessment in Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML).
Aims: To analyze, by using MFC, immunophenotypic changes that occur during hypomethylating (HMT) agent therapy in MDS and CMML.
Methods: This study included MDS and CMML adult patients (pts) under hypomethylation, and used FAB, WHO, IPSS and IWG classifications. Two bone marrow samples were taken, the first one at diagnosis, and the second, after the 4th cycle of treatment. An eight-color MFC and a specific MDS panel validated by Euroflow and Infinicyt® program were applied. Granulocytic maturation was classified into 3 groups according to precise stops or blocking maturation observed in myeloblasts (stage 1), in promyelocytes or metamyelocytes (stage 2), and without any blocking (stage 3). Blasts count (CD34), peripheral cytopenia, clonal cytogenetic evolution, AML progression, and mortality rate were also studied.
Results: Twenty-six pts with MDS (16) and CMML (10) were assessed. Response to HMT were: CR 9 (35%), PR 5 (19%), and No Response (NR) 12 (46%); the mean number of cycles received was 9 (range 4-22). CD34 precursors study confirmed an increase in NR group: 5.66% (range 0.26 - 15.85), while PR and CR groups showed 2.46% (range: 0.61 - 6.59%) and 1.68% (range 0. 13 - 9.38) respectively. Responders showed a drop in blocking maturation and an increase in mature neutrophils (from 44 to 66%), and those NR patients kept blocking maturation (from 75 to 62%). Non Responder CMML patients increased blocking maturation, specifically in monocyte lineage (from 33 to 50%), and there were no changes during treatment in CR or PR CMML patients.
Summary and Conclusion: The use of MFC proved to be objective and reproducible by standards. Immunophenotypic changes during HMT treatment in MDS and CMML were closely correlated with clinical response according to IWG.

P922

CLINICAL VALUE OF MONOSOMAL KARYOTYPE AND COMPLEX KARYOTYPE AS PROGNOSTIC PARAMETER IN PATIENTS WITH IPSS INTERMEDIATE-2 AND HIGH RISK MYELODYSPLASTIC SYNDROMES TREATED WITH AZACITIDINE

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Background: Azacitidine (AZA) is standard care in patients with MDS not allow allogeneic stem cell transplantation. Rencetly, chromosomal abnormality (CA) including complex karyotype (CK) or monosomal karyotype (MK) is known to be associated with clinical outcome in myelodysplastic syndrome (MDS).
Aims: The present study was investigated which prognostic factor including CAs would predict the response and survival in patients with international prognostic scoring system (IPSS) higher risk (intermediate-2, and high risk) MDS treated with AZA.
Methods: Patients with IPSS higher risk MDS treated with AZA were enrolled. CK was defined as the presence of three or more numerical or structural CAs. MK was defined as the presence of two or more distinct autosomal monosomies or single autosomal monosomy with at least one additional structural CA.
Results: A total of 243 patients were enrolled. Median follow-up time was 24.2 months Median age was 65 years. Median cycle of AZA treatment was 6 cycles and overall response rate was 57.2 %. CK was present in 124 patients and MK was 90 in patients. -5/del(5q) was identified in 124 patients, and -7/del(7q) was 35 patients. In the whole patients, bone marrow blast 15 or more percent, and CK were associated with poorer response (BM blast 15 or more percent, *p*=0.038; CK, *p*=0.007) and OS (BM blast 15 or more percent, p<0.001; CK, p<0.001), independently. Although MK in CK group was not associated with the outcome, non-MK status in non-CK group reflected favorable OS (*p*=0.005). According to number of CAs, the group including more than 3 CAs was associated with worst OS (group including less than three CAs *vs*. only three CAs, *p*=0.001; group more than three CAs *vs*. only three CAs, *p*=0.001).
Summary and Conclusion: CK, but not MK was an important prognostic parameter associated with worse outcome. However, MK in non-CK status may predict poor survival. In Addition, more high number of CAs was associated with poorer survival.

P923

COEXPRESSION OF CD7 AND LINEAGE INFIDELITY OF MYELOID PRECURSORS CORRELATES WITH RESPONSE TO AZACITIDINE IN IPSS HIGH RISK MDS PATIENTS

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Background: Flow Cytometry (FC) has been recommended by ELN guidelines as an important co-criterion in diagnosis of myelodysplastic syndromes (MDS). Very recently, the absence of immunophenotypically aberrant myeloid progenitors has been correlated with good response to Epo and azacitidine (AZA). In general, association of FC with other parameters such as cytogenetics, morphology, response to therapies, has been evaluated as a FC scoring considering different immunophenotipical anomalies. However, little is known about a specific FC feature in poor prognosis MDS patients (i.e complex karyotypes and non-responders to AZA therapy).
Aims: In this study, we evaluated the FC features of 56 high risk MDS patients undergoing AZA therapy. We stratified them according to cytogenetic subgroups to evaluate possible differences in FC parameters and their predictive role for AZA response.
Methods: We evaluated 56 consecutive patients with higher-risk MDS (IPSS: Int-1, n=8 ; Int-2: n=33; High: n=15) treated with AZA (75 mg/m2/d x 7d, q28d). FC and cytogenetics were studied prior to AZA treatment and at the first evaluation post-therapy. Response to AZA was evaluated according IWG 2006 criteria. We classified our patients in four cytogenetic subgroups: chromosome 7 anomalies (n=5), complex karyotype(n=11), trisomy 8 (n=10), other intermediates (n=4) and normal karyotype (n=26). FC features analyzed were: 1) percentage of granulocytes, monocytes, erythrocytes and myeloid progenitors, 2) maturation pattern of granulocytes, monocytes and erythrocytes, 3) expression of lineage infidelity markers on granulocytes, monocytes and myeloid progenitors, 4) upper or lower expression of normal myeloid markers on all populations and abnormal (often lower) granularity in granulocytes, expressed as low SSC. Acquisition of flow data was performed on a FACS Canto II (Becton Dickinson).
Results: We observed that the expression of lineage infidelity markers (T/NK lineage markers, such as CD7, CD56 or TdT) on myeloid progenitors was a common feature on all patients with complex karyotype. After 6 months of AZA therapy, 40% of the patients achieved a response to AZA and 60% did not. A constant feature observed in non-responder patients was the expression of T/NK lineage infidelity markers. In particular, CD7 was expressed by myeloid progenitors half of these patients. This characteristic was present prior and after AZA treatment. This is in line with what observed recently (Alhan, et al 2014): moreover, in our group of MDS cases, generally aberrant markers on progenitor cells were significantly more frequent in non-responders patients. Abnormal maturation pattern on granulocytes and erythrocytes, together with an abnormal granularity on granulocytes (SSC low) were equally present in non-responder and responder cases.
Summary and Conclusion: We confirm here that the presence of abnormal myeloid progenitors is associated with unfavorable response to AZA in higher risk MDS. In particular, we observed that all non-responder MDS patients expressed lineage infidelity markers on myeloid progenitors, and indeed CD7 was the most frequent marker detected. This observation deserves further analysis and physio-pathological interpretation. We also demonstrated that MDS cases presenting with complex karyotype constantly expressed T/NK markers.

P924

A PHASE II STUDY EXPLORING THE FEASIBILITY OF AZACITIDINE AND LENALIDOMIDE USE (COMBINATION *vs* SEQUENTIAL TREATMENT) FOR HIGHER-RISK MYELODYSPLASTIC SYNDROMES (IPSS RISK: HIGH OR INT-2)

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Background: Azacitidine (AZA) is able to induce hematologic responses in 50-60 % of patients (pts) with Myelodysplastic Syndromes (MDS) and moreover to prolong survival in higher risk MDS pts. However, there is a clear unmet need to improve the clinical outcome of high-risk MDS pts, as the duration of therapeutic response to AZA is limited, with a median survival advantage of only 9.5 months. Lenalidomide (LEN), approved for low-risk MDS with del(5q), also showed some activity either in MDS without del(5q), or in del(5q) acute myeloid leukemia (AML). Recently, several studies have evaluated the efficacy and safety of combining, in high-risk MDS pts, AZA with LEN, either administered concurrently *(Sekeres, 2010; 2012)*, or sequentially *(Platzbecker, 2013)*, in both cases showing promising results, although in a limited number of pts.
Aims: To evaluate the feasibility of the combination AZA + LEN *vs* the sequential use of AZA and LEN in high risk MDS (IPSS score risk : High or INT-2) pts. Primary endpoint: ORR, defined as the Rate of Complete Remission (CR), Partial Remission (PR) and Hematological Improvement (HI), following the International Working Group (IWG) criteria *(Cheson, 2006)*.
Methods: This is a randomized, phase II, multicenter, open label study, including pts with MDS (according to WHO 2008 classification) with International Prognostic Scoring System (IPSS) risk High or Intermediate-2, without previous treatment with AZA or LEN. **ARM 1 (combined treatment)**: AZA: 75 mg/m^2/day (days 1-5) I.C. + LEN: 10 mg/day (days 1-21), orally, every 4 weeks. **ARM 2 (sequential treatment):** AZA: 75 mg/m^2/day (days 1-5) I.C. + LEN: 10 mg/day (days 6-21), orally, every 4 weeks. The treatment for both arms was planned for 8 cycles (32 weeks) in the absence of disease progression or unacceptable toxicity. A sample size of 44 pts was planned.
Results: From March 2013, 34 pts (19 males), with a median age of 72 (48-83 yrs) were enrolled, from 13 hematologic italian Centers. At baseline, WHO diagnosis was: Refractory Cytopenia with Multilineage Dysplasia (RCMD): 3 pts; Refractory Anemia with Excess of Blasts-1 (RAEB-1): 8 pts; RAEB-2: 21 pts; MDS-unclassified (MDS-U): 2 pts; IPSS-risk was: Intermediate-2: 27 pts; High: 6 pts; not determined (N.D.) (because of lack of cytogenetic data): 1 pt. Karnofsky Performance Status was: 100%: 12 pts; 90%: 9 pts; 80%: 7 pts; 70%: 2 pts; 60%: 4 pts. 21 pts were transfusion-dependent at baseline. 15 pts were randomly assigned to ARM 1, and 19 pts to ARM 2. At the time of this analysis, 15/34 pts (44.1%) completed ≥ 6 cycles of treatment, and are evaluable for response. 11/15 pts (73.3%) showed a favourable response to treatment, following IWG criteria: 2 pts achieved CR, 2 pts attained PR, and 7 pts showed HI, while the 4 non responder pts maintained a Stable Disease (SD). Responder pts were 7/8 (87.5%) in ARM 1, and 4/7 (57.1%) in ARM 1, respectively. Median time to response: 2 (2-7) months. 4 pts showed a >100% increase of platelet count after 1st cycle. A significant toxicity (grade >2) was observed in 10/34 (29.4%) pts. 14/34 pts (41.2%) had a dose reduction of LEN because of hematologic or non-hematologic toxicity.
Summary and Conclusion: Our results, although preliminary, seem to confirm the feasibility of the combination AZA + LEN in high-risk MDS pts. More data are needed in order to compare the efficacy and safety of combined *vs* sequential treatment. Moreover, a possible relationship with signal transduction pathways will be evaluated.

P925

IMPACT OF GRAFT-VERSUS-HOST DISEASE ON OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR LOW-RISK MYELODYSPLASTIC SYNDROMES

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a treatment option for low-risk myelodysplastic syndromes (MDS) patients with severe cytopenia or poor prognostic factors. A previous study reported that low-risk MDS patients had a low relapse rate and the primary cause of transplant failure among these patients was non-relapse mortality (NRM). It is thus considered that the graft-*versus*-MDS effect is limited and the development of graft-*versus*-host disease (GVHD) results in a worse transplant outcome. However, the impact of GVHD on allo-HSCT for low-risk MDS patients remains unclear.
Aims: With this background, we conducted a retrospective analysis to elucidate the influence of GVHD on outcome for low-risk MDS patients receiving allo-HSCT.
Methods: Clinical data were collected from the registry database of the Japan

Society for Hematopoietic Cell Transplantation. Low-risk MDS was defined as refractory anemia or refractory anemia with ring sideroblasts according to the FAB classification. Patients with low-risk MDS aged over 15 years, who underwent allo-HSCT for the first time between January 1993 and December 2011, were extracted from the database the Japan Society for Hematopoietic Cell Transplantation. Patients who did not achieve neutrophil engraftment were excluded. Effects of acute and chronic GVHD on OS, relapse and NRM were analyzed using landmark analysis at days 60 and 150, respectively. To evaluate the association of patient characteristics with GVHD, Cox proportional hazards model was used for multivariate analyses.
Results: There were 431 patients with low-risk MDS who received first allo-HSCT. Of those, 397 patients achieved neutrophil engraftment. The median age was 44 years (range 16-72 years). There were 232 males and 165 females. Grade II-IV acute GVHD and chronic GVHD were observed in 152 and 154 patients, respectively. Grade II-IV GVHD significantly increased NRM (p<0.001), leading to worse OS (p<0.001). The 3-year OS, cumulative incidence of relapse and NRM in patients with and without grade II-IV acute GVHD were 64.0, 2.6 and 31.1% and 76.9, 7.0 and 15.0%, respectively. Chronic GVHD did not affect OS, relapse and NRM. The 3-years OS, cumulative incidence of relapse and NRM of patients with and without chronic GVHD were 77.6, 5.1 and 17.2% and 80.4, 3.6 and 14.0%, respectively. In multivariate analysis, grade II-IV acute GVHD was associated with high NRM and poor OS and chronic GVHD was not associated with OS, relapse and NRM after adjusting for age, gender, donor source, HLA mismatch, conditioning regimen, GVHD prophylaxis, previous transfusion amount, gender mismatch, ABO mismatch and disease duration before allo-HSCT. Multivariate analysis revealed that predictive factors for grade II-IV acute GVHD were donor source, unrelated donor bone morrow and intermediate or poor cytogenetic risk category.
Summary and Conclusion: This study showed that grade II-IV acute GVHD had an adverse impact on allo-HSCT for low-risk MDS patients. These results suggest intensive GVHD prophylaxis might be required after allo-HSCT for low-risk MDS patients.

P926

IMPACT OF TP53 MUTATION ON OUTCOME OF MDS PATIENTS UNDERGOING ALLOGENEIC TRASPLANT

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Background: Although new agents have been approved for the treatment of MDS, the only curative approach for these patients is allogeneic hematopoietic stem cell transplantation (HSCT). Nevertheless, in these patients this aproach only obtain 40-60% of overall survival. Several prognostic factors have demostrated to impact on trasplant outcome (blast percentage, cytogenetics, age, etc...). Somatic mutations in MDS have recently been analyzed in order to confirm clonality and also prognostic impact in those patients. In this regard, *TP53* mutated gene is present in MDS in less than 10% of patients and is associated with advanced disease and high risk features.
Aims: Impact on outcome of the mutational status of *TP53* have not been analized and is the purpose of the present study.
Methods: We have retrospectively analyzed the results of HSCT in 67 MDS patients (out of a 100 patient series) from 4 centers in Spain in order to define if the mutational status of *TP53* gene could have impact on clinical evolution after transplantation. The samples from these patients were collected 1 month prior to transplant. The genomic DNA from mononuclear bone marrow cells was screened for somatic mutations in *TP53* gene. The study was performed by NGS on a GS Junior Instrument (Roche Applied Science) according to an amplicon sequencing design. For each sample, seven exons (5-11) were amplified from 280 ng of DNA with preconfigured primer plates provided within the IRON II study network. Data were analyzed for sequence alignment and variant detection using the Sequence Pilot software version 3.5.2 (JSI Medical Systems) and GS Amplicon Variant Analyzer software, versions 2.7 and 2.9 (Roche Applied Science). Minimun coverage of sequenced exons was 100 reads and the sensitivity of variant detection was set to a lower limit of >2% for bidirectional reads. Overall survival (OS) and relapse-free survival (RFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparisons of Kaplan-Meier curves and multivariate analyses were performed using logistic Cox regression analysis. P value was considered significant if it was less than 0.05 and the multivariate analysis included all variables that in the univariate analysis had P value less than 0.1. All calculations were done using SPSS 18.0.
Results: Median age of patients was 51 years (range 22-70 years), 75% of them were "de novo" MDS. Regarding IPSS risk, 47% of them were included in the low and intermediate-1 risk while 53% were in the intermediate-2 and high-risk category. Seventy one percent of patients had received treatment (Azacitidine 35% and intensive chemotherapy 65%) before transplant. Disease status at transplant was stable disease in 30% (no treatment), CR in 24%, PR in 13%, NR in 21% and progression in 10%. The source of the HSCT was PB in 94% of patients, 66% received matched related donor and HLA was identical in 81% of the patients. Most of them received reduced intensity conditioning regimen (68%). Mutational status of *TP53* was tested in the pre-transplant evaluation, 11 patients (21%) were mutated and 42 (79%) were not mutated. After a median follow up of 1.4 years, median OS at 1 and 2 years was 64 and 54% and RFS was 78% and 76%, respectively (median not reached). Mutational status of *TP53* at the time of the transplant setting trends to impact on survival in the univariate analysis, median OS for patients with mutated gene was 0.6 years *vs* not reached for patients without mutated *TP53* (OS at 1y 62% *vs* 49% for patients without *vs* mutated *TP53* gene, p=0.2). In the multivariate analysis, *TP53* status, cytogenetics, IPSS-R, FAB and previous treatment impact on OS. Regarding relapse free survival, the presence of mutated *TP53* also trend to have an impact in the univariate analysis, RFS of 86% among patients without mutated *vs* 60% for patients with mutated gene (p=0.2).
Summary and Conclusion: The present study suggests that mutational disease status pre-transplant (*TP53* gene) as well as disease characteristics could have a significant impact on transplant outcome. Uploaded analysis of 100 patients and mutational status at different time points could be ready to be presented at the meeting.

P927

ISOCHROMOSOME 17Q10 IN MYELOID NEOPLASMS IS FREQUENTLY ASSOCIATED WITH MULTIPLE MUTATIONS AND TRANSFORMATION TO ACUTE MEGAKARYOBLASTIC LEUKEMIA

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Background: Isochromosome 17q, or "i(17q)," which results in a loss of the short arm 17p and duplication of the long arm 17q, represents the most common isochromosome in human neoplasms. It's generally established that i(17q) confers poor prognosis and high-risk of leukemia transformation in myeloid neoplasms, but limited knowledge is available regarding either the subsequent leukemia subset or the associated molecular genetic consequences.
Aims: Herein we performed a retrospective analysis on 10 cases of myeloid neoplasms with i(17q10) to shed light to the converging features of this rare neoplasm.
Methods: The patients of myeloid neoplasms harboring i(17q10), except CML and de novo AML, were collected for retrospective analysis upon review of the clinical and cytogenetics databases at nanfang hospital from JAN 2003 to MAY 2012. Diagnosis of MDS, MPN and MDS/MPN were based on WHO classification. The diagnosis of AMKL was established on the basis of the FAB criteria by studies of cell morphology, cytochemistry and further confirmed with immunophenotyping by flow cytometry or immunocytochemistry staining. Integrated genetic mutations profiling analysis were performed on preserved DNA specimens from bone marrow with conventional Sanger sequencing approach on PCR amplification of genes of interest.
Results: Herein we presented 10 cases of i(17q10)-positive myeloid neoplasm, including 3 cases of myelodysplastic syndrome (MDS) and 7 cases of acute leukemia secondary to MDS, myeloproliferative neoplasm (MPN) and MDS/MPN. I(17q10) was sole cytogenetic abnormality in 7 of 10 cases. Marked megakaryocytic hyperplasia, dysplasia and myelofibrosis were frequently observed in bone marrow smear and biopsies. Five of 7 secondary leukemia cases were diagnosed as acute megakaryoblastic leukemia (AMKL) by flow cytometry and immunohistochemical analysis. Integrated genomic mutation analysis on 10 DNA samples revealed frequent *TET2* (n=7), *SRSF2* (n=4), *CEBPA* (n=3), *JAK2V617F* (n=2), *SH2B3* (n=2), *IDH1* (n=1), *TP53* (n=1), *DNMT3A* (n=1) and *WT1* (n=1). A median time to blast transformation was 12 months (range 2-84). These cases showed poor response to anthracycline-based chemotherapies, with a median overall survival of 16.5 months (range 8-90).
Summary and Conclusion: Our results showed i(17q10)-positive myeloid neoplasms is frequently associated with multiple mutations and transformation to AMKL and it deserves more researches.

P928

COMBINED ASSESSMENT OF WT1 AND BAALC EXPRESSION LEVELS IMPROVES RISK STRATIFICATION IN MYELODYSPLASTIC SYNDROMES

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Background: In patients with myelodysplastic syndromes (MDS) several validated prognostic scores, such as IPSS and R-IPSS,are available to assess the risk of AML progression and predict overall survival (OS) as well as leukemia-free survival (LFS). A number of molecular aberrations can be identified in MDS. However, differently from AML, none of current prognostic indexes takes into account molecular profile at diagnosis. WT1 expression has often been evaluated in acute leukemias and MDS. High WT1 expression levels on bone marrow at diagnosis have been reported to identify MDS patients who are at high risk of progression to AML. BAALC (Brain And Acute Leukemia Cytoplasmic) hyper-expression has been associated with a poor prognosis in AML patients, whereas its prognostic value in MDS is not yet clearly defined.
Aims: The aim of our study was to determine if combined assessment of WT1 and BAALC expression levels at diagnosis could be predictive of leukemic evolution.
Methods: We selected 86 patients with available WT1 and BAALC expression levels on BM samples at diagnosis. According to IPSS score, 22 patient were considered low-risk, 27 intermediate-1 and 28 intermediate-2 or high risk. Patients underwent different treatment schedules including supportive care, erythropoietin, hypomethylating and immunomodulating agents, according to their risk group. Median follow-up was 20 months (range 4 -121 months). Leukemia-free survival (LFS) was calculated from the diagnosis until last follow-up or documented leukemic progression as defined in literature. LFS was estimated using the Kaplan–Meier method. All Real-Time PCR were performed on DNA Engine 2 (Opticon®, MJ Research®). WT1 copy number/Abl copy number $1000x10^4$ was used as cut-off value for high WT1 expression, a level of $1000x10^4$ BAALC copy number/Abl copy number was set as cut-off for BAALC hyper-expression.
Results: Twenty-nine leukemic evolutions were observed. Median LFS was 34 months. The probability of leukemic evolution was significantly affected by karyotype, IPSS and R-IPSS scores, diagnosis according to WHO classification, and molecular profile at diagnosis. According to our data WT1 and BAALC combined expression levels could further enhance prognostic stratification. In IPSS Int-1, Int-2/high and in R-IPSS high risk groups, low levels of expression resulted in significantly lower probability of leukemic progression, whereas high levels predicted poor outcome. Furthermore, in patients assigned to IPSS unfavorable prognostic groups, low levels of WT1 and BAALC seem to predict a significantly longer LFS. In the univariate analysis LFS duration was significantly affected by WT1 and BAALC expression levels, IPSS and R-IPSS scores, karyotype and WHO classification at diagnosis. A multivariate Cox Regression model showed that LFS duration was significantly influenced only by molecular profile at diagnosis and R-IPSS risk group ($p<0.001$ and $p<0.01$, respectively).

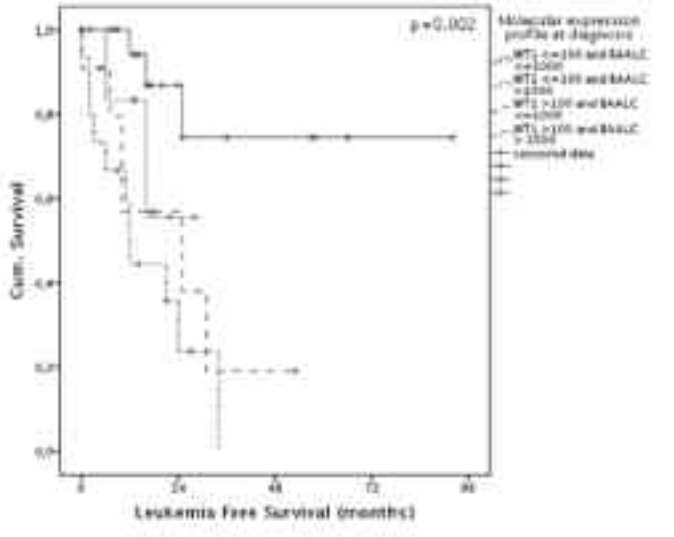

Figure 1.

Summary and Conclusion: In MDS patients combined WT1 and BAALC expression levels on bone marrow samples at diagnosis is a reliable predictor of risk of AML progression. This can improve risk stratification especially in intermediate and high risk groups and may lead to a risk tailored therapy.

P929

IMPACT OF HIGH-DOSE RADIATION EXPOSURE ON NAGASAKI ATOMIC BOMB SURVIVORS WITH MYELODYSPLASTIC SYNDROMES TREATED WITH AZACITIDINE

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders that may arise de novo or secondarily after treatment with chemotherapy and/or radiation therapy for other cancers or, rarely, after environmental exposure. High-dose radiation exposure (such as that resulting from an atomic bomb explosion or nuclear reactor accident) increases an individual's risk of developing MDS. The risk of developing MDS increases at least 45 years after acute radiation exposure, along with the relative risk of acute myeloid leukemia–related mortality. Azacitidine (AZA), a hypomethylating agent, is the standard therapy for MDS in Japan. However, no reports have investigated the efficacy of AZA in atomic bomb survivors with MDS.
Aims: We retrospectively evaluated 33 patients (including 13 atomic bomb survivors) diagnosed with MDS between April 2011 and April 2013 at Nagasaki Genbaku Hospital. All patients received AZA. The primary objective was to estimate the overall survival (OS) rates.
Results: The Table summarizes baseline patient characteristics and response rates. The median age of Nagasaki atomic bomb survivors (A) was 74 (range, 68–84) years, whereas that of patients who did not experience an atomic bomb explosion (non-A) was 68 (range, 46–87) years. The median numbers of administered AZA cycles were 5.4 (range, 1–19) in A patients and 8.2 (range, 1–21) in non-A patients. According to the World Health Organization diagnostic criteria, the following diagnoses were made: refractory cytopenia with multilineage dysplasia (RCMD) in 7 (54%) and 13 (65%), refractory anemia with excess blasts-1 (RAEB)-1 in 2 (15%) and 5 (25%), and RAEB-2 in 4 (31%) and 2 (10%) A and non-A patients, respectively. International Prognostic Scoring System scores were Int-1 in 5 (38%), Int-2 in 6 (46%), and High in 2 (15%) A patients, and Int-1 in 8 (40%), Int-2 in 10 (50%), and High in 2 (10%) non-A patients. Cytogenetic risk was intermediate in 3 (23%) and poor in 3 (23%) A patients, and intermediate in 2 (10%) and poor in 10 (50%) non-A patients. The best curative effect was evaluated and overall response rates (ORR) (CR + marrow CR + PR) were 38% in A patients and 40% in non-A patients. There was no clear difference in the background characteristics except for age (P=0.0258) between A and non-A patients. Although the ORR did not differ (P=0.2635), the median OS in November 2013 was significantly different between the 2 groups (13 months for A *vs*. undefined for non-A patients, P=0.0429).

Table 1.

		Atomic bomb survivors (A) n = 13	Non-atomic bomb survivors (non-A) n = 20	*P* value
Age (median)		68–84 (74)	46–87 (68)	0.0258
WHO subtype	RCMD	7 (54%)	13 (65%)	0.3051
	RAEB-1	2 (15%)	5 (25%)	
	RAEB-2	4 (31%)	2 (10%)	
IPSS at diagnosis	Int-1	5 (38%)	8 (40%)	0.2857
	Int-2	6 (46%)	10 (50%)	
	High	2 (15%)	2 (10%)	
Cytogenetics	Good	7 (54%)	8 (40%)	0.0029
	Intermediate	3 (23%)	2 (10%)	
	Poor	3 (23%)	10 (50%)	
Blood transfusion dependence	+	10 (77%)	16 (80%)	1.0000
	-	3 (23%)	4 (20%)	
Response rate	CR	5 (38%)	7 (35%)	0.2635
	marrow CR	1 (8%)	1 (5%)	
	PR	3 (38%)	0 (0%)	
	SD	4 (62%)	12 (60%)	
AZA cycle (median)		5.4 (1–19)	8.2 (1–21)	0.1860

Summary and Conclusion: These data demonstrate that despite the same ORR, the OS of Nagasaki atomic bomb survivors with MDS was significantly shorter compared to that of patients with MDS who did not experience an atomic bomb explosion. A certain influence of atomic bomb exposure is suggested; however, further studies to clarify the cause of this phenomenon are warranted.

P930

TREG CELLS NUMBER FROM HIGH-RISK MYELODISPLASTIC SYNDROME PATIENTS IS INCREASE, BUT ITS SUPRESSION HABILITY IS IMPARED

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Background: The Myelodysplastic Syndromes (MDS) are characterized by clonal proliferation of bone marrow cells with inefficient production and its pathogenesis is still unknown. Studies show abnormal immune function and also describe some patients with autoimmune phenomena. In the last years, special attention in immunological evaluation has been devoted, which may be related to the pathogenesis of MDS.
Aims: The study aimed to evaluate a broad scrutiny of regulatory T cells function and markers in healthy controls and patients with MDS, associating with autoimmune manifestations and prognostic scores.
Methods: Peripheral and bone marrow (BM) T regulatory (Treg) cells of 37 patients and 18 healthy controls (HC) were obtained. Treg were sorted by flow citometry and co cultured for 7 days with T effector cells (Teff) in different concentration to evaluate cell-mediate suppression and quantify IL2, IL4, IL6, IL10, IL17, TNFa and IFNg production in culture supernatant by CBA. Genetic expression of Foxp3 and IRF-1 was performed by qRT-PCR in peripheral and BM Treg cells. Proteic expression of Foxp3 and IRF-1 was performed by immunohistochemistry in BM Treg cells.
Results: MDS group presented median age of 74 years (23–93y) and gender ratio (M:F)=1:1.6. Median hemoglobin was 9.6g/dL (5.3-14.8g/dL) and 36.8% of the patients were transfusion-dependents. 35.2% showed altered karyotype. According to the WHO classification (2008), 17 patients were CRDM, 3 CRDM-SA, 2 AR, 7 RARS, 3 RAEB-I, 4 RAEB-II and 1 patient was 5q-syndrome. There was a trend toward a higher number of Treg in MDS group compared to HC ($p=0.14$). IPSS score low/intermediate 1 risk patients ($p<0.043$) and WPSS ($p<0.025$) and IPSS-R ($p<0.007$) scores intermediate/high risk patients presented a higher number of Treg cells. There was no difference in Treg number regarding the presence of altered karyotype ($p=0.46$). HC presented higher number of Teff cells compared to MDS patients ($p<0.01$). Treg cell-mediate suppression was decreased in the high-risk group compared to the low-risk ($p<0.01$). The expression of IRF-1 exons 2 ($p<0.01$) and 4/5 ($p<0.01$) proved to be decreased in MDS patients Treg cells compared to HC. Additional results (cytokine production, immunohistochemistry analysis and Foxp3 genetic expression) will be described later.
Summary and Conclusion: This study describes a decrease of Treg cell-mediate suppression was in the high-risk patients, showing an inadequate function in tolerance mechanism. However, paradoxically there was a higher Treg cells number in the same group. Although the last finding is in consonance to previous papers, explaining a higher tolerance to the neoplastic clone and leading to disease progression, we postulate that it could be secondary to suppression impairment. On the other hand, the reduced expression of IRF-1 in MDS patients decreases its tumor suppressor power and may also cause disease progression. IRF-1 is involved in several immune functions and higher expression was described in MDS patients with autoimmune manifestations. Associated with this feature was described, in mice, that IRF-1 plays inhibition of Foxp3 expression in Treg cells, which may suggest that decreased expression of IRF-1 in patients with high-risk MDS, for example, may lead to an increase in the number of Tregs but that the change in cell suppression may have other intrinsic mechanism involved. In summary, we originally described a decreased tolerance mechanism in high-risk MDS patients, represented by a lower Treg cell-mediate suppression.

P931

METHYLATION PATTERNS IN PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SINDROMES AND SECONDARY ACUTE MYELOID LEUKEMIA TREATED WITH HIPOMETHYLANT AGENTS (HIGH-RISK MDS 2009 PROTOCOL OF CETLAM GROUP)

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Background: Myelodysplastic syndromes (MDS) are a group of hematologic disorders characterized by ineffective hematopoiesis and increased risk of transformation to acute myeloid leukemia (AML). Aberrant DNA methylation is the dominant and most well-studied epigenetic alteration in MDS. Various genes, including cell cycle regulators, apoptotic genes, and DNA repair genes, are epigenetically silenced and have roles in pathogenesis and transformation to leukemia. Clinical response of MDS and AML to drugs that change the aberrant hipermethylation, such as 5-aza-2´-deoxicitidine and 5-azacitidine (AZA), suggests that this hipermethylation has an important role in the disease and it is not a secondary effect to other mechanisms.
Aims: The aim of this study was to define the methylation pattern of DNA at diagnosis in patients with high-risk MDS and secondary AML treated with the same protocol (high-risk MDS 09 of CETLAM Group) in order to determine if there are some predicting pattern of relapse or response to AZA treatment.
Methods: Genomic DNA was obtained from bone marrow of 74 patients at diagnosis, 70 samples at follow-up and 12 control samples (bone marrow donors). Genome-wide DNA methylation profiling was performed using the Illumina Infinium HD methylation array (450K). We have used RnBeads program to find predicting pattern of relapse or response to AZA treatment. This program allow to remove patient related SNPs.
Results: The global methylation dendogram suggests that control samples had the most similar methylation pattern. Diagnosis and follow-up samples cluster for patients and not for cytological group or treatment response. Although we have not found significant differences in the methylation patterns of the patients at diagnosis *versus* the same patient after treatment, we have been able to observe a decrease in the state of global methylation in the samples that have received AZA. No methylation pattern at diagnosis was associated to those patients who respond to AZA.
Summary and Conclusion: The methylation arrays are a good methodology to study the methylation pattern of cells. It would be necessary to increase the number of patients with MDS treated with AZA and their follow-ups, to confirm that the hipermethylation decrease along the treatment with AZA and the responders patients keep this hipomethylation whereas the nonresponders patients lose this hipomethylation.

Myeloma and other monoclonal gammopathies - Biology 2

P932

HIF-1A INHIBITION BLOCKS THE CROSS TALK BETWEEN MULTIPLE MYELOMA PLASMA CELLS AND TUMOR MICROENVIRONMENT

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Background: Multiple Myeloma (MM) is a clonal B-cell malignancy characterized by accumulation of malignant plasma cells (PCs) within the bone marrow (BM) in close contact with stromal cells (SCs) which secrete growth factors and cytokines, promoting tumor cell growth and survival. The rapid progression of MM is dependent upon cellular interactions within the BM microenvironment, and novel agents targeting this interaction appear to be promising therapeutic strategies for the treatment of MM tumor expansion.
Unlike most other organs, the BM microenvironment is physiologically hypoxic, a pre-requisite for normal BM hematopoiesis. It is well established that hypoxia is an important selective force in the evolution of tumor cells and a stabilization of HIF-1α protein has been documented in several human cancers. While the role of hypoxia in the pathogenesis of hematologic malignancies has yet to be elucidated, recent animal studies have shown that changes in oxygen levels within the BM microenvironment support the survival and expansion of MM cells. Furthermore, some drugs active in MM, such as Bortezomib and Lenalidomide, are believed to exert their effects in part by interfering with hypoxia-induced signaling cascades.
Aims: Given the importance of the BM microenvironment in MM pathogenesis, we investigated the possible involvement of HIF-1α in the PCs-BMSCs interplay.
Methods: A panel of MM cell lines (MM1.S, U266, OPM-2, RPMI8226) and primary samples from MM patients were cultured *in vitro* in the presence of clinically achievable doses of EZN-2968 (a small 3rd generation antisense oligonucleotide against HIF-1α, to inhibit HIF-1α functions) in normoxia ($p0_2$ 21%) culture conditions.
Results: We have already shown that EZN-2968 is highly specific for HIF-1α mRNA and it results in a long lasting and time dependent inhibition of HIF-1α protein level. Herein, we provide evidence that the interaction between MM cells and BMSCs is drastically reduced upon HIF-1α down-modulation. Notably, we showed that upon exposure to HIF-1α inhibitor, neither the incubation with IL-6 nor the co-culture with BMSCs were able to revert the anti-proliferative effect induced by EZN-2968. Moreover, we observed that EZN-2968 down-modulates cytokine-induced signaling cascades after a short incubation, and seems to induce a negative modulation of those transcripts previously shown to reflect the activation state of specific tumor cell pathways (cell proliferation and survival). This observation was also supported by gene expression profile experiments. One of the key finding of our study is that PC attachment to the extracellular matrix protein was markedly reduced in the presence of EZN-2968. The effects of HIF inhibition on MM cell adhesion are quite intriguing, since MM pathogenesis is dependent upon the interaction of MM cells with the SCs.
Summary and Conclusion: Taken together, these results strongly support the concept that HIF-1α plays a critical role in the interactions between BMSCs and PCs in MM. We conclude that HIF inhibition may be an attractive therapeutic target for MM.

P933

THE NOTCH PATHWAY CONTROLS MULTIPLE MYELOMA CROSSTALK WITH THE OSTEOCLASTOGENIC NICHE

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Background: Multiple myeloma (MM) is an incurable hematological tumor stemming from malignant plasma cells that accumulate in the bone marrow (BM) and establish interactions with BM stroma, promoting tumor survival and bone disease due to unbalanced bone deposition and resorption. The Notch family consists of 4 receptors, Notch1 to 4, which once activated, act as transcription factors. The activation is triggered by Jag1-2 and DLL1-3-4 ligands. Notch plays a key role in bone tissue remodeling and skeletal development. Notch signaling is deregulated in MM and plays a role in MM pathogenesis by modulating tumor cell biology, as well as pathological interactions with BM niche. The myeloma-associated alteration of Notch signaling consists in the aberrant expression of the ligands Jag1 and Jag2 by MM cells, resulting in Notch signaling activation in both tumor cells and BM stroma cells.
Aims: The aim of this work was to investigate the role of Notch signaling in MM-driven osteoclastogenesis. To address this issue we assessed:
• The role of Notch in osteoclast (OCL) differentiation and activity;
• The contribute of different Notch isoforms in OCL development;
• The involvement of Notch pathway in MM cell osteoclastogenic properties.
Methods: Cells were maintained in complete DMEM medium with 10% heat inactivated FBS. DAPT was added to the medium at a final concentration of 50μM. Recombinant mouse RANKL was used at the final concentration of 50ng/ml. Jagged1 recombinant peptide was used at 0.5μg/ml. anti-RANKL neutralizing antibody was used at 0.1μg/ml. OCL differentiation of Raw264.7 cells was induced by treating them with mRANKL or co-culturing with MM cells or their conditioned medium (CM). After 5-7days cells were stained using the TRAP Kit and counted. For bone resorption assay, Raw264.7 cells were cultured on Osteo Assay Surface plates under differentiation conditions. After 7-10 days, the plates were washed in 5% sodium hypochlorite solution. Images of the resorbed areas on the plates were captured and the percentage of resorbed area was measured by using the Wimasis image analysis software (Wimasis GmbH). Select RNAi™ siRNA system (Invitrogen) was used according to the manufacturer's guidelines for the selective knock-down of Jag1 and Jag2. Transfection was performed by electroporation using two plasmid carrying intracellular Notch1 (ICN1) and Notch2 (ICN2). Total RNA was isolated using TRI-Reagent. cDNA was prepared through MMLV reverse transcriptase, then quantitative PCR (qPCR) was performed by Maxima SYBR Green qPCR Master Mix. ELISA Assay was performed using biotin-conjugated goat anti-human RANKL (Merck-Millipore) and Streptavidin-HRP-labeled secondary antibody.
Results: We demonstrates that Notch signaling drives MM cell-induced osteoclastogenesis. The underlying molecular mechanisms is based on MM cell-derived Jagged ligands ability to efficiently drive osteoclastogenesis by contemporaneously activating Notch signaling on tumor cells and osteoclasts. Notch signaling activation in MM cell promote the release of the osteoclastogenic receptor activator of NF-kB ligand (RANKL). RANKL, in turn, promotes within OCL precursors Notch2 signaling which drives osteoclastogenesis completion by promoting the transcription of osteoclastogenic master genes, such as *Tartrate-resistant acid phosphatase* (TRAP) and *Receptor Activator of Nuclear Factor κ B (*RANK) and the autonomous secretion of RANKL by OCL precursors. Remarkably, MM-induced osteoclastogenesis can be disrupted by silencing Jagged1 and Jagged2 Notch ligands in MM cells.
Summary and Conclusion: Our findings make Jagged1 and Jagged2 new promising therapeutic targets to hamper MM-associated bone disease and co-morbidities, lacking the toxicity of the currently used drugs which contemporaneously affect the signaling of all Notch receptors.

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JAGGED-INDUCED NOTCH SIGNALING PROMOTES ENDOGENOUS AND BONE MARROW-MEDIATED DRUG RESISTANCE IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) represents 10% of all hematological malignancies. Malignant plasma cells accumulate in the bone marrow. Although in the last 10 years new drugs such as immunomodulators or proteasome inhibitors increased patients' survival, MM remains still incurable mainly due to the development of endogenous or BM mediated drug resistance. Therefore it is crucial to find new therapeutic targets. The deregulated expression of two Notch ligands, Jagged1 and 2, activates Notch pathway both in MM cells and in bone marrow stromal cell (BMSC) which express Notch receptors. Several Notch downstream effectors are involved in MM cell growth, survival and proliferation, *i.e.* IL-6, SDF-1alfa, CXCR4, NF-kB, VEGF and IGF.
Aims: The aim of this study was to evaluate the role of Notch signaling in endogenous and BMSC-promoted drug resistance in MM, focusing on the contribution of the chemokine axis CXCR4/SDF-1alfa.
Methods: The human MM cell lines, U266 and OPM2, were cultured alone in complete RPMI-1640 medium or co-cultured with murine (NIH3T3) or human (HS5) BMSC cell lines in DMEM medium supplemented with 10% V/V FBS. U266 and OPM2 cells were either kept in suspension for 24 hours at 3 x 10^5/mL or plated on BMSC monolayer for 24h, then treatments with drugs (1-2 mM Mitoxantrone, 5-8 nM Bortezomib or 100-30 mM Melphalan) or 50μM AMD3100 were applied for additional 24 hours. Apoptosis assay: HS5 cells were colored with PKH26 red fluorescent dye (Sigma-Aldrich) before co-culturing to allowed flow-cytometric detection of MM cells co-cultured with BMSCs. At the end of the treatment, cells were stained with Annexin V-FITC (ImmunoTools) and processed with Cytomics FC500 software (Beckman Coulter). RNA interference: specific knock-down of Jagged1 and 2 was obtained by transient expression of specific siRNAs for Jagged1-2 (Stealth Select RNAiTM siRNA system, Life Technologies). MM cell lines were seeded at 350.000 cells/ml and,

after 24h, Jagged1 and 2 genes were simultaneously silenced. Every 48h cells were diluted and transfected again. Quantitative PCR reactions were carried out on a 7500 Fast Real-time PCR system (Applied Biosystems) using the Maxima™ SYBR Green/ROX qPCR Master Mix (Dasit).
Results: RNA interference for Jagged-1 and 2 in OPM-2 and U266 cells resulted in the reduced expression of anti-apoptotic genes such as SDF-1alfa, CXCR4, Bcl-XL, Bcl-2, Survivin and ABCC1. At the same time, MM cells with reduced levels of Jagged-1 and 2 showed an increased sensivity to different drugs commonly used in MM therapy such as Bortezomib, Mitoxantrone and Melphalan. By co-culturing MM cell lines and BMSCs in the presence or the absence of chemotherapeutic agents we observed that BMSCs were able to protect MM cells from apoptosis. We investigated the underlying mechanism showing that MM cells and BMSC interaction resulted in the activation of Notch signaling in both cell types. MM cells-driven Notch signaling activation in BMSCs resulted in the increased expression of soluble growth factors relevant for MM cell growth and survival, such as SDF-1alfa and VEGF. On the other side, BMSCs increased in MM cells the expression of several anti-apoptotic genes, *i.e.* Bcl-XL, Bcl-2, Survivin and ABCC1. Interestingly, Jagged-1 and 2 silencing in MM cells could reverse all gene expression changes and BMSC protective effect. Finally, the CXCR4 antagonist AMD3100 could partially reverse the protective effect of BMSCs to drugs-induced apoptosis in MM cells, suggesting that Jagged ligands deregulation observed in MM is necessary to BM-promoted drug resistance by activating the SDF1alfa/CXCR4 chemokine signaling.
Summary and Conclusion: The evidence that anti-Jagged-1 and 2 siRNAs affect endogenous and BMSC-induced drug resistance in MM cells suggests that a Jagged-directed approach could be effective in MM therapy alone or in a combined treatment with commonly used drugs.

P935

NOTCH PATHWAY PROMOTES MULTIPLE MYELOMA CELL IL-6 INDEPENDENCE

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Background: Multiple myeloma (MM) is a malignant plasma cells (PC) disorder accounting for approximately 10% of hematologic cancers. Even though advanced chemotherapeutic regimens have increased the median time of survival to 5 years after diagnosis, myeloma remains incurable. Once immortalized, the survival and proliferation of myeloma cells strictly depend on a complex interaction with the bone marrow (BM) microenvironment, which is mediated both by adhesion molecules and production of several cytokines, especially interleukin-6 (IL-6). Following MM progression, at the stage of plasma cell leukemia, the malignant PC acquires autonomous proliferative ability, becomes indipendent on growth factors like IL-6 and is no longer confined in the BM. Several recent evidences point to a possible role for Notch signaling in mediating critical events in MM progression. The Notch pathway is highly conserved and plays a crucial role in cell-fate decision, tissue patterning and morphogenesis. Recently, Notch receptors and ligands have been shown to be upregulated during MM progression and their signaling positively regulates cell proliferation, drug resistance and BM infiltration.
Aims: The ability of Notch signaling to regulate proliferation and survival pathways (*i.e.* NF-kB, AKT, Myc, and the same IL-6) prompted us to study if its up-regulation during MM progression may play a role in the acquirement of IL-6 independence. To this end we used two opposite approaches. Specifically, we verified if Notch signaling upregulation in IL-6 dependent cell lines promotes their independence and assessed if, upon Notch inihibition, IL-6 independent MM cell lines lost self-sufficient proliferation.
Methods: Cell culture and cell growth analysis: HMCL CMA03, INA-6 and XG-1 were maintained in complete RPMI-1640 medium supplemented with 10% V/V FBS and IL-6 10, 2.5 or 1 ng/mL, respectivetly. OPM2, CMA03/06 and U266 cell lines were cultured in the same conditions without IL-6 addition. The number and viability of cells were assessed by means of trypan blue exclusion assay. The Notch inihibitor, DAPT, was added to the medium at the final concentration of 50mM. Soluble Jagged1 was used at 5mg/mL.
Flow cytometry analysis: Apoptosys analysis was perfomed by AnnexinV-FITC/Propidium Iodide staining. Cell cycle analysis was performed by Propidium Iodide staining.
Real time-PCR: Quantitative PCR reactions were carried out using the Maxima™ SYBR Green/ROX qPCR Master Mix.
Results: To evaluate if Notch pathway upregulation is involved in the development of IL-6 independence in MM cells, we activated the Notch signaling in three MM cell lines, CMA03, INA-6 and XG-1, strictly dependent on IL-6. At this purpose, MM cells were cultured with the soluble form of the Notch ligand Jagged1. We demonstrated that Jagged1 stimulation partially rescued the reduced cell growth due to IL-6 withdrawal. On the other hand, three different IL-6 independent cell lines, CMA03/06, OPM2 and U266, treated with a gamma-secretase inhibitor (DAPT) which causes Notch pathway blockade, displayed a significant decrease in cell growth. Remarkably, this effect could be reverted by the addition of IL-6 in the culture medium. The mechanisms underlying Notch-IL-6 crosstalking was partially investigated. Preliminary results indicate that Notch signalling is required for MM cell autonomous IL-6 production.
Summary and Conclusion: The present results suggest that Notch pathway activation may contribute to the transition from IL-6-dependent to IL-6-independent MM cell growth. Furthermore, the inhibition of the Notch patwhay may lead to a decrease in MM cells proliferation in part due to the reduction of IL-6 expression. Even though studies are necessary to identify further mechanisms of IL-6 independence possibly involving other Notch downstrem pathways, these preliminary results support the rationale for a Notch-directed approach in plasma cell dyscrasias.

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PROTEASOME INHIBITORS MODULATE OSTEOCYTE DEATH AND AUTOPHAGY IN MULTIPLE MYELOMA

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Background: Cell death and autophagy are the main cellular processes involved in the regulation of bone remodeling by osteocytes. Recently we have demonstrated that an increased osteocyte death is involved in multiple myeloma (MM)-induced osteolysis through the upregulation of osteoclast recruitment.
Aims: Because proteasome inhibitors including Bortezomib (BOR) are known to be able to target osteoblasts in this study we have investigated the potential effect of these drugs on osteocytes and their cell death and autophagy.
Methods: Firstly the effect of the proteasome inhibitors BOR and MG262 on osteocyte viability was evaluated *in vitro* in murine osteocytic cell line MLO-Y4 and in the human pre-osteocytic one HOB-01. Both cell lines were co-coltured for 48 hours in the presence or absence of the human myeloma cell lines (HMCLs) RPMI8226 and JJN3, placed in a transwell insert in the presence or the absence of BOR or MG262. Moreover the effect of proteasome inhibitors on dexamethasone (DEX)-induced MLO-Y4 death, obtained at high doses (10^{-5}-10^{-6}M), was checked in combination with PTH(1-34). To evaluate the presence of autophagy and apoptosis in osteocytes, we checked the expression of both autophagic marker LC3 and apoptotic marker APAF-1 by confocal microscopy in the co-colture system with MLO-Y4 and RPMI-8226. Finally we performed a retrospective histological evaluation on bone biopsies of a cohort of 31 newly diagnosis MM underwent to different treatments including BOR-based regimen. Bone biopsies were obtained at the diagnosis and after an average time of 12 months of treatment. Osteocyte viability was evaluated in a total of 500 lacunae per histological sections.
Results: The *in vitro* treatment with BOR or MG262 significantly blunted MLO-Y4 and HOB-01 cell death. Similarly, DEX-induced MLO-Y4 death was reduced by proteasome inhibitors. Interestingly, we found that both proteasome inhibitors potentiated the PTH (1-34) short-term effects on DEX-induced osteocyte death. Prevalence of autophagic cell death compared to apoptosis was observed in this system. In line with these data, we showed that neither the HMCLs nor treatment with DEX increase the apoptotic death and caspase 3 activation in both MLO-Y4 and HOB-01 cell lines. BOR treatment increased the basal level of LC3 indicating a pro-survival and protective function of autophagy against the BOR-induce stress. On the contrary, when the cells undergo to a stronger stress such as in the presence of HMCLs or by treatment with high dose of DEX we found that both proteasome inhibitors blocked autophagic cell death in osteocytes. In the *in vivo* study we found a significant increase of the number of viable osteocytes in MM patients treated with BOR-based regimen as compared to those treated without BOR (% median increase: +6% *vs*. +1.30%; p=0.017). Patients treated with BOR alone showed the highest increase of osteocyte viability, as compared to those either treated without BOR (+11.6% *vs*. +1.3%, p=0.0019) or treated with BOR plus DEX (+11.6% *vs*. +4.4%, p=0.01). On the other hand, any significant difference was not observed in patients treated with Thalidomide (THAL) or Immunomodulatory drugs (IMiDs) than in those untreated with these drugs (p=0.7). A multiple regression non-parametric analysis showed that BOR had a significant positive impact on osteocyte viability (p=0.042) whereas THAL/IMiDs as well as Zoledronic acid (ZOL) treatments have not (p=0.2). BOR also counterbalanced the negative effect of DEX treatment (p=0.035).
Summary and Conclusion: Our data suggest that proteasome inhibitors blunted osteocyte cell death induced by MM cells and DEX through the modulation of the autophagy and potentiated the effect of PTH. Overall our *in vitro* and *in vivo* data support the use of BOR to improve bone integrity in MM patients.

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THE METABOLIC STRESS INDUCED BY CYCLIN D1 IMPACTS CELL ADHESION, MIGRATION AND CELL ADHESION-MEDIATED DRUG RESISTANCE (CAM-DR) IN MYELOMA CELLS

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Background: The interactions of multiple myeloma (MM) cells with their microenvironment are crucial in MM pathogenesis. Direct interaction between MM cells and bone marrow cells activates pleiotropic signaling pathways that mediate growth, survival, drug resistance, migration of MM cells and angiogenesis. But the molecular profiles of MM could also induce alterations at the transcriptional and proteomic levels that mediate the protective effects of the bone marrow microenvironment on MM cells.
Aims: In this study, we induced constitutive cyclin D1 expression in MM cell line and assessed how cyclin D1 impacts microenvironment interactions of MM cells.
Methods: Cyclin D1 expression were induced in RPMI 8226 cell line and checked by flow cytometry and western blotting. A gene expression profiling assay was performed on cyclin D1 positive *versus* control clones and confirmed by quantitative PCR. Cell adhesion was assessed by fluorescence after adhesion to stroma cells or fibronectin. Chemotaxism was determinated by boyden chamber assay and chemokine secretion by human cytokine antibody arrays. Protein intracellular localization and F-actin stabilization were confirmed by immunofluorescence staining and western blotting. Apoptosis was quantified by flow cytometry after APO2.7 staining. Student's *t*-test was used to determine the significance of differences between two experimental groups. Data were analyzed with a two-sided test. $p<0.05$ was considered significant.
Results: The constitutive expression of cyclin D1 in RPMI 8226 cell line increased cell adhesion to stromal cells and fibronectin, enhanced chemotaxism and chemokine secretion. Global gene expression analysis revealed that cyclin D1 expression was associated with the modification of genes controlling DNA synthesis and metabolism. Unexpectedly, the expression of genes encoding adhesion molecules was not modified. Cyclin D1 expression induced the downregulation of cyctochrome c oxidase subunit VII (*COX7*) expression and the upregulation of adenylate cyclase 1 (*ADCY1*) and glutathione S-transferase omega (*GSTO*) expression. Consequently, cyclin D1 disrupted the redox balance by inhibiting mitochondrial activity and modifying intracellular ATP/ADP and NADPH/NADP+ ratios. This metabolic stress activated the ERK and p70S6K pathways, stabilized F-actin stress fibers and in turn, enhanced cell adhesion to fibronectin and stromal cells. Pomalidomide is an immunomodulator actively tested in MM. Control and cyclin D1-expressing RPMI 8226 clones were resistant to pomalidomide treatment. However, the CAM-DR of cyclin D1-expressing cells was specifically inhibited after pomalidomide treatment opening new perspectives for cyclin D1-expressing MM patients.
Summary and Conclusion: Our data demonstrated for the first time that metabolism regulates microenvironment interactions of MM cells and that pomalidomide could enhance drug sensitivity by inhibiting metabolic stress-induced cell adhesion.

P938

CYCLIN D1 CONSTITUTIVE EXPRESSION SENSITIZES MULTIPLE MYELOMA CELLS TO BORTEZOMIB BY INHIBITING HEAT SHOCK PROTEINS

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Background: Multiple myeloma (MM) is characterized by genetic instability with frequent translocations in more than 50 % of cases. Gene expression profiling assays have segregated MM patients into 7 groups exhibiting different prognostic values and responses to treatment. Cyclin D1 expression is associated with a good prognosis and a prolonged overall survival.
Aims: For understanding its mechanism of action and identifying therapeutic targets, we introduced cyclin D1-GFP fusion protein in MM cell lines characterized by adverse prognostic markers such as *MAF* or *MMSET* overexpression.
Methods: We generated from MM cell lines several clones expressing either cyclin D1-GFP or GFP. Cellular viability and apoptosis were measured by MTT assay and flow cytometry after APO2.7 staining. Regulators of the unfolded protein response and apoptosis were evaluated by real-time PCR and western blotting. Student's *t*-test was used to determine the significance of differences between two experimental groups. Data were analyzed with a two-sided test. $p<0.05$ was considered significant. Combination indexes were calculated according to the Chou and Talalay's method.
Results: In all cell lines tested, the presence of cyclin D1 was associated with increased apoptotic response after treatments with either dexamethasone or bortezomib but not lenalidomide. The expression of cyclin D1 enhanced a caspase-dependent apoptosis belonging to both intrinsic and extrinsic pathways. It also increased endoplasmic reticulum-stress leading to the induction of apoptosis-mediated unfolded protein response (UPR). Besides cyclin D1 co-immunoprecipitated with heat shock proteins (HSP) and inhibited their anti-apoptotic effects. We futher showed that HSP70 rather than HSP90 inhibitors synergized with anti-myeloma drugs to trigger apoptosis in coculture cellular models and in MM primary cells.
Summary and Conclusion: Ours results strongly suggest that HSP 70 inhibitors should be included in clinical protocols to improve the response to treatment of refractory or relapsed patients.

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SERUM LEVELS OF DICKKOPF-1 PREDICT PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTAT BUT ITS PROGNOSTIC VALUE IS OVERCOME BY THE USE OF NOVEL DRUGS

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Background: Dickkopf-1 glycoprotein (DKK-1) is one of the key regulators of bone homeostasis. It is highly expressed in multiple myeloma and its overexpression leads to the development of myeloma bone disease by the inhibition of Wnt signalling pathway, thus inhibiting osteoblasts and activating osteoclasts.
Aims: The aim of our study was to compare the relationship of serum levels of DKK-1 to other parameters of bone marrow microenvironment, and to assess the potential prognostic value of DKK-1 in patients treated with or without the support of autologous stem cell transplantation, and in patients treated with thalidomide and bortezomib based regimens.
Methods: We assessed 128 patients with newly diagnosed symptomatic multiple myeloma at our department during 2008-2013. Serum levels of DKK-1 were measured at the time of diagnosis before therapy, and correlated to serum levels of thymidine kinase (TK), osteocalcin (OC), bone fraction of alkaline phosphatase (bALP), parathormone (PTH), C-terminal telopeptide type I collagen (ICTP), procollagen I intact N-terminal (PINP), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), interleukin-6 receptor (IL-6R), syndecan-1 (SYN), vascular endothelial growth factor (VEGF), osteoprotegerin (OPG), endostatin (END), macrophage inhibitory protein 1α and 1β (MIP-1α, MIP-1β), and angiogenin (ANG). Also, we evaluated progression free survival and overall survival with regard to DKK-1 levels and therapeutic approach used.
Results: Spearman correlation analysis revealed weak positive relationship between serum levels of DKK-1 and TK (r=0,274; p=0,045); there was no other significant correlation in comparison of all other selected parameters, *i.e.* DKK-1 and OC, bALP, PTH, ICTP, PINP, IGF-1, HGF, IL-6R, SYN, VEGF, OPG, END, MIP-1α, MIP-1β and ANG. In the whole cohort of patients, DKK-1 was not found a suitable predictor of progression, the area under curve (AUC)=0,540 (<0,75). The Cox regression analysis revealed that DKK-1 is not a suitable prognostic factor of PFS and OS for the whole cohort of patients, RR=0,96 (95%CI: 0,57-1,62; p=0,876). In the subgroup of patients treated using autologous stem cell transplantation (ASCT, n=30), ROC analysis found DKK-1 as a convenient progression predictor with AUC=0,861 (>0,75). The optimal cut-off level was 9399ng/l (91% sensitivity, 74% specificity). Cox regression analysis followed by Kaplan-Meier analysis confirmed significant shorter PFS in patients with DKK-1<9399ng/l than in patients with DKK-1 ≥9399ng/l (2,6 *vs* 4,2 years, p=0,008). Due to short follow up we were not able to assess OS as the patients did not reach median OS yet. There was no significant difference in either OS or PFS in patients treated with thalidomide based regimens (n=62) or bortezomib based regimens (n=49).
Summary and Conclusion: Our data suggest that serum levels of DKK-1 are independent prognostic factor in MM patients treated with autologous stem cell transplantation. Patients with low DKK-1 had shorter PFS than patients with high serum levels of DKK-1. The use of novel biological drugs, however, overcomes its prognostic significance, suggesting a distinct interference of these drugs with bone marrow microenvironment. Supported by the grant IGA MZ CR NT 14393, NT14400, NT12451-5 and NT12215-4.

P940

IMPACT OF EXONIZED ALUS ON SOLUBLE RANKL MRNA TRANSLATIONAL DYNAMICS

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Background: Alu retrotransponsons (Alus) are known to exert a broad evolutionary impact by affecting both the splicing motifs and the expression patterns of genes. TNFSF11, the gene encoding for RANKL, generates by alternative splicing two mRNA variants. TNFSF11 variant 1 (NM_003701) contains five exons and encodes for the membrane-bound form of RANKL. TNFSF11 variant 2 (NM_033012), including a total of seven exons, contains a

set of alternative 5′ exons; interestingly, this variant lacks the exon encoding for both the 5′ untranslated region (5′ UTR) as well as the intracellular and transmembrane domains of membrane-bound RANKL. Originally identified in two distinct squamous cell carcinoma cell lines, it has been suggested that variant 2 is predominantly expressed in malignant cell types. Recently, novel transcript variants of TNFSF11 were identified while it was shown that the longest open reading frame of variant 2 can be directly translated into the soluble form of RANKL, a protein linked causatively to induction of bone loss at distant sites in the skeleton. Importantly, translational dynamics of TNFSF11 variant 2 remain, yet, shadowy.

Aims: Provide, via bioinformatics analysis, novel insights on TNFSF11 variant 2 (soluble RANKL mRNA) translational dynamics; *id est* introduce this variant as a molecule of low translational efficiency. Highlight that a, yet unprecedented, shorter TNFSF11 variant could likely be translated into soluble RANKL in a more efficient manner.

Methods: Translational efficiency of TNFSF11 variant 2 was estimated with regard to the size of the 5′ UTR, the tendency of this region for forming stable secondary structures and the number of pseudosignals; 5′ UTR tendency for forming stable stem-loop structures was accessed via *pknotsRG-mfe* program. Soluble RANKL mRNA sequence was scanned for the presence of exonized Alus by *BLAST* and *Repeat Masker*. Search for uORFs was performed with the *ORF Finder* program; search parameters were set as previously suggested [Calvo *et al.,* 2009]. Alignment of corresponding sequences was schematically reproduced with the *Multalin* program.

Results: TNFSF11 variant 2 (Figure 1A, upper panel) represents a molecule of, likely, low translational efficiency, especially as compared to variant 1 (Figure 1B, 1C). Exons 1B and 1C of TNFSF11 variant 2 contain exonized Alus, of sense and antisense orientation, respectively (Figure 1A, upper panel). The exonized Alus potentially synergize in the formation of a complex stem-loop structure that could significantly impair translational efficiency, as indicated by the value for the secondary structures' minimum free energy (MFE) (Figure 1B, right panel). An evolutionary-induced single-nucleotide variation of the integrated AluSz element allows for the mutational gain of an upstream AUG trinucleotide (Figure 1A, green boxes) that demarcates the longest out of the five upstream open reading frames (uORFs) present in soluble RANKL mRNA. Both the start and stop codons of this uORF are conserved among higher primates (Figure 1A, lower panel) while, according to dbSNP, are not polymorphic in human. In addition, out of the 12 known polymorphic sites included within this uORF, all representing single nucleotide substitutions, none induces an in-frame nonsense codon; all the above could suggest an important biological role of this uORF, mainly in the context of post-transcriptional regulation. An alternative splicing event, exon 1C skipping, allows for the generation of a shorter TNFSF11 transcript variant (Figure 1D). Originally identified in the human osteoblast-like cell line Saos-2 and the human T cell line Jurkat Clone E6 [Walsh *et al.*, 2013], this variant contains also the primary ORF that encodes for the full length extracellular domain of RANKL (soluble RANKL). However exclusion of exon 1C results in, markedly, shortening the length and increasing the MFE of the 5' UTR while disrupts the uORF, described above.

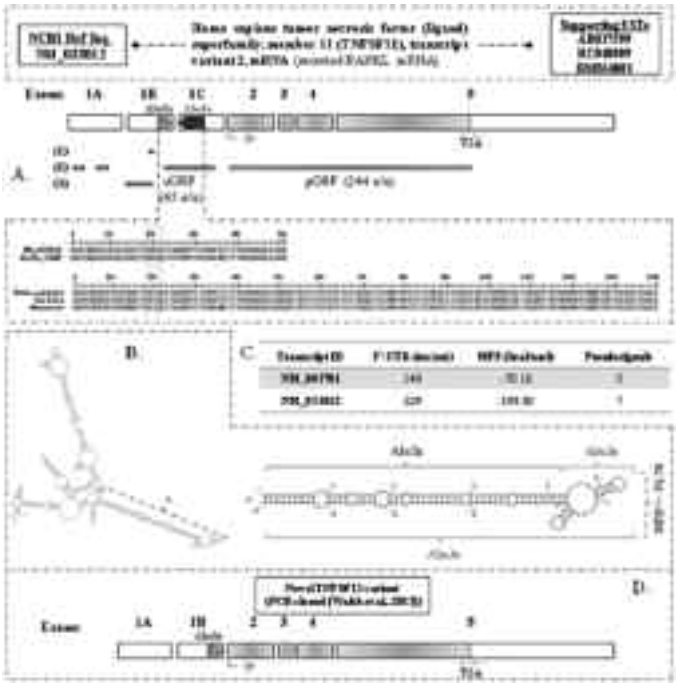

Figure 1.

Summary and Conclusion: Intriguingly, natural selection seems to have induced the assembly of stringent biological mechanisms that could significantly impair the translation of TNFSF11 variant 2. However a novel TNFSF11 variant, partially bypassing these constraints, could efficiently encode for soluble RANKL; *id est* this novel variant could be the one encoding for soluble RANKL, instead of TNFSF11 variant 2 as previously assumed.

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FIBROBLAST ACTIVATION PROTEIN PROTECTS BORTEZOMIB INDUCED APOPTOSIS IN MULTIPLE MYELOMA CELLS THROUGH B-CATENIN SIGNALING PATHWAY

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Background: Multiple myeloma(MM), a malignant plasma cells proliferative disease, is characterized by increased blood calcium level, renal insufficiency, anemia, and bone lesions(CRAB). The coevolution of MM cells with bone marrow stromal cells plays an important role in MM cells growth, survival and drug resistance. Fibroblast activation protein(FAP), a vital transmembrane protein which is expressed in 90% epithelial tumor stroma, has been reported to be involved in mediating drug resistance, tumor progression, immune evasion, invasion and metastasis of tumor cells. However, the expression and function of FAP in MM is still less known.

Methods: Bone marrow mesenchymal stem cells(BMMSCs) from MM patients, normal donors and the cell line of human BMMSCs were analyzed for expression of the FAP protein by semiquantitative real time-polymerase chain(qRT-PCR), flow cytometry(FCM) and immunofluorescence(IF). Tumor cell-conditioned medium (TCCM) from supernatant of MM cell lines were added to BMMSCs or MM cells coculture with BMMSCs to observe the effect of TCCM or MM cells stimulation for the expression of FAP. We further studied the function and mechanism of FAP in bortezomib induced apoptosis of myeloma cells by silencing FAP with small interfering RNA(siRNA). Apoptotic cells of MM cells were detected by APC-CD138/annexin V-FITC using flow cytometry analysis. Western blotting was used to elucidate the signaling pathway that FAP may be involved in mediating apoptosis of MM cells induced by bortezomib.

Results: There was no significant difference in the expression of FAP in hBMMSCs isolated from MM patients and normal donors($p \square 0.05$) as determined by qRT-PCR, and FCM. BMMSCs stimulated by TCCM or coculture with MM cells displayed an elevated expression of FAP as detected by qRT-PCR($p \square 0.05$). In the presence of 30nM bortezomib, MM cell lines RPMI8226 or CAG cells co-cultured with BMMSCs in which FAP was knockdown or not by siRNA for 48h demonstrated that FAP is capable of protecting RPMI8226 and CAG cells induced by bortezomib from apoptosis as determined by FCM(NC siRNA *vs* FAP siRNA, $p \square 0.05$). Further study showed that the activity of β-catenin was significantly elevated in RPMI8226 and CAG cells after co-cultured with BMMSC in the presence of bortezomib. Knockdown FAP can reduce the expression of β-catenin and its downstream target proteins, such as c-myc, survivin, cyclin D1 in RPMI8226 and CAG cells detected by western blot. The activation of β-catenin need MM-BMMSCs direct contact other than separated by transwell.

Summary and Conclusion: Taken together, our data indicated that the expression level of FAP was no difference between the BMMSCs isolated from MM patients and normal donors. The expression of FAP can be increased by TCCM stimulation or coculture with MM cells. Further study demonstrated that FAP can protect MM cells from apoptosis induced by bortizomib, which is likely through β-catenin signaling pathway. This protection need direct contact with BMMSCs.

P942

PRECLINICAL ANTIMYELOMA ACTIVITY OF THE ALKYLATING-HDACI FUSION MOLECULE EDO-S101 THROUGH DNA-DAMAGING AND HDACI EFFECTS

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Background: Alkylators such as melphalan are part of the backbone treatment of young and also elderly MM patients. Moreover, novel ones such as bendamustine have been used in several combinations with either proteasome inhibitors or IMIDs. EDO-S101 is a novel drug resulting from the fusion of a molecule of bendamustine with a vorinostat one, with the aim of increasing the efficacy of the alkylator through the HDACi-mediated chromatin relaxation that would make DNA more accessible to the damaging effect of bendamustine.

Aims: To study the efficacy and mechanism of action of EDO-S101 in human myeloma cell lines (HMCL) either alkylator sensitive and resistant, in freshly isolated MM patient cells and in a xenograft model of human MM.
Methods: Sensitivity to EDO-S101 was assessed by MTT in HMCLs and by flow cytometry in freshly isolated cells from 5 MM patients. *in vivo* efficacy was analyzed in a xenograft plasmocitoma model of MM1S in CB-17 SCID mice. For the evaluation of the mechanism of action several techniques were used to analyze apoptosis and changes in the cell cycle profile. Western blot and immunohistochemistry in tumors excised from treated mice were also employed. Calcusyn program was used to calculate synergy or additive effect with other anti-MM drugs.
Results: Among a panel of 6 HMCLs representative of MM heterogeneity, high sensitivity to EDO-S101 was observed in all of them independently of p53 status (IC50 values ranging from 1 to 5μM). Furthermore EDO-S101 was also active in cells isolated from MM patients, with median IC50 of 5 μM (ranging from 1,8 to 8 μM) and, interestingly, EDO-S101 could overcame alkylators-resistance as it was active in melphalan resistant cells (U266-LR7 and RPMI8226-LR5). Regarding the *in vivo* efficacy, three weekly doses of EDO-S101 (60 mg/Kg iv) were able to significantly decrease tumor growth (time to reach 1.000 mm^3 of 20 days for the control group as compared with 60 days for the treated mice) and also to prolong survival ($p<0.05$). Importantly, EDO-S101 was also active in mice bearing big tumors (3.000 mm^3). With regards to toxicity, only some body weight loss was observed in the treated mice. Regarding the mechanism of action, EDO-S101 induced apoptosis *in vitro* as assessed by Annexin-V staining, loss of mitochondrial membrane potential and cleavage of caspases 8, 9, 7, 3 and PARP. Cell cycle profile showed an S and G2M arrest. Specifically, EDOS-101 induced apoptosis was dependent of a dual alkylating and HDACi mechanisms. As far as the alkylating mechanism is concerned, EDO-S101 induced double strand breaks (DSBs) as demonstrated by comet assay by immunofluorescence and by an increase in pH2AX by western blot, both *in vitro*, and *in vivo* by immunohistochemistry in tumors from treated mice. As a result, an activation of the DNA damage checkpoints was also demonstrated with clear increases in p53, p-CHK1 and p-CHK2. As mentioned, an HDACi effect could also be proven, as an increase in the acetylation of histones H3 and H4 were observed both *in vitro* and *in vivo*. Finally, among different combinations tested, EDO-S101, was synergistic when combined with bortezomib and with dexamethasone in double and also in triple combination (EDO-S101 + bortezomib + dexamethasone).
Summary and Conclusion: The *in vitro*, *ex vivo* and *in vivo* efficacy of EDO-S101 through an alkylator and a HDACi effect, provides the rationale for the investigations of this compound in clinical trials in MM. Probably a potential clinically relevant combination would be that including bortezomib + dexamethasone.

P943

SAR650984, A HUMANIZED ANTI-CD38 ANTIBODY, POTENTLY TARGETS CANCER CELLS THROUGH MULTIPLE MECHANISMS

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Background: CD38 is a type II transmembrane glycoprotein with both ectozyme activity and receptor function that has been implicated in cancer cell adhesion, signal transduction and calcium signaling. CD38 is highly expressed at the surface of many hematological cancer cells. SAR650984 is a humanized IgG1 antibody targeting CD38 in early clinical development to treat patients with $CD38^+$ hematological malignancies. Several mechanisms of action of SAR650984 have been identified including antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct cell death induction (ASH 2008 Abstract #2756). In addition, SAR650984 displays potent inhibition of recombinant CD38 ADP-ribosyl cyclase activity in a biochemical assay (AACR 2013, Abstract #4735).
Aims: To further characterize the mechanism of direct cell death induction and extracellular nucleotide modulation by SAR650984.
Results: The direct effects of SAR650984 on cancer cells were evaluated in a panel of multiple myeloma (MM) and diffuse large B-cell lymphoma (DLBCL) cells. In cancer cells with high CD38 expression levels, Annexin V positive staining increased significantly after SAR650984 treatment, which correlated with strong cell proliferation inhibition. Cell death induced by SAR650984 was further confirmed by detection of released cell death biomarker HMGB1 and Cyclophilin A in culture medium. SAR650984 induced cell death was independent of caspase activation and BCL2 family protein alterations but associated with homotypic cell adhesion, which was similar to the reported activity of anti-CD20 type II antibody GA101 (Blood, 117:4519, 2011). These findings reveal that unlike other anti-CD38 antibodies, SAR650984 is able to directly induce novel mode of cell death in the absence of cross-linker or immune cells which potentially will lead to improved tumor cell killing *in vivo*. To explore the impact of SAR650984 on CD38 ecto-enzymatic activities in cancer cells, we have developed a robust LC-MS-based cellular CD38 enzymatic assay that monitors the depletion of CD38 substrates nicotinamide adenosine dinucleotide (NAD) and the production of cyclic adenosine diphosphoribose (cADPR) and adenosine diphosphoribose (ADPR) from CD38 cyclase and CD38 NAD glycohydrolyase activities, respectively. Preliminary results demonstrate that in response to the treatment of SAR650984, CD38 cyclase activity in multiple myeloma and lymphoma cancer cells was significantly inhibited by more than 90% as shown by a rapid and dose-dependent decrease in the rate of both substrate NAD depletion as well as extracellular cADPR production. Interestingly, a significant reduction of intracellular cADPR (over 60%) was also observed after SAR650984 treatment. Emerging data suggest that nucleotides such as NAD and cADPR are important regulators of cell growth, survival and immune responses. The altered levels of nucleotides resulting from CD38 enzymatic inhibition by SAR65084 in tumor microenvironment therefore could contribute to the anti-tumor efficacy of the antibody. Additional studies to elucidate this activity are currently ongoing.
Summary and Conclusion: SAR650984 demonstrated unique properties of direct cell death induction and potent CD38 enzymatic inhibition on cancer cells in addition to its complement and immune cells mediated cytotoxicity. These results provide new insights toward understanding the multiple antitumor mechanisms of SAR650984.

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NETWORK OF MICRO RNA AND EPIGENETICS ARE ASSOCIATED WITH THE PROGRESSION OF MGUS AND MULTIPLE MYELOMA

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Background: Micro RNAs (miRs) are small non-coding RNAs of 19-25 bases in length having ability to modulate gene expression and involved in carcinogenesis. Transcriptional silencing of tumor suppressor genes (TSG) in cancer cells is often associated with DNA methylation and histone deacetylation carried out by epigenetic modifiers DNMTs and HDACs. miRs can regulate DNMTs expression and promoters of several miRs are also hypermethylated suggesting network of miR and epigenetics in cancer cells.
Aims: To understand the interaction of miRs and epigenetics in multiple myeloma (MM) progression, we investigated correlation between miR and epigenetic modifiers expressions and promoter methylation of TSG and miRs in plasma cells in MM and MGUS.
Methods: Purified plasma cells of BM obtained from 71 of MM patients 29 of MGUS patients and 11 of control patients are subjected to the study after informed consent. This study was approved by IRB, MiRs and epigenetic modifiers' mRNA values were determined by real time PCR with Taqman probe or SYBR green and $2^{-\Delta\Delta}$method. Methylation status of DNA were determined by methylation specific PCR (MSP).
Results: We found significant reduction of miRs 15a (mean values; 52.36, 85.95, 110.71; MM, MGUS and control respectively, $p<0.001$), 15b (55.05, 79.97, 103.86, $p<0.001$), 16a (55.53, 87.62, 101.14, $p<0.001$), 29a (55.91, 84.19, 97.14, $p<0.001$), 29b (58.14, 83.67, 103.00, $p<0.001$), 29c (10.91, 14.94, 59.50, $p<0.001$), 34a (103.11, 132.78, 1617.25, $p<0.001$), 34b (21.73, 71.43, 56.73, $p<0.001$), 34c (1771.02, 12816.17, 7830.79, $p<0.001$) along with elevation DNMT1, 3A and HDAC3, 5, 7, 9 in MM. DNMT1, 3A were elevated in MM than in MGUS and control subjects ($p=0.03$). DNMT3B expression was not different among subjects. In the MM cell lines, miR 29a and 29b expression were inversely correlated with DNMT1 mRNA expression ($r=0.96$, $p=0.0003$: $r=0.86$, $p=0.014$). In the patient samples, DNMT1 was inversely correlated with miR15a, miR15b, miR16, miR29a, miR29b ($r=-0.435$, $p=0.003$; $r=-0.341$, $p=0.02$, $r=-0.332$, $p=0.03$, $r=-0.419$, $p=0.005$, $r=-0.407$, $p=0.006$), DNMT3A was inversely correlated with miR15a, miR29a, miR29b, miR29c ($r=-0.365$, $p=0.02$; $r=-0.315$, $p=0.04$; $r=-0.371$, $p=0.01$; $r=-0.315$, $p=0.04$), DNMT3B was inversely correlated with miR15a, miR15b, miR29a, miR29b ($r=-0.418$, $p=0.005$; $r=-0.385$, $p=0.01$; $r=-0.353$, $p=0.02$; $r=-0.358$, $p=0.02$). HDAC3, 7, 9 expression were elevated in MM than in MGUS ($p=0.03$, $p=0.001$, $p=0.001$) and normal subjects (0.18, 1.1, 20.8) ($p=0.01$, $p=0.017$, $p=0.012$). HDAC1, 3, 7 were inversely correlated only with miR15a ($r=-0.25$, $p=0.039$, $r=-0.27$, $p=0.025$, $r=-0.25$, $p=0.038$). The sequential samples during the treatment showed opposite direction change between miRs and epigenetic modifiers, associating with refractoriness. The rate of methylation in TSG was higher in MM and interestingly putative tumor suppressor miR 34a, b/c were also methylated. There were significant positive correlations among miRs expressions; miR29a-29b $r=0.832$, 29a-29c $r=0.616$, 29a-34a $r=0.448$, $p<0.001$, 29a-34b $r=0.309$, $p=0.001$, miR29a-miR34c $r=0.414$, $p<0.001$, miR29b-29c $r=0.659$, 29b-34a $r=0.500$, $p<0.001$, 29b-34b $r=0.297$, $p=0.002$, 29b-34c $r=0.392$, $p<0.001$, 29c-34a $r=0.432$, 29c-34b $r=396$, $r=0.655$, 29c-34c $r=0.398$, $p<0.001$. Covariance structure analysis revealed the complex network between miRs and epigenetics. When the cell lines were treated with 5 deoxy-azacytidine, miR34a, b, c expression levels increased after the treatment.
Summary and Conclusion: We found significant reduction of miRs expression

in MM and the reduction in part associated with methylation. Correlations in between miR expression, methylation and DNMT imply an important role of them in MM progression. Our study suggests the existence of network between miR and epigenetics, and further investigation would be valuable for understanding the nature of disease progression.

P945

CHARACTERIZATION OF SERUM AND GLUCOCORTICOID-INDUCIBLE KINASE 3 (SGK-3) AS A POTENTIAL ONCOGENIC ALTERNATIVE TO SIGNALLING VIA AKT IN MULTIPLE MYELOMA

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Background: We have previously shown that multiple myeloma (MM) tends to segregate in two groups: those that heavily depend on oncogenic Akt activity for their survival, and those that are relatively Akt-independent (Zöllinger et al, Blood, 2008). SGK-3 has been implicated as mediator of phosphoinositide-3-kinase (PI3K)-dependent, but Akt-independent signalling in breast cancer (Vasudevan *et al.*, Cancer Cell, 2009). These observations provide a rationale to investigate if SGK-3 might serve either as stand-alone or as combination therapeutic target downstream of PI3K in (particular subgroups of) MM.
Aims: Analysis and characterization of the potential functional roles of SGK-3 in multiple myeloma.
Methods: SGK-3 expression analyses at mRNA and protein levels were conducted in phospho-Akt-positive and -negative MM cell lines (n=10) and in CD138-purified primary myeloma samples (n=10). SiRNA-mediated knockdown of SGK-3 was performed by electroporation and subsequent purification of affected cells. Cell death and proliferation measurements were conducted with SGK-3 knockdown cells with and without further addition of pharmacologic inhibitors of potentially interconnected oncogenic signalling pathways.
Results: SGK-3 was found expressed in all MM cell lines and in primary MM cells. Electroporation proved a powerful means to achieve extensive transient siRNA-mediated SGK-3 knockdown (>95%) in MM cell lines (AMO-1, U-266, JJN-3, L-363, MM.1S) and the effect lasted for at least five days before protein levels began to recover. SGK-3 knockdown did not lead to substantial changes in the levels of phosphorylated Akt (positions Thr308 and Ser473), or of other PI3K-downstream targets, such as phospho-GSK-3beta, phospho-PRAS40 or phospho-FOXO3A in either phospho-Akt-positive (JJN-3, L-363, MM.1S) or phospho-Akt-negative (AMO-1, U-266) MM cells. SGK-3 depleted MM cells were not noticeably impaired regarding either proliferation/metabolism (AlamarBlue assay) or survival (annexin V-positivity determined by FACS analysis) when compared to cells treated likewise but transfected with an innocuous siRNA against EGFP. We are currently investigating if in addition to SGK-3 blockade the parallel pharmacologic and/or siRNA-mediated inhibition of other signalling pathways either directly downstream of PI3K (Akt, Bruton tyrosine kinase) or indirectly connected to it (MEK/MAPK, JAK/STAT3) provides a means for enhanced anti-myeloma activity, and the respective results will be presented.
Summary and Conclusion: We have so far not found evidence for a prominent direct role of SGK-3 in the survival and/or proliferation of either phospho-Akt-positive or phospho-Akt-negative MM cells. Although our results are exclusively based on SGK-3 knockdown studies (no specific pharmacological SGK-3 inhibitors exist), this is in clear contrast to the situation with Akt (specifically: Akt1 and Akt2; Zöllinger, Blood, 2008) and also PI3K-alpha (Hofmann, BJH, in the press) where siRNA-mediated target depletion can result in substantial induction of MM cell apoptosis. While an indirect role of SGK-3 in interconnection with other oncogenic signaling pathways is still being investigated, our preliminary data would argue against SGK-3 as a significant potential therapeutic target in MM.

P946

ORAL STATIN USE ASSOCIATED WITH REDUCED RISK OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA: A POPULATION BASED NESTED CASE-CONTROL STUDY

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Background: Several studies have suggested statin-mediated inhibition of the mevalonate pathway is tumour-suppressive, with a pronounced effect in haematological malignancies reported. To date, there have been few investigations of the associations between statin use and monoclonal gammopathy of undetermined significance (MGUS) and/or multiple myeloma (MM). As all cases of MM arise from MGUS, preventing the development of MM provides an opportunity to combat global increases in incidence with a well-tolerated class of drugs.
Aims: To investigate the association between prior oral statin use and risk of MGUS and MM using the UK Clinical Practice Research Datalink (CPRD).
Methods: Conditional logistic regression models were used to estimate odds ratios (OR) and associated 95% Confidence Intervals (CI) excluding 12 months prior to MGUS/MM diagnosis. Findings were adjusted for a number of potential confounders including age at diagnosis, comorbidities, prior cancer, number of GP consultations and lifestyle variables.
Results: In total, 4,654 MGUS and 3,801 MM patients matched to 23,101 and 18,991 controls respectively were identified. In adjusted analyses, statin users were found to have a 19% reduced risk of developing MGUS (OR, 0.81; 95% Confidence Interval (95% CI), 0.74-0.88) and a 23% reduced risk of developing MM (OR, 0.77; 95% CI, 0.69-0.85). Only lipophilic statins were significantly associated with a reduced risk of both MGUS and MM. A dose response relationship between prior statin use and risk of both MGUS and MM was also evident.
Summary and Conclusion: Statin usage was associated with a reduced risk of developing both MGUS and MM in this large population-based study. Statins may therefore have a role to play in preventing progression of MGUS to MM or associated lymphoproliferative malignancies. Further investigation and clinical trials are however needed to confirm this.

P947

CHRONIC INFECTION, A NEGLECTED CAUSE OF DEVELOPMENT OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) AND MYELOMA

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Background: Chronic infection can lead to B-cell malignancy via the direct transformation of infected B-lymphocytes or indirectly via cell transformation consecutive to chronic antigen-driven stimulation; the two mechanisms may occur simultaneously [1,2]. Numerous studies have established that viruses (Epstein-Barr virus (EBV), human herpes virus 8, hepatitis C virus (HCV)) or bacteria (*H. pylori*) can induce lymphoma and chronic lymphocytic leukemia (CLL). In contrast, the role of chronic infection in the pathogenesis of myeloma is rarely investigated. Yet monoclonal immunoglobulins (mc Ig) that arise in HCV-positive myeloma patients typically target the virus [2]. Thus HCV infection may lead to monoclonal gammopathy of undetermined significance (MGUS) and eventually, myeloma.
Aims: The present study shows that mc Ig also target EBV, *H. pylori* and Varicella Zoster Virus (VZV) and that HCV is not the only germ involved.
Methods: In order to study the specificity of mc Ig, we designed a new assay based on a multiplexed infectious antigen microarray (MIAA) that combines representative epitopes of a panel of germs that included HCV, EBV, *H. pylori* and 5 other germs, spotted in triplicate on nitrocellulose-coated slides [3]. Slides were first incubated with either serum or purified mc Ig, then with an infrared dyed (IRD)-labeled secondary antibody, and fluorescence signals were detected and quantified.
Results: The specificity of purified mc Ig from 110 patients diagnosed with MGUS (n=34) or myeloma (n=76) was analysed using the 8-germs MIAA assay. We found that 17.6% of MGUS and 23.6% of myeloma patients presented with a mc Ig that was specific for an antigen from HCV (10 cases), EBV (11 cases), *H. Pylori* (2 cases) or VZV (1 case). In contrast, none of the mc Ig studied targeted CMV, a virus against which a majority of individuals possess antibodies. Gathering our previous studies [1,2], 10/11 HCV-positive patients had mc Ig directed against HCV, either the core (n=7) or NS-4 (n=3) proteins. Interestingly, EBV-specific mc Ig all targeted a single antigen. Regarding EBV-positive patients, the mc Ig was specific in 17.7% cases (11/62), and in all cases the mc Ig targeted Epstein Barr nuclear antigen (EBNA). For 7% (2/28) of *H. pylori*-positive patients, the mc Ig targeted different antigens of *H. Pylori*. Only one mc Ig targeted glycoprotein E of VZV.
Summary and Conclusion: Altogether, 24 of the 110 MGUS and myeloma patients (21.8 %) examined presented a mc Ig specific for a pathogen (HCV, EBV, *H. pylori* or VZV). Thus as described for lymphoma and CLL, chronic infection can also induce MGUS and eventually, trigger the pathogenic processes that lead to myeloma. Efforts should be made to identify the subsets of patients with mc Ig specific for HCV, EBV, *H. pylori* and VZV, preferably at the MGUS stage, as anti-infection treatment could cure MGUS and prevent progression toward myeloma.

P948

IMMUNE IMPAIRMENTS IN MULTIPLE MYELOMA BONE-MARROW MESENCHYMAL STROMAL CELLS

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Background: In multiple myeloma, bone marrow mesenchymal stromal cells play an important role in pathogenesis and disease progression by supporting myeloma cell growth and immune escape. Previous studies suggested that direct and indirect interactions between malignant cells and mesenchymal stromal cells result in constitutive abnormal immunomodulatory capacities in mesenchymal stromal cells.
Aims: The aims of this study were to further study and to investigate the mechanisms that underlie these MM BM-MSC abnormalities.
Methods: We analyzed MM BM-MSCs expression of a variety of adhesion molecules and immune effectors and compared the results to MSCs from healthy donors (HD BM-MSCs). We measured the concentrations of immunoregulatory cytokines in co-cultures of T cells and MM BM-MSCs. Finally, we evaluated the fate of T cells co-cultured with MM BM-MSCs.
Results: We demonstrated that myeloma mesenchymal stromal cells exhibit abnormal expression of CD40/40L, VCAM1, ICAM-1, LFA-3, HLA-DR and HLA-ABC. We observed an overproduction of IL-6 and a reduced secretion of IL-10 when myeloma mesenchymal stromal cells were co-cultured with T lymphocytes compared to co-cultures with healthy donors mesenchymal stromal cells. An increased Th17/Treg ratio was observed when T cells were co-cultured with myeloma mesenchymal stromal cells compared to co-cultures with healthy donors mesenchymal stromal cells.
Summary and Conclusion: Our observations demonstrated that altered immunomodulation capacities of myeloma mesenchymal stromal cells were linked to variations in their immunogenicity and secretion profile. These alterations lead not only to a reduced inhibition of T cell proliferation but also to a shift in the Th17/Treg balance. We identified factors that are potentially responsible for these alterations, such as IL-6, VCAM-1 and CD40/40L which could also be associated with myeloma pathogenesis and progression.

P949

MALAT-1, A LONG NON-CODING RNA, IS OVER-EXPRESSED IN MULTIPLE MYELOMA AND IS A PREDICTOR OF EARLY PROGRESSION

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Background: The pathogenesis of multiple myeloma involves complex genomic and epi-genomic events.
Aims: The purpose of this study was to investigate the role and clinical significance of long non-coding RNA in multiple myeloma.
Methods: Forty long non-coding RNAs (length >5000 nucleotides) selected from a bio-informatics database were used. The clinical characteristics and associations of these long non-coding RNAs were analyzed
Results: Initial screening revealed that a long non-coding RNA, MALAT-1, showed consistent and significant changes between newly-diagnosed and post-treatment patients (mean ΔC_T: -5.26±0.85 *vs*. -3.74±1.38; p=0.02). In the validation test, MALAT-1 was over-expressed in newly-diagnosed patients with myeloma originating from bone marrow compared with extramedullary myeloma (mean ΔC_T: -5.58±0.21 *vs*. -3.83±0.23; p<0.01). In the patients with myeloma originating from bone marrow, the expression of MALAT-1 changed dynamically during treatment and follow-up. The expression of MALAT-1 decreased significantly after induction treatment and then increased significantly when the disease was in progression. The magnitude of the change in MALAT-1 after treatment was clinically significant. The patients with early progression had a significantly smaller change in MALAT-1 compared with the patients with late progression (mean ΔC_T change:1.25±1.12 *vs*.2.37±0.81, p<0.01). A cut-off value of MALAT-1 change(ΔC_T change:1.5) was obtained, and could predict the risk of early progression even in VGPR or CR status (OR 5.44, 95% CI 1.78-16.63; p<0.01).
Summary and Conclusion: MALAT-1, a non-coding RNA with very long length, was over-expressed in patients with myeloma originating from bone marrow, and may play a role in the pathogenesis of this disease. In addition, MALAT-1 may serve as a molecular predictor of early progression.

P950

PREVALENCE AND PROGNOSIS SIGNIFICANCE OF THE MYD88 (L265P) MUTATION IN IGM MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING WALDENSTRÖM MACROGLOBULINEMIA

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Background: Waldenström macroglobulinemia (WM) is a clonal lymphoproliferative disorder characterized by the accumulation of lymphocytes and plasma cells producing an excess of IgM monoclonal immunoglobulin protein, resulting in symptoms either from bone marrow infiltration or from blood alterations due to the paraprotein. *MYD88* (L265P) is a highly prevalent somatic mutation in WM patients. WM is usually preceded by an IgM monoclonal gammopathy of undetermined significance (MGUS) and/or smoldering WM (SMW). It has been reported that *MYD88* mutation is present in almost half of IgM MGUS patients.
Aims: To ascertain the prevalence of *MYD88* (L265P) mutation in IgM MGUS, SMW and MW patients, evaluating also the clinical, biological and prognostic impact of this mutation in a series of patients from a single institution.
Methods: We studied ninety-eight patients (45F/53M; median age 70 years, range 33 to 92) diagnosed with an IgM MGUS, SMW or MW between May 1982 and November 2011. According to the Mayo Clinic guidelines, SWM was defined by a bone marrow lymphoplasmacytic infiltration over 10% and/or a serum IgM monoclonal protein ≥30 g/L, with no evidence of end-organ damage or symptoms attributed to the lymphoproliferative disorder. Patients with less tumoral burden were classified as IgM MGUS, and those with any evidence of symptomatic disease attributed to the monoclonal gammopathy were diagnosed with WM. Genomic DNA was isolated from bone marrow slides using a commercial kit (Qiagen). *MYD88* (L265P) mutation was analyzed using a quantitative real-time PCR technology (qBiomarker Somatic Mutation PCR Assay, Qiagen) using ARMS® (Amplification Refractory Mutation System) primer-based allele discrimination with negative and positive controls in each plate. Sensitive detection of the intended mutation is as low as 1% mutant sample on a wild-type sample background. Time to progression (TTP) and overall survival (OS) were measured in MGUS and SWM patients from diagnosis until progression to symptomatic gammopathy or death, respectively.
Results: Fifty-six patients (57.1%) were classified as IgM MGUS, 30 (30.6%) as SWM and 12 as symptomatic WM (12.2%). Light-chain isotype was mainly kappa (63.3%), followed by lambda (31.6%) and few biclonal cases (5.1%). After a median follow-up of 4 years for alive patients (range, 3 months to 25 years), only 5.4% patients with IgM MGUS as compared to 23.3% with SWM developed symptomatic WM (p=0.029). Most of these patients progressing to symptomatic WM carried the *MYD88* (L256P) mutation (2 out of 3 MGUS; 6 out of 7 SWM). Median TTP was longer in patients with MGUS than those with SWM (21 *vs*. 12 years; p=0.001). Median OS of patients with MGUS and SWM was significantly longer than WM cases (19.9 and 17.2 *vs*. 5 years; p<0,001). MYD88 mutation was found in 88.3%, 80% and 23.6% of patients diagnosed with WM, SWM and MGUS, respectively (p<0,001). Regarding our 86 patients with asymptomatic IgM monoclonal gammopathy, we observed no difference in bone marrow plasma cell infiltration but a higher lymphocytic infiltration in mutated compared with wild-type MYD88 patients (20% *vs*. 9%; p<0.001). MYD88 carriers were also significantly older (p=0.02), had higher ESR (p=0.025) and showed a trend towards higher serum M-spike (p=0.07). TTP was shorter in mutated *vs*. wild-type patients (median not reached *vs*. 22 years, p=0.067) (Figure). However, MYD88 status did not influence survival in our series.

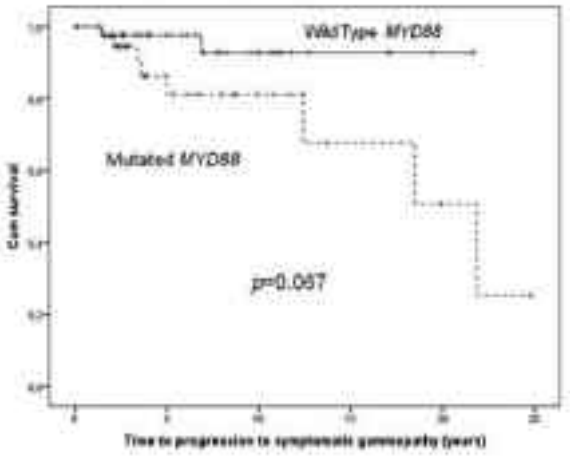

Figure 1. Time to progression from asymptomatic IgM monoclonal gammopathies (MGUS and smoldering Waldenström macroglobulinemia) to symptomatic disease, according to the presence of L265P mutation in MYD88.

Summary and Conclusion: We observed a high prevalence of *MYD88* L265P mutation in asymptomatic IgM gammopathies, in agreement with previous series, as well as a significantly higher tumour burden in patients carrying the mutation. In our series with a long follow-up, patients with asymptomatic IgM gammopathies carrying the mutation showed a trend towards a shorter time to progression than patients with wild-type genotype. Our results support the potential role of *MYD88* status to identify asymptomatic IgM gammopathies at higher risk of progression to symptomatic disease.

P951

INHIBITION OF NAMPT AUGMENTS IBRUTINIB INDUCED KILLING IN MYD88 L265P EXPRESSING WALDENSTROM`S MACROGLOBULINEMIA CELLS

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Background: Whole-genome sequencing studies have revealed that >90% of Waldenström macroglobulinemia (WM) patients exhibit a recurrent somatic mutation in MYD88 (L265P). The presence of MYD88 L265P distinguishes WM from overlapping IgM secreting lymphoproliferative disorders including marginal zone lymphoma, chronic lymphocytic leukemia and multiple myeloma (MM), wherein MYD88 L265P is either absent, or infrequent (<10%). MYD88 is an adaptor molecule for Toll-like and IL-1 receptors which promotes IRAK1/4/TRAF6 as well as BTK mediated NFκB signaling. The BTK inhibitor, Ibrutinib, has shown remarkable pre-clinical and clinical activity in WM. Since nicotinamide phosphoribosyl transferase (Nampt) is a key enzyme involved in NAD+ directed NFkB signaling in MM cells, we evaluated its role in WM growth and survival, and sought to clarify its potential as a target for WM related therapy, alone and in combination with Ibrutinib.
Aims: In this study, we aim to identify a new potential therapeutic target in MYD88 L265P-expressing WM cells.
Methods: The expression levels of Nampt transcript (probe ID 217739_s_at) as well as its prognostic role was evaluated by analysis of microarray databases in WM patients. A panel of WM cell lines as well as purified WM-LPCs cells obtained from WM patients was used in the study. The antitumor effect of the Nampt inhibitor, FK866, alone and combined with BTK inhibitor, ibrutinib, was investigated by Annexin-V/propidium iodide staining. Intracellular NAD+ level and NF-kB activity were quantified by specific assays. Mechanistic studies were performed with thymidine incorporation, western-blotting, lentivirus-mediated shRNAs and immunofluorescence assay. Synergistic interactions were assessed by CalcuSyn software.
Results: Proteomic analysis of WM cell lines and primary tumor cells showed wide overexpression of Nampt. Using sRNAi against Nampt, we observed significant decrease in WM cell growth (BCWM.1 and MWCL-1) confirming a pivotal role in tumor growth. Treatment of WM cells with the NAD+ depleting agent FK866 with ibrutinib induced synergistic anti-WM cell death in both WM cell lines and primary WM LPCs with a CI<1. This effect was associated with: 1) more robust inhibition of NFkB signaling; 2) activation of caspase-8, caspase-9, caspase-3, and PARP and 3) enhanced intracellular NAD+ depletion. Lastly, knockdown of Nampt significantly enhanced the anti-WM effects of ibrutinib which can be rescued by ectopic overexpression of Nampt.
Summary and Conclusion: Our *in vitro* findings demonstrate that Nampt has a crucial role in MYD88 L265P-expressing WM cell biology. Indeed, intracellular NAD+ levels represent a major determinant in promoting constitutive NFkB activation observed in MYD88 L265P expressing WM cells. Consistent with these findings, the anti-WM effect of Nampt inhibition was enhanced by BTK inhibition in a synergistic fashion. Our studies therefore provide the rationale for clinical protocols evaluating Nampt inhibitors such as FK866 together with ibrutinib to improve clinical outcome in WM.

P952

MOLECULAR PROFILING OF PATIENT-DERIVED MYELOMA XENOGRAFTS

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Background: Multiple myeloma (MM) is caused by clonal expansion of malignant plasma cells within the bone marrow caused by acquired genetic mutations within myeloma cells. Interactions with bone marrow stromal cells (BMSCs) contribute to disease progression. Dynamic interplays lead to dysregulation of key processes resulting in treatment failure. For overcoming these problems, development of new therapeutic agents for MM is urgently required in a preclinical model that reproduces *in vivo* environment.
Aims: We tried to establish clinically relevant human myeloma xenograft that can contribute to the understanding of MM cells' biologic behaviors. We also intended to characterize genomic profile of these xenograft.
Methods: To establish patient-derived MM xenograft, mononuclear cells obtained from MM patient's bone marrow were directly injected via tail vein in a NRG/SCID mouse. Fourteen weeks after the injection, tumor developed at subcutis of the mouse. The engraftment of MM cells into mouse bone marrow (BM) was also observed. We separated cells from these two sites (subcutis and BM). Molecular characterization of patient tumors and xenografts was performed using whole exome sequencing.
Results: After the separation of cells from these two sites (subcutis and BM) we performed various analysis including genomic profiling. In cytogenetic analysis, karyotype of newly established both MM xenograft showed tetraploidy which is different from the karyotype of the patient (duploidy) indicating clonal evolution. In FASC analysis, the expression of CD138 and CD45 was detected in both xenografts. Whole exome sequencing showed that less than 2% of genes had recurrent variations between patient tumors and their respective xenografts. Comparison analysis showed that unique 28 somatic mutations from xenografts growing at subcutis and unique 29 somatic mutations from xenografts growing at bone marrow were found, respectively. These unique mutations govern the biologic behaviours of MM cells such as the sites of tumor growths. These genes are largely responsible for the progression of MM. Finally, analysis of successive passages of the same MM cells showed that sequential mouse-to-mouse tumor grafts did not affect genomic rearrangements or patterns of somatic mutations, suggesting stability of genetic alterations of these models over time. These xenograft models, therefore, represent a valid model for preclinical investigation of new therapeutic agents.
Summary and Conclusion: Xenograft of different sites from a single patient showed differences in karyotype and genomic profile. Xenograft modeling as used in our study would help understanding the biology of multiple myeloma.

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ANTI-TUMOR ACTIVITY OF SELINEXOR (KPT-330), AN ORAL SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE), ± DEXAMETHASONE IN MULTIPLE MYELOMA PRECLINICAL MODELS & TRANSLATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Selinexor (KPT-330) is a potent, oral selective inhibitor of exportin 1 (XPO1) mediated nuclear export (SINE) that restores tumor suppressor proteins (TSPs) to the nucleus, causing their activation, inducing apoptosis of neoplastic cells. Selinexor also controls the nuclear export of the glucocorticoid receptor and may enhance the anti-tumor effects of dexamethasone (DEX). Selinexor has previously shown activity in patients (pts) with heavily pretreated multiple myeloma (MM) in an ongoing Phase 1 study. The main side effects of selinexor are anorexia, nausea, and fatigue. DEX has clear anti-myeloma activity and could mitigate the main side effects of selinexor.
Aims: Here we investigate the combination of selinexor with DEX in preclinical models and present updated results of selinexor ± DEX in patients with MM.
Methods: MM1.S human MM cells were used for nonclinical studies. MTT assay was used to measure *in vitro* cell survival. Xenograft studies involved subcutaneous tumors in NOD-SCID mice. Pts with advanced, relapsed/refractory (rel/ref) MM were dosed with oral selinexor at doses of 3 to 60mg/m^2 (8-10 doses/4 wk cycle) ± DEX (20mg PO 2X/wk), as part of a broad Phase 1 program in advanced hematological malignancies. Appetite stimulants and anti-emetics were used as supportive care.
Results: Selinexor or DEX alone inhibited MM1.S *in vitro* proliferation with nanomolar potency and the combination was additive. In the MM1.S MM xenograft model, suboptimal doses of selinexor (7.5 mg/kg PO QODX3/wk) or DEX (1 mg/kg IP QDX5/wk) induced tumor growth inhibition (TGI) of 25% and 32%, respectively. The selinexor/DEX combination was synergistic with 82% TGI. Thirty-four MM pts (18 M, 16 F; median age 61 yrs; median prior regimens: 5.6 (1-13); ECOG 0/1/2: 5/28/1) received selinexor across 10 dose levels (3 to 60mg/m^2) an additional 4 pts received selinexor 45mg/m^2+DEX 20mg. Cycle 1 Grade 3/4 adverse events (AEs) in >1 pt included: thrombocytopenia (6pts), nausea (3pts), and neutropenia (3pts). The most common Cycle 1 Grade 1/2 AEs for 8 / 10 doses were GI-related including nausea (71% / 70%), fatigue (54% / 50%), anorexia (42% / 50%) & vomiting (42% / 20%). Dosing is ongoing at 60mg/m^2 twice weekly and MTD has not been reached. No clinically significant cumulative drug toxicities have been noted. Tumor biopsies confirmed nuclear localization of TSPs, reduced MM proliferation, cell cycle arrest, apoptosis and reduced osteoclast differentiation following selinexor treatment. Response was evaluable in 28 MM pts on selinexor alone: Partial Response 1 pt (3%), Minor Response 4 pts (14%), Stable Disease 17 pts (61%), Progressive Disease 6 pts (21%). Several pts have continued on monotherapy with selinexor >8 months. Four pts have received combined selinexor + DEX with no unexpected toxicities to date; anti-tumor effects are pending.
Summary and Conclusion: Combined treatment of selinexor with DEX enhanced antitumor activity *in vitro* and were synergistic in a MM mouse model. Oral selinexor treatment is generally well tolerated and has prolonged single agent disease control in heavily pretreated pts with progressive, rel/ref MM. Clinical results show single agent selinexor activity and preliminary observations suggest improved tolerability with selinexor and "low dose" DEX. Additional patients are being enrolled on the combination.

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COMPARISON OF THE N-LATEX AND FREELITE ASSAYS FOR SERUM FREE LIGHT CHAIN: CLINICAL PERFORMANCE IN AL AMYLOIDOSIS

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Background: The measurement of circulating free light chain (FLC) is essential in the diagnosis, prognostic stratification, and evaluation of response to therapy in light chain (AL) amyloidosis. In this disease FLC are not only a clonal marker, but they are the pathogenic agent causing organ damage. For more than 10 years this has been done with an immunonephelometric assay based on polyclonal antibodies (Freelite). Recently, a new assay based on monoclonal antibodies (N latex FLC) has been marketed in Europe and Australia.
Aims: To evaluate the performance of the N latex FLC assay in the diagnosis, prognostication of survival, and assessment of response to therapy in AL amyloidosis.
Methods: We compared the clinical performance of the assays in 426 patients with newly-diagnosed AL amyloidosis from the Pavia Amyloidosis Research and Treatment Center (353 patients) and from the Limoges Centre de Référence des Amyloses Primitives et des Autres Maladies de Dépôts d'Immunoglobuline Monoclonale (73 patients). All the patients gave written informed consent.
Results: We found poor agreement between the two methods (84% for k and 88% for l), with median normalized (percent) differences between Freelite and N latex FLC measurements increasing with the concentration of clonal FLC, particularly in patients with k clones. The diagnostic sensitivity of the Freelite (82%) and N latex FLC (84%) assays was similar, and both improved to 98% in combination with serum and urine immunofixation, indicating that the identification of amyloidogenic FLC requires the combination of high-sensitivity assays including urine immunofixation. The concentration of FLC measured with both methods had prognostic significance, with slightly different cutoffs (180 mg/L for the Freelite test, and 165 mg/L for N latex FLC). The dFLC percent reduction best predicting survival was 50% with the Freelite assay, as expected; whereas it was lower, *i.e.* 33%, with the N latex FLC test. Response, defined as a >50% dFLC decrease by Freelite and by a >33% dFLC decrease by N latex FLC, translated in a significant survival advantage. With these cutoffs, the concordance of the two assays in the discrimination of responders was 84% (95%CI 78-89%).
Summary and Conclusion: The two assays have similar diagnostic and prognostic performance. With the N latex FLC method variations after treatment are smaller. The two assays are not interchangeable and follow-up should be done with either one. New response criteria are needed for the N-latex assay.

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CYSTATIN C BASED ESTIMATION OF GLOMERULAR FILTRATION RATE IN AL AMYLOIDOSIS

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Background: Cystatin C-based eGFR (eGFR-cy) has been proven superior to creatinine based eGFR (eGFR-cr) in predicting death and progression to end stage renal disease in general populations and in patients with chronic kidney disease. The kidney is involved in approximately 70% of patients with AL amyloidosis, and is responsible for significant morbidity and treatment limitations.
Aims: To evaluate the performance of different formulas for estimation of eGFR in AL amyloidosis.
Methods: We compared eGFR-cr, eGFR-cy, and the estimation based on both creatinine and cystatin C (eGFR-cr/cy) in 362 consecutive newly-diagnosed (between 2004 and 2012) patients with renal AL amyloidosis. All the patients gave written informed consent. Sixty-five percent had also cardiac involvement (cardiac stage III in 30%). Patients who died off-dialysis were censored for the analysis of renal survival.
Results: Median (interquartile range) creatinine was 1.11 mg/dL (0.65-1.62 mg/dL) and cystatin C 1.2 mg/L (1.0-1.8 mg/L). Forty-nine percent of patients died and 15% initiated dialysis. Median survival was 43 months. There was no difference between the equations in predicting progression to dialysis (Table). However, Cystatin C-based estimations were predicors of patients' survival, while eGFR-cr had no impact on survival. Subjects with eGFR-cy<90 mL/min had shorter survival (median 28 months *vs*. not reached, P<0.001). Comparing patients (n=33) with eGFR-cr but not eGFR-cy<90 mL/min with patients (n=39) with eGFR-cy but not eGFR-cr<90 mL/min there was no difference in progression to dialysis, while the latter had shorter survival (P<0.001). At multivariate analysis cardiac stage III (HR 3.34, P<0.001) and eGFR-cy (but not eGFR-cr)<90 mL/min (HR 2.51, P=0.001) were independent prognostic determinants.

Table 1. ROC analysis based on progression to dialysis at 2 years

Formulas	AUC	95%CI
eGFRcr/MDRD	0.86	0.79/0.93
eGFRcr/CKDEPI	0.85	0.78/0.92
eGFRcy	0.83	0.76/0.90
eGFRcr/cy	0.85	0.78/0.92

Summary and Conclusion: The different eGFR formulas perform similarly in assessing risk of progression to dialysis. However, eGFR-cy is an independent marker of patients' survival.

P956

A PROFILE OF 140 LONG-TERM SURVIVORS WITH SYSTEMIC AL AMYLOIDOSIS

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Background: Diagnosis of AL amyloidosis is widely regarded to incompatible with long term survival consequently, very little has been reported on the long term outcome in this group of patients. The mayo group reported a 5 year survival of 16% for patients diagnosed between 1966 and 1987 and the Italian group reported a median survival of 46 months.
Aims: We report the largest series of long term surviving patients with systemic AL amyloidosis from the UK National Amyloidosis centre (NAC).
Methods: All patients with systemic AL amyloidosis seen at the NAC, diagnosed between 1979-2003, who had survived for longer than 10 years from diagnosis were included in this study. AL amyloidosis was confirmed histologically in all patients with exclusion of hereditary amyloidosis. Organ involvement and responses were defined as per the international consensus criteria.
Results: 913 patients with AL amyloidosis were seen at NAC upto August 2003. 140 (15%) patients survived for longer than 10 years of which 111(79%) had renal, 34 (24%) had cardiac and 46 (33%) had liver involvement. 32 patients lived >15 years, 5 for >20 years and the longest surviving patient is alive at 34 years after diagnosis. Median age at diagnosis was 54.9yrs (28.9-76.3) and the male:female ratio was 0.89. The median creatinine was 91umol/L (47-850) and 24 hour proteinuria was 4.91g (<0.15-27.30g), LV septal wall thinness of 10mm(8-19) and ALP of 89IU/L (36-1814). 68% patients had nephrotic range proteinuria. 4 presented with end stage renal failure (ESRF) and 1 has had a renal transplant at presentation. Median number of lines of treatment was 2 (1-7). 49 (37%) patients had autologous stem cell transplant (ASCT), of which 5 patients had 2 ASCTs and 2 had allograft. There were also 17 renal, 3 cardiac and 1 liver transplantation. 36 (31%) patients developed ESRF (73% established on HD and 28% on PD) and the median time to ESRF was 4.18 years (0.01-16.4 years). Of the 17 patients who underwent renal transplant, the mean time to transplant was 5.7 years (0.5-16.76 years) from diagnosis. 2 patients had amyloid recurrence in the graft. Median OS was 18.3yrs. Patients with cardiac involvement had a poorer outcome compared to the non-cardiac cohort (14.7 years *vs* 22 years, p=0.005). Those who had achieved a VGPR or better had a significantly better median OS than non responders (not reached *vs* 13.1yrs respectively, p=0.001, figure 1) and those who had required 2 or less lines of treatment had a significantly superior outcome compared to those requiring multiple therapies (22yrs *vs* 15.9yrs, p=0.001). Those who underwent ASCT at any time also had a better median OS compared to those treated with chemotherapy alone (NR *vs* 15.9, p=0.018).

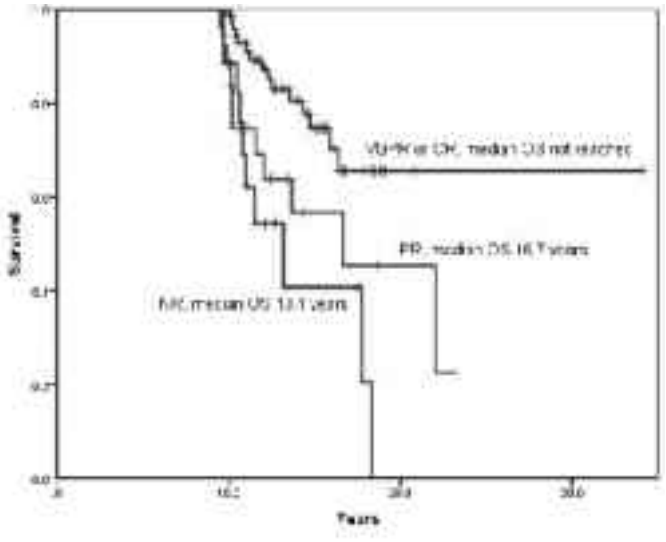

Figure 1.

Summary and Conclusion: In conclusion long terms survival is possible for 16% of patients with AL amyloidosis and the majority (80%) of these have renal involvement. Despite surviving over 10 years, patient with cardiac involvement continue to have an inferior outcome compared to those without. Achieving a deep clonal response improves survival and on multivariate analysis, ASCT at any time and<2 line of total therapy remained significant independent factors of survival.

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FIRST-LINE THERAPY FOR MULTIPLE MYELOMA (MM): RESULTS FROM THE SECOND INTERIM ANALYSIS OF THE REAL-WORLD, INTERNATIONAL, NON-INTERVENTIONAL EMMOS STUDY

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Background: Objective data on country-to-country variation in therapies for MM are lacking.
Aims: The prospective, observational EMMOS study (NCT01241396) aimed to collect data on MM treatments at different disease stages in routine clinical practice.
Methods: Consenting adult patients (pts) initiating any new line of MM therapy, regardless of treatment line at inclusion or type of chosen therapy, were eligible. A multi-staged site/pt recruitment model was applied to minimize selection bias; enrolment was stratified by country, region, and practice type. At baseline, pts' medical and disease features, treatment history, and current remission status were documented. Prospective data, including information on treatment, efficacy, and safety, were electronically captured every 3 mos until 3 yrs after the last pt enrolled. Responses were physician-assessed after each cycle; no predefined response criteria were mandated. Retrospective data on adverse drug reactions (ADRs) were collected, and treatment-emergent adverse events (AEs) were recorded prospectively. Here we present results of the second interim analysis, focusing on first-line therapy in pts who had not yet undergone autologous stem cell transplantation (ASCT) at the time of data extraction.
Results: At data cut-off (30 Apr 2013), 2447 pts had been enrolled in 22 countries in Europe and Africa, including 1642 non-ASCT pts. Median follow-up was 20 mos from diagnosis and 12 mos from study entry. Median age at study entry was 69 yrs (range, 32–91). 49% of pts were male and 63% were diagnosed in 2011–12. 38%, 32%, 17%, and 13% were enrolled at academic, regional, private, and local clinics, respectively. 67% had bone lesions, and 18% had severe renal impairment. Of 348 pts with cytogenetic testing, 7% were positive for del 17p and 7% for t(4;14). First-line therapy data were available for 1596 non-ASCT pts of whom 1104 (69%) received novel agent-based therapy, including 775 (49%) with bortezomib (208 pts prior to study entry; 567 pts during the prospective phase). Most common first-line regimens received

were bortezomib-melphalan-prednisone (VMP, 18%), bortezomib-dexamethasone (VD, 12%), melphalan-prednisone alone (MP, 12%), and melphalan-prednisone-thalidomide (MPT, 7%). In pts aged<65 yrs (n=622), the most common first-line regimens were MP, VD, and vincristine-doxorubicin-dexamethasone (each 11%), and cyclophosphamide-dexamethasone-thalidomide (10%), and in ≥65 yrs (n=971), VMP (27%), VD (13%), MP (12%), and MPT (11%). Best response by first-line therapy is shown in the table. ORRs appeared higher with bortezomib-based than with non-bortezomib-based first-line therapy. Retrospective data for 208 pts who had received first-line bortezomib showed that 23% had ≥1 ADR, mainly nervous system (13%) and gastrointestinal disorders (5%); 3% had ≥1 serious ADRs. Of 567 pts who received first-line bortezomib in the prospective phase, 69% had ≥1 AE, including 14% thrombocytopenia, 8% leucopenia, 7% diarrhea, and 6% peripheral neuropathy; 26% had ≥1 serious AE and 6% died due to AEs.

Table 1.

First-line combination regimen received (n [%] of all pts receiving regimen)	All response-evaluable pts (n=1274)		Pts aged <65 yrs (n=603)		Pts aged ≥65 yrs (n=771)	
	ORR (≥PR), %	≥VGPR, %	ORR (≥PR), %	≥VGPR, %	ORR (≥PR), %	≥VGPR, %
Bortezomib + IMiD-based (n=62 [5%])	79	48	78	60	80	43
Bortezomib, non-IMiD-based (n=590 [43%])	78	36	77	37	78	35
IMiD, non-bortezomib-based (n=272 [21%])	66	28	66	32	66	25
Other combinations (n=350 [31%])	53	12	54	13	52	10

IMiD, immunomodulatory drug (thalidomide or lenalidomide); ORR, overall response rate; VGPR, very good partial response; PR, partial response.

Summary and Conclusion: Data from this real-world study appear to reflect results from the clinical trial setting. First-line bortezomib, received by 49% of non-ASCT pts, produced substantial response rates with no new safety signals. Ongoing analyses will provide longer follow-up and additional data about MM treatments (*e.g.* sequencing, ASCT pts) in routine clinical practice.

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SUBCUTANEOUS (SC) VERSUS INTRAVENOUS (IV) BORTEZOMIB IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA (MM): SUBANALYSIS OF PATIENTS WITH RENAL IMPAIRMENT IN THE PHASE 3 MMY-3021 STUDY

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Background: The randomized, open-label phase 3 MMY-3021 study (NCT00722566) in 222 patients (pts) with relapsed MM determined non-inferiority of SC *vs* IV bortezomib (Btz) dosing, with an improved safety profile (Moreau P, *et al.* Lancet Oncol 2011;12:431–40).
Aims: This subanalysis in pts with renal impairment investigated the effect of SC *vs* IV Btz dosing on response rates (CR+PR by EBMT), time to response (TTR), rates of renal impairment reversal, long-term outcomes, and safety profiles.
Methods: Consenting adults with measurable MM and 1–3 prior lines were randomized 2:1 (stratified by ISS and number of prior lines) to receive eight 21-d cycles of Btz 1.3 mg/m^2 d 1, 4, 8, 11 by SC or IV injection. Within each arm, pts were categorized into subgroups: baseline creatinine clearance (CrCl) ≤50 mL/min (moderate to severe renal impairment) or >50 mL/min (mild or no renal impairment). Renal impairment reversal was defined as achievement of post-baseline CrCl >60 mL/min.
Results: Of 148/74 pts randomized to SC/IV Btz, 33/13 (22%/18%) had CrCl ≤50 mL/min. Baseline characteristics for SC *vs* IV pts in the CrCl >50 and ≤50 mL/min groups were generally well balanced except for median age (71 *vs* 78 yrs), ISS stage III disease (52% *vs* 77%), and 1 prior therapy (73% *vs* 62%) in the CrCl ≤50 mL/min group. In pts with CrCl ≤50 *vs* >50 mL/min, the median number of cycles received was 8 *vs* 8 for pts on SC Btz, and 3 *vs* 8 for pts on IV Btz. ORR in SC/IV pts with CrCl ≤50 mL/min was 53%/31% (RR 1.73; CR 6%/0%). In pts with CrCl >50 mL/min, ORR for SC/IV was 52%/57% (RR 0.92; CR 15%/15%). Median TTR for SC/IV pts was 2.3/3.3 mos (HR 1.205) and 3.5/3.5 mos (HR 1.021) for pts with CrCl ≤50 and >50 mL/min, respectively (Figure). In pts with CrCl ≤50 mL/min, renal impairment was reversed in 10/33 (30%) *vs* 2/13 (15%) pts with SC *vs* IV Btz. IV arm results were consistent with unpublished data from the APEX study (renal impairment reversal in 8/62 (13%) pts on IV Btz). After 17.3 (SC) and 17.8 (IV) mos' follow-up, median PFS for SC *vs* IV pts with CrCl ≤50 mL/min was 8.6 *vs* 6.3 mos (HR 0.388), and 9.5 *vs* 9.6 mos (HR 0.949) for pts with CrCl >50 mL/min. For SC *vs* IV pts with CrCl ≤50 mL/min, 1-yr overall survival was 70% *vs* 46%, compared with 78% *vs* 85% for pts with CrCl >50 mL/min. In pts with CrCl ≤50 mL/min, 70% receiving SC *vs* 77% IV had Gr ³3 adverse events (AEs); most common Gr ≥3 AEs were neutropenia 21% *vs* 8%, thrombocytopenia 15% *vs* 8%, anemia 18% *vs* 8%, and asthenia 3% *vs* 15%. In pts with CrCl >50 mL/min, 54% *vs* 69% had Gr ³3 AEs with SC *vs* IV dosing. In the SC *vs* IV arms, serious AEs occurred in 39% *vs* 46% of pts with CrCl ≤50 mL/min, and in 35% *vs* 33% of pts with CrCl >50 mL/min; AEs leading to treatment discontinuation occurred in 24% *vs* 23% of pts with ≤50 mL/min and in 22% *vs* 28% of pts with CrCl >50 mL/min. In pts with CrCl ≤50 mL/min, 2 pts (6%) on SC and 3 pts (23%) on IV Btz died due to AEs. In pts with CrCl ≤50 mL/min, peripheral neuropathy (PN) occurred in 45% *vs* 31% pts in the SC *vs* IV arms, consistent with lower Btz exposure in the IV arm; Gr ³3 PN occurred in 9% *vs* 8% pts.

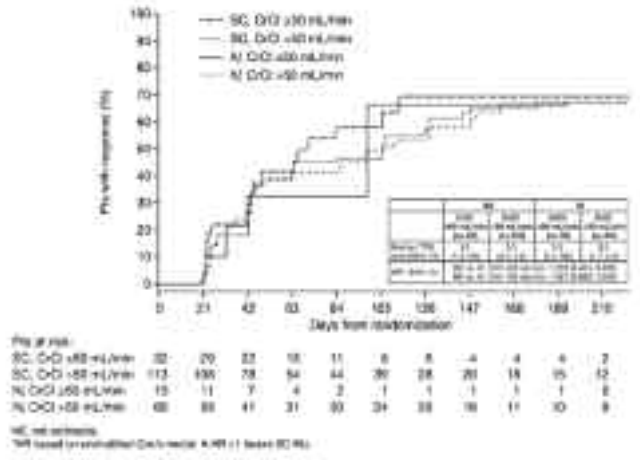

Figure 1.

Summary and Conclusion: In the MMY-3021 study, SC Btz dosing in pts with renal impairment was associated with a more rapid TTR and higher response rates than with IV dosing, and more patients receiving the planned number of cycles. This was accompanied by higher rates of renal impairment reversal with SC Btz.

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A PHASE II SINGLE-ARM SAFETY STUDY OF ELOTUZUMAB IN COMBINATION WITH THALIDOMIDE AND LOW DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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Background: Elotuzumab (E) is a humanized monoclonal IgG1 antibody targeting the cell surface glycoprotein SLAMF7 (CS1, CRACC, CD319, 19A24), which is highly expressed (>95%) on multiple myeloma (MM) cells. Thalidomide, in combination with dexamethasone (Td) is a therapy used in relapsed disease and is associated with grade ≥3 nonhematologic toxicities in 63% of patients. The most common toxicities associated with Td include thromboembolic events, fatigue, constipation, and neuropathy (Rajkumar V. J Clin Oncol 2006;24:431-36).
Aims: To evaluate the safety and tolerability of adding elotuzumab to Td (E-Td) in MM patients with relapsed and/or refractory MM.
Methods: Patients were treated with E-Td in 28 day cycles. During cycle 1 and

2, a weekly loading dose of E was given at 10 mg/kg IV on days 1, 8, 15, and 22, with a dose escalation of T from 50 to 200 mg po qhs. From cycle 3 and beyond, E was given on a biweekly schedule on days 1 and 15. Dexamethasone was given weekly at 40 mg po. Patients who did not achieve ≥partial response by cycle 5 or progressed between cycles 2 and 5 could add cyclophosphamide (C) 50 mg po qd to E-Td (E-TdC). Treatment was continued until disease progression, unacceptable toxicity, or death. The primary endpoint was the proportion of patients who experienced ≥1 severe (≥Grade 3) nonhematological toxicity. Secondary and exploratory endpoints included additional safety parameters and efficacy by IMWG criteria.

Results: Forty patients signed informed consents and were treated. Median age was 64 years, with 63% males. The median time from diagnosis to treatment was 5.0 years (range 0.25-14.4 years). The median number of prior therapies was 3 (range 1-8). Seventeen (43%) patients received ≥4 prior therapies. Prior treatments included: bortezomib 39 (98%), melphalan 30 (75%), lenalidomide 29 (73%), vincristine 20 (50%), cyclophosphamide 19 (48%), doxorubicin 19 (48%), BCNU 18 (45%), and thalidomide 8 (20%). The number (%) of patients refractory or intolerant to prior bortezomib, lenalidomide or both were 24 (60%), 22 (55%), and 14 (35%), respectively. Median duration of study treatment was 4 months (range 0.1-15 months). Eleven patients received E-TdC, primarily due to progressive disease between cycles 2 and 5. Grade ≥3 nonhematologic events were reported in 62.5% of patients, and the most common events (≥20%) were asthenia (35%), peripheral edema (25%), fever (25%), respiratory tract infection (23%), neuropathy (20%), back pain (20%), and constipation (20%). Grade 3-4 neutropenia, anemia, and thrombocytopenia were noted in 13%, 21%, and 11%, respectively. Six patients experienced an infusion reaction; however, no patient discontinued due to these events. The clinical benefit rate was 58% (7 MR, 9 PR, 4 VGPR, 2 CR, and 1 sCR). Among patients with a PR or better, 63% maintained their response at 1 year.

Summary and Conclusion: Elotuzumab can be safely combined with Td or TdC. The grade ≥3 nonhematological toxicities observed in this study are consistent with toxicities observed with Td alone. Minimal incremental toxicity was observed with the addition of E to Td or TdC except for E-related infusion reactions which responded to supportive care and did not lead to discontinuation of therapy. Although the trial was performed in more heavily pretreated MM population in comparison with the results reported for Td trials, the clinical benefit rate is encouraging.

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EFFECTS OF SINGLE-AGENT BORTEZOMIB (BTZ) AS POST-TRANSPLANT CONSOLIDATION ON MULTIPLE MYELOMA (MM)-RELATED BONE DISEASE: FIRST RESULTS FROM A MULTICENTER, RANDOMIZED PHASE 2 STUDY

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Background: Lytic bone destruction is a debilitating manifestation of MM. In addition to its anti-MM activity, data suggest that Btz inhibits osteoclastogenesis and stimulates osteoblast activity, exerting positive effects on bone metabolism that may be bone-specific and independent of the effects on MM. This is the first randomized study of Btz with a bone-specific endpoint.

Aims: This study is evaluating the effects of Btz consolidation *vs* observation (Obs) on bone health in newly diagnosed MM patients (pts) who underwent prior high-dose therapy with autologous stem cell transplant (HDT-ASCT) (NCT01286077). Primary endpoint was baseline to end-of-treatment (EOT) change in bone mineral density (BMD). Secondary endpoints included changes in bone biomarkers, skeletal events, new bone lesions, and safety.

Methods: Consenting adults with newly diagnosed MM who had achieved partial response or better (≥PR) after single/double HDT-ASCT were eligible. Pts were randomized 1:1 (stratified by age [<65 *vs* ≥65 yrs] and baseline bisphosphonate [BPP] use), to 4 x 35-d cycles of Btz 1.6 mg/m^2 IV on d 1, 8, 15, 22, or Obs alone. BMD was measured in spine/femur at baseline, after 2 cycles, and at EOT by dual energy X-ray absorptometry; data were centrally assessed. Serum samples were acquired at these same time points for bone biomarker and M-protein measurement. For pts with missing assessments, a last observation carried forward (LOCF) approach was used. Response was assessed by IMWG 2009 criteria. Adverse events (AEs) were graded by NCI-CTCAE v3.0. Analysis was mainly by intent-to-treat (ITT), including all randomized pts who received ≥1 dose of Btz (Btz arm), and in pts who had a baseline and ≥1 post-baseline BMD assessment.

Results: Between July 2009 and May 2012, 106 pts from 8 countries were randomized (52 Btz, 54 Obs). The ITT population included 104 pts (51 Btz, 53 Obs); median age 58 *vs* 57 yrs, 12% *vs* 15% aged ≥65 yrs, 65% *vs* 58% male, 77% *vs* 74% baseline BPP use. A similar proportion of pts in the Btz (71%) and Obs (66%) arms had received Btz-based induction prior to HDT-ASCT. Post-HDT-ASCT, rates of complete response [CR]/PR were 16%/82% (Btz) and 15%/83% (Obs). 78% *vs* 85% of pts completed Btz consolidation and Obs, respectively. Baseline characteristics were generally well balanced between arms, but 69% (Btz) *vs* 75% (Obs) of pts received BBPs; 39% *vs* 60% zoledronic acid and 29% *vs* 19% pamidronic/ibandronic/clodronic acid. Median baseline to EOT change in BMD is shown in the table; no differences were observed between the arms regardless of age or baseline BPP use. Further, there were no differences between the arms in median baseline to EOT change in bone biomarkers (Table), or incidence of skeletal events or number of new bone lesions. Post-consolidation rates of CR/PR/progressive disease [PD] were 22%/69%/8% (Btz) and 11%/66%/21% (Obs). Rates of grade ≥3 AEs were 10% (Btz) *vs* 6% (Obs) and serious AEs 12% (Btz) *vs* 6% (Obs); no new safety signals for Btz were observed.

Table 1.

Percentage change from baseline to EOT*	Btz		Obs	
BMD†	N evaluable	Median (IQR)	N evaluable	Median (IQR)
Spine	38	+1.89 (+0.17, +3.38)	38	+1.19 (-0.01, +3.73)
Femur (neck)	43	+0.38 (-1.24, +2.03)	45	+0.29 (-1.56, +1.88)
Femur (total)	43	+0.74 (-0.25, +1.84)	45	+1.77 (-0.48, +2.92)
Bone biomarkers‡	N evaluable	Median (IQR)	N evaluable	Median (IQR)
C-terminal cross-linking telopeptide of type I collagen (CTX-I)	48	-29.60 (-62.63, 0)	49	-26.64 (-49.69, 0)
C-terminal cross-linking telopeptide of type I collagen generated by MMPs (CTX)	48	-29.75 (-42.56, -12.21)	49	-25.79 (-38.44, -4.14)
Dickkopf homolog 1 (DKK-1)	48	-12.14 (-34.88, +48.91)	49	-8.94 (-49.62, +43.00)
Bone specific alkaline phosphatase (BALP)	48	-13.94 (-28.30, +13.10)	49	-8.67 (-28.94, +10.68)
Osteocalcin (Oc)	48	-26.21 (-52.79, -8.69)	49	-30.71 (-40.36, -12.80)

EOT, end of treatment; IQR, interquartile range.
*Noncompleting LOCF.
†Analyzed in pts with a baseline assessment and at least one post-baseline assessment of BMD (N=95).
‡Analyzed in the ITT population (N=104).

Summary and Conclusion: Single-agent Btz consolidation and Obs produced comparable changes in BMD and bone biomarkers. Btz consolidation was generally well tolerated, and appeared to improve response depth relative to post-ASCT response and reduce PD rates. Prior Btz-based induction, prior HDT-ASCT, and concomitant BPP use represent possible confounding factors in this analysis. Long-term follow-up is ongoing.

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BENDAMUSTINE AND PREDNISONE IN COMBINATION WITH BORTEZOMIB (BPV) IN THE TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED/UNTREATED MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a generalized malignant disease of plasma cells. In Germany, the incidence of myeloma is approximately 8 cases per 100,000 of the population with around 6200 newly diagnosed patients each year (Robert Koch-Institut 2013). The introduction of thalidomide, lenalidomide and bortezomib into standard therapy has had a positive effect on the complete response rate, progression free survival and overall survival of MM patients.

Aims: Bortezomib is a proteasome inhibitor that has shown important clinical efficacy either as a single agent or in combination in patients with multiple myeloma (MM). In the present protocol, bortezomib was combined with other active substances like bendamustine and prednisone, in order to assess the efficacy and toxicity of this combination therapy in patients with newly diagnosed/untreated MM.

Methods: Between June 2006 and October 2013, 49 patients with newly diagnosed/untreated MM were treated with bendamustine 60 mg/m^2 on days 1 and 2, bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11, and prednisone 100 mg on days 1, 2, 4, 8 and 11 (BPV). Patients were divided into three groups: group A (n=19) consisted of patients with normal renal function or mild dysfunction (eGFR ≥ 60 ml/min), group B (n=15) patients with moderate or severe renal dysfunction (eGFR 15–59 ml/min) and group C (n=15) patients with renal failure/dialysis (eGFR<15 ml/min).
Results: A median number of two (range 1 – 5) BPV treatment cycles were given to the patients. The majority of the patients (n=40, 82 %) responded after at least one cycle of BPV-therapy with 5 sCR, 9 nCR, 12 VGPR, and 14 PR. Five patients had MR, 3 stable and 1 progressive disease. The BPV regimen caused a rapid decrease in the M-protein, with 13 (27 %) patients reaching the best response after the first cycle and a further 20 (41 %) patients after the second cycle. The median time to first hematological response (≥ PR) was 14 days, and the median time to best response was 42 days. Importantly, the rapidity of response of patients in group A with normal renal function or mild renal dysfunction was not different from that of the patients in group B with moderate or severe renal dysfunction and group C with renal failure/dialysis. After a median observation time of the surviving patients of 13 months, PFS at 12 months was 92 % and OS 94 % for patients with normal renal function or mild renal dysfunction as well as 83 % and 93 % for patients with moderate or severe renal dysfunction. Outcome for these patients was slightly better, albeit not statistically significant, compared to patients with renal failure/dialysis with a PFS, and OS of 66 % (p=0.08) and 73 % (p=0.05), respectively. The regimen was well tolerated with few significant haematological and nonhaematological side effects. The most common grade 3/4 hematological toxicities were leukocytopenia (n=12, 25 %), neutropenia (n=3, 6 %), thrombocytopenia (n=11, 22 %), and anemia (n=8, 16 %). There was a moderate difference in leukocytopenia (p=0.09) and neutropenia (p<0.01) between group A/B and group C, with correspondingly more moderate to severe infections in group C (p<0.01).
Summary and Conclusion: These results indicate that this BPV-combination is feasible, effective and well tolerated in patients with newly diagnosed MM.

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WHOLE-BODY LOW-DOSE CT (WBLDCT) IS USEFUL FOR DETECTION, STAGING, RESPONSE EVALUATION AND FOLLOW-UP IN MYELOMA PATIENTS

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Background: Conventional skeletal Xray is still considered the standard approach for the evaluation of bone disease in MM due to its wide availability and cost-effectiveness, although several studies have demonstrated that. Whole body low-dose TAC (WBLDTC) has higher sensitivity, reducing the risk of false negative. It can detect extraosseous disease; it is well tolerated also by compromised patients because it does not need painful positions and acquisition time is short (few minutes). When a low-dose tecnique was developed, whole body TAC could be introduced in the clinical practice.
Aims: We report a group of 318 patients studied from diagnosis with WBLDCT.
Methods: A WBLDCT was performed at diagnosis and repeated at the end of induction therapy (including transplantation), at relapse, and, in the observational period every 12-18 months. In our WBLDCT protocol, the overall dose delivered to each patient was 4.2 mSv.
Results: WBLDCT was positive in 191/318 patients at diagnosis. The distribution of the observed osteolytic lesions (OLs) is reported in table 1. Among patients with a positive WBLDCT at diagnosis, in 94/191 patients this represented the only reason to classify MM as symptomatic. On the contrary, in 97/191 patients, one or more concomitant CRAB symptoms were present at diagnosis. In 149/191 patients a WBLDCT at the end of induction therapy was available. Of these, 29/149 showed an improvement; 109/149 were unchanged; 11 showed a progressions of bone disease. In 7 out of these last, this was the only criterion of progression. In 127 pts initial WBLDCT was negative: 81/127 pts had a symptomatyic MM and received induction therapy and 46 pts had an asymptomatic MM. Among treated patients, 67 were re-evaluated with a WBLDTC at the end of induction therapy: 3 of them showed a progression of bone disease (1 progression defined only by bone disease), and 64 were unchanged. Among patients with asymptomatic myeloma, three were lost to follow-up, 31 remained asymtomatic after a median of 34 months (range 2-89), 12 progressed to symtomatic myeloma after a median of 32 months (range 8-60). In 11 patients a WBLDCT was performed at the time of evolution: in 8 patients WBLDTC was positive and in 3 of them this was the only reason to define progression. In 27 patients unexpected parenchymal lesions were seen, in most cases parenchymal lung lesions, generally interpreted as infectious; 3 patients had asymptomatic kidney masses. In three cases "abdominal lymphadenopathies" were observed: two of them regressed after therapy; in one case the lesions were still present at restaging and, when biopsied, they were shown to be extramedullary disease of myeloma.

Table 1.

Bone segment	Number	(% of positive TC)
Skull	100	49,8
Spine	161	80,1
Sternum	64	31,8
Ribs	98	48,8
Claviculae	57	28,4
Scapulae	52	25,9
Humeri	51	25,4
Femuri	62	30,8
Pelvis	94	46,8
Total	739	

Summary and Conclusion: In conclusion whole-body low-dose CT protocol is feasible and reliably evaluates bone disease at diagnosis and in follow-up. Its higher sensitivity allows earlier detection of bone and extramedullary disease.

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PANOBINOSTAT IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE (VPD) AS INDUCTION THERAPY IN PATIENTS WITH MULTIPLE MYELOMA CANDIDATES TO AUTOLOGOUS TRANSPLANT

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Background: Autologous transplant (tx) is considered the gold standard for young patients (pts) with multiple myeloma (MM). Novel agents containing schemes has significantly improved pre-tx good response rate (≥VGPR). The combination of panobinostat plus bortezomib and dexamethasone, effective in heavily pre-treated MM patients, hasn't yet been tested as pre-tx induction.
Aims: To test the efficacy in terms of pre-tx ≥VGPR rate of the three drugs combination panobinostat, bortezomib and dexamethasone (VPD) in previously untreated symptomatic MM pts.
Methods: This is a phase II multicenter study approved by the local ethic committees, registered on EudraCT registry (I.D. *2011-005847-29)* and conducted according to the Helsinki Declaration. All patients provided written informed consent. Pts with untreated symptomatic MM ≤65 years with measurable disease and no significant comorbidities were eligible. Treatment schedule included 4 VPD cycles with panobinostat 20 mg given orally on days 1, 3, 5, 8, 10, 12, bortezomib 1.3 mg/m2 by intravenous bolus on days 1, 4, 8, and 11 and dexamethasone 40 mg by intravenous bolus on days 1, 4, 8, 11 of each 21-day cycle. All patients were planned to undergo Tx with melphalan at 200 mg/m^2. Pts were monitored for efficacy at the beginning of each VPD cycle. Adverse events were graded according to the NCI CTCAE, Version 4.0 (http://ctep.cancer.gov). These are results of the interim analysis performed after the enrollment of sixteen out of 65 originally planned patients, which led to early closure of the study.
Results: Sixteen pts were analyzed. **One patient withdrawn informed consent during the first cycle and thus was not evaluable.** Eleven out 15 evaluable patients (73%) completed the VPD induction phase; 4 patients (27%) completed VPD cycles at full dose; 4 patients went off study for toxicity (27%). Overall response rate after 4 VPD cycles was 66.7% (1 CR+ 3 VGPR+6 PR); only 26.7% patients achieved ≥VGPR, with no benefit with respect to good response rate previously reported for bortezomib combinations (61%>71% ≥VGPR) (Cavo et al, 2010; Corso et al, 2010; Rosiñol et al, 2012). **Regarding toxicity, s**ixty-three adverse events of any grade were observed: 17.5% (N=11) were hematologic and 82.5% (N=52) non hematologic. The median number of adverse events per patients was 3.5 (range 2-15) with 20% of patients experienced ≥6 adverse events. Common grade 3/4 adverse events included neutropenia (20%), thrombocytopenia (20%) and diarrhea (20%). No grade 3/4 peripheral neuropathy was observed. Due to the low rate of **≥VGPR** achieved at the end of VPD induction the study was closed in advance. **Main reason for** results **lower than expected** has been identified in high rate of dose

reduction/delays (80%) and discontinuation (27%). In I cycle, 10 pts received VPD at full doses, 4 pts required delay/reduction, 1 pts went off study for toxicity. In II cycle, 8 pts received full doses, 4 pts required dose delay/reduction, 2 pts went off study for toxicity. In III cycle, 7 pts received full doses, 4 pts required dose delay/reduction, 1 pts went off study for toxicity. In IV cycle, 6 pts received full doses, 5 pts required dose delay/reduction.
Summary and Conclusion: Given the low rate of good quality response observed in this study after VPD induction, the combination of panobinostat+bortezomib+ dexamethasone might not be suitable for transplant canditates MM patients, in whom reaching a fast and profound response before transplant is crucial.

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EXPOSURE-SAFETY-EFFICACY ANALYSIS OF ORAL IXAZOMIB CITRATE (MLN9708) IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA (MM): PHASE 3 DOSE SELECTION FOR MAINTENANCE POST AUTOLOGOUS STEM CELL TRANSPLANT

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Background: Ongoing phase 3 trials of ixazomib citrate with lenalidomide and dexamethasone in MM are enrolling patients (pts) at a dose of 4 mg weekly (one dose level below the maximum tolerated dose [MTD] of 5.5 mg). A phase 3, randomized, placebo-controlled, double-blind study of oral ixazomib citrate maintenance therapy in MM pts following autologous stem cell transplant is planned. The balance of benefit *vs*. risk is paramount for maintenance therapy when pts already have a clinical response to high-dose therapy, will likely be symptom-free from their disease, and will not have had prior exposure to ixazomib citrate before starting maintenance.
Aims: To identify optimal ixazomib citrate dose to balance benefit *vs*. risk for evaluation in a phase 3 trial of ixazomib citrate maintenance therapy in MM pts following autologous stem cell transplant.
Methods: Safety (S) and efficacy (E) data from pts enrolled in a phase 1/2 study of weekly single-agent ixazomib citrate in relapsed/refractory MM (NCT00963820) were used (N=44); the ixazomib citrate dose range investigated was 1–8.9 mg. The metric of exposure was AUC per day (derived from individual clearance values using population pharmacokinetics) for both exposure (Ex)/S and Ex/E logistic regression analyses. Ex/S analysis was done on 7 adverse events (AEs): hematologic (H) (anemia, thrombocytopenia, neutropenia) and non-hematologic (non-H) (fatigue, rash, peripheral neuropathy, diarrhea). The non-H AE data were categorized into grade ≥2 *vs*. grade ≤1 groups while H AE data were grouped into grade ≥3 *vs*. grade ≤2. The data were categorized in this way as maintenance treatment should have a tolerable AE profile and contribute to acceptable quality of life (QoL). Different cut-offs were used for H and non-H AEs because grade 3 H AE may have less impact on QoL and be more manageable than grade 2 non-H AE (diarrhea). For Ex/E, data were categorized as: ≥stable disease (SD) *vs*. progressive disease (PD). Clinical benefit rate including SD achieved in relapsed/refractory pts may be a meaningful predictor of expected response in a maintenance setting. The logistic regression analyses were done using SPLUS software version 8.1.
Results: The $t_{1/2}$ of ixazomib after multiple dosing ranged from 4 to 8 days, based on an analysis of data from 6 ongoing phase 1 studies, supporting a weekly dosing schedule. Statistically significant relationships to Ex ($p<0.05$) were observed for 5 AEs (fatigue, rash, diarrhea, thrombocytopenia, neutropenia) and clinical benefit rate (≥SD) (Figure). At a starting dose of 3 mg weekly (54% of MTD), the model predicts ~33% ≥SD, and incidence of grade ≥2 non-H AEs (rash 16%, diarrhea and fatigue 19% each) and grade ≥3 H AEs (neutropenia 10%, thrombocytopenia 22%). Further, the 3 mg dose is within the therapeutic range and represents one dose level below the starting dose used in ongoing phase 3 trials in relapsed/refractory and previously untreated MM.

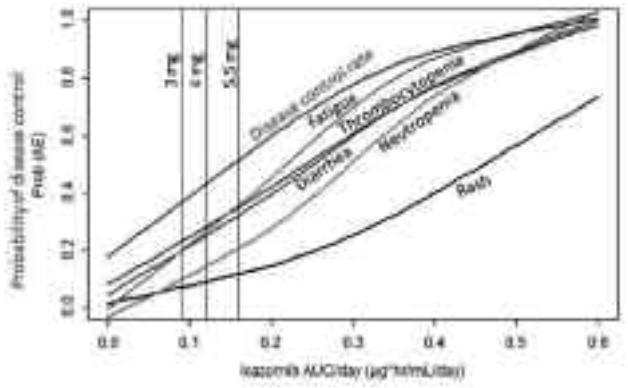

Figure 1.

Summary and Conclusion: The Ex/response relationships indicate that a favorable benefit *vs*. risk may be achieved at doses of 3 mg and 4 mg, below the MTD. Therefore, in the maintenance therapy study, pts will initiate ixazomib citrate at a once-weekly dose of 3 mg, increased to 4 mg if acceptable tolerability after 4 cycles, to provide maximum clinical benefit.

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PHASE 3 STUDY OF POMALIDOMIDE + LOW-DOSE DEXAMETHASONE VS. HIGH-DOSE DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: MM-003 SUBANALYSIS OF ELDERLY PATIENTS (>65 AND >70 YEARS OF AGE)

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Background: Overall survival (OS) is markedly shortened in patients (pts) with relapsed/refractory multiple myeloma (RRMM) who have exhausted bortezomib (BORT), lenalidomide (LEN), or thalidomide treatment (Tx) (Kumar SK, *et al*. *Leukemia*. 2012). Pomalidomide (POM) + low-dose dexamethasone (LoDEX) has demonstrated clinical benefit in pts ≤ 65 yrs and >65 yrs (Jagannath S. EHA. 2013; Leleu X. *Blood*. 2013). MM-003, a randomized phase 3 study, demonstrated significant progression-free survival (PFS) and OS benefits for POM + LoDEX *vs*. high-dose dexamethasone (HiDEX), despite half of HiDEX pts subsequently receiving POM (San Miguel J. *Lancet Oncol*. 2013).
Aims: This subanalysis of MM-003 examined pt outcomes based on age.
Methods: Pts had to be refractory to last prior Tx (progressive disease [PD] during Tx or within 60 days) and exhausted BORT and LEN after ≥ 2 consecutive cycles of each alone or in combination. Pts were randomized 2:1 to receive 28-day cycles of POM 4 mg on days 1-21/28 day cycle + DEX 40 mg (20 mg for pts >75 yrs) weekly or DEX 40 mg (20 mg for pts >75 yrs) on days 1-4, 9-12, and 17-20. Tx continued until PD or unacceptable adverse events (AEs). The primary endpoint was PFS. Secondary endpoints included OS, overall response rate (ORR; ≥ partial response), and safety. All pts provided informed consent.
Results: 455 pts were randomized to POM + LoDEX (n=302) or HiDEX (n=153). This analysis focused on pts ≤ 65 yrs *vs*. >65 yrs since only 8% of pts were >75 yrs; an efficacy comparison between pts ≤ 70 yrs *vs*. >70 yrs was added as an exploratory analysis. Pts ≤ 65 yrs were more likely to have prior stem cell transplant (91% *vs*. 45%), to have better renal function (CrCl ≥ 60 mL/min: 78% *vs*. 51%), and less advanced disease (ISS stage III: 28% *vs*. 37%). All groups had a median of 5 prior Tx, and the median follow-up was 15.4 mos. Consistent with the overall population, POM + LoDEX resulted in significantly extended PFS (Figure A) and favorable OS (Figure B) across all age subgroups. The ORR was significantly higher for POM + LoDEX *vs*. HiDEX in all pts (32% *vs*. 11%), and in all age subgroups: ≤ 65 yrs (32% *vs*. 11%), >65 yrs (33% *vs*. 11%), ≤ 70 yrs (31% *vs*. 13%), and >70 yrs (35% *vs*. 7%); *P*<.001 for all comparisons. The most common grade 3-4 AEs for pts ≤ 65 yrs (POM + LoDEX *vs*. HiDEX) were neutropenia (51% *vs*. 22%), anemia (35% *vs*. 41%), and infections (34% *vs*. 20%). For pts >65 yrs, neutropenia (45% *vs*. 13%), anemia (30% *vs*. 37%), and infections (31% *vs*. 30%) were also the most frequent grade 3-4 AEs. Overall, grade 3-4 deep vein thrombosis or pulmonary embolism was infrequent (POM + LoDEX *vs*. HiDEX): 1% *vs*. 0% (≤ 65 yrs) and 2% *vs*. 0% (>65 yrs). Grade 3-4 peripheral neuropathy was also infrequent: 1% *vs*. 3% (≤ 65 yrs) and 2% *vs*. 0% (>65 yrs). Discontinuation due to AE was low in pts ≤ 65 yrs (POM + LoDEX: 6%; HiDEX 10%) and >65 yrs (POM + LoDEX: 13%; HiDEX: 11%). Median duration of POM Tx was 4.4 mos and 4.0 mos in pts ≤ 65 yrs and >65 yrs respectively and relative POM dose intensity was 90% for both age groups.

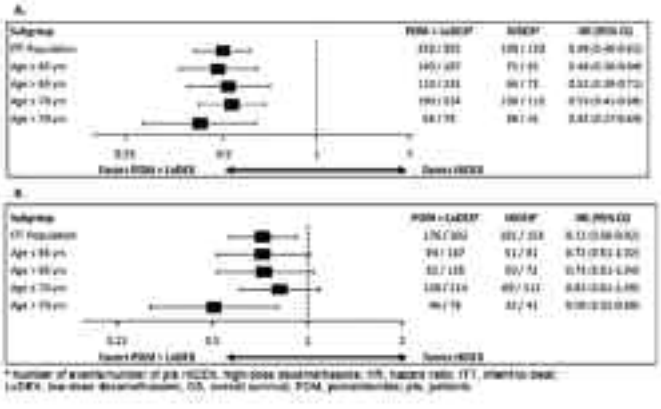

Figure 1.

Summary and Conclusion: PFS and OS benefits of POM + LoDEX were similar to those of the overall pt population and favored POM + LoDEX in the age subgroups analyzed. Tolerability profiles were consistent regardless of age group. POM at 4 mg is an appropriate starting dose for pts aged >65 yrs. These data support considering POM + LoDEX as a standard Tx option in RRMM pts of any age.

P966

CYTOGENETICS AND LONG-TERM SURVIVAL IN MM-003, A PHASE 3 TRIAL OF POMALIDOMIDE + LOW-DOSE DEXAMETHASONE VS. HIGH-DOSE DEXAMETHASONE IN REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA

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Background: Patients (pts) with relapsed/refractory multiple myeloma (RRMM) have few treatment (Tx) options and short overall survival (OS) after exhausting novel agents. Pts with high-risk cytogenetic abnormalities have shorter OS (Kumar SK. *Leukemia.* 2012). The immunomodulatory agent pomalidomide (POM) combined with low-dose dexamethasone (LoDEX), showed significant progression-free survival (PFS) and OS benefits in RRMM pts who failed bortezomib (BORT) and lenalidomide (LEN; San Miguel J. *Lancet Oncol*. 2013).
Aims: Update survival data and address the impact of cytogenetics on outcomes.
Methods: Pts were refractory to last prior Tx (progressive disease [PD] during Tx or within 60 days) and failed BORT and LEN after ≥ 2 consecutive cycles of each (alone or in combination). Pts were randomized 2:1 to receive 28-day cycles of POM 4 mg D1-21 + LoDEX 40 mg (20 mg for pts >75 yrs) weekly or HiDEX 40 mg (20 mg for pts >75 yrs) on D1-4, 9-12, and 17-20. Tx continued until PD or unacceptable adverse event (AE). The primary endpoint was PFS; other endpoints included OS, overall response rate (ORR; ≥ partial response), duration of response (DoR), safety, and cytogenetics. Modified high-risk cytogenetics were defined as the presence of del(17) and/or t(4;14) by conventional or FISH analysis at the time of screening.
Results: 455 pts were randomized to POM + LoDEX (n=302) or HiDEX (n=153). Modified high-risk cytogenetics were present in 77 POM + LoDEX pts (25%) and 35 HiDEX pts (23%). 44 POM + LoDEX pts had del(17p) and 44 had t(4;14). Median follow-up was 15.4 mos. POM + LoDEX significantly extended PFS and OS *vs*. HiDEX (Figure), despite 56% of HiDEX pts receiving subsequent POM. ORR was significantly higher (32% *vs*. 11%; *P*<.001), and median DoR was significantly longer (7.5 *vs*. 5.1 mos; *P*=.031) for POM + LoDEX *vs*. HiDEX. By multivariate analysis, Tx with POM + LoDEX, normal serum albumin levels, and normal lactate dehydrogenase (LDH) levels correlated with longer OS. Longer (>12 mos *vs* ≤ 3 mos) duration of POM + LoDEX Tx and OS were associated with better ECOG PS, absence of plasmacytoma, lower ISS stage, and normal LDH, hemoglobin, and platelet levels. ORR and median PFS advantages were seen for POM + LoDEX *vs*. HiDEX for both modified high-risk (ORR: 25% *vs*. 9%, *P*=.071; PFS: 3.8 *vs*. 1.1 mos, HR=0.44, *P*<.001) and standard-risk cytogenetics (ORR: 35% *vs*. 10%, *P*<.001; PFS: 4.2 *vs*. 2.3 mos, HR=0.55, *P*<.001). Within the POM + LoDEX arm, PFS was similar for modified high-risk *vs*. standard-risk cytogenetics (3.8 *vs*. 4.2 mos, *P*=.217) but differed between pts with t(4;14) *vs*. del(17p) (2.8 *vs*. 4.6 mos, *P*=.011). Median OS favored POM + LoDEX *vs*. HiDEX (modified high risk: 9.9 *vs*. 4.9 mos, HR=0.67, *P*=.092; standard risk: 14.0 *vs*. 9.0 mos, HR=0.85, *P*=.38). OS was shorter for POM + LoDEX pts with modified high-risk *vs*. standard-risk cytogenetics (9.9 *vs*. 14.0 mos, *P*=.041) and did not vastly differ but favored del(17p) *vs*. t(4;14) (12.6 *vs*. 7.5 mos, *P*=.133). The most frequent grade 3-4 AEs for POM + LoDEX *vs*. HiDEX were neutropenia (49% *vs*. 17%), anemia (33% *vs*. 39%), and infections (33% *vs*. 25%). Grade 3-4 DVT/PE (1% *vs*. 0%) and peripheral neuropathy (1% *vs*. 1%) were infrequent. Discontinuation due to AEs was low: 9% *vs*. 10%.

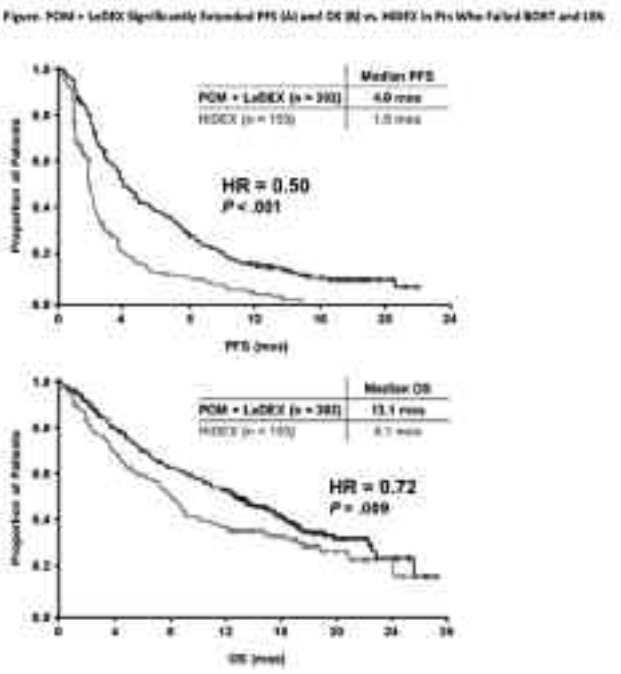

Figure 1. POM + LoDEX Significantly Extended PFS (A) and OS (B) vs. HiDEX in Pts Who Failed BORT and LEN

Summary and Conclusion: This analysis confirms the significant PFS and OS benefits for POM + LoDEX *vs*. HiDEX, which were consistent across cytogenetic groups. Median PFS for pts receiving POM + LoDEX was not impacted by cytogenetics. POM + LoDEX is a new standard Tx option in pts with RRMM who failed BORT and LEN.

P967

OVERALL SURVIVAL OF PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA: ADJUSTING FOR CROSSOVER IN THE MM-003 TRIAL FOR POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE VS. HIGH-DOSE DEXAMETHASONE

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Background: Multiple myeloma is a chronic and an incurable relapsing haematological malignancy. After exposure to treatments (Txs) such as thalidomide, bortezomib, and lenalidomide, patients (pts) with relapsed and refractory multiple myeloma (RRMM) have very poor overall survival (OS); one multinational study reported a median OS of 9 months (N=286; Kumar et al,

2012). MM-003 was an open-label, randomized, phase 3 trial undertaken to compare pomalidomide plus low-dose dexamethasone (POM + LoDEX; n=302) *vs*. high-dose dexamethasone (HiDEX; n=153) in the Tx of RRMM pts who had received prior bortezomib and lenalidomide (San Miguel et al, 2013). Median progression-free survival (PFS) and OS were significantly longer with POM + LoDEX than with HiDEX. Median OS in MM-003 was 12.7 and 8.1 months in the POM + LoDEX and HiDEX groups, respectively (intent-to-treat [ITT] analysis; HR=0.74; *P*=.0285). Following disease progression approximately 50% of pts from the MM-003 HiDEX arm crossed over to POM + LoDEX or to POM monotherapy.

Aims: In the presence of extensive crossover, conventional ITT survival analysis methods are biased, therefore, our study aims to estimate the OS difference between POM + LoDEX and HiDEX after adjusting for crossover.

Methods: A 2-stage Weibull method (Latimer et al, 2013) was used to estimate counterfactual adjusted OS data for the HiDEX arm. First, a data set containing only information for HiDEX pts from the point of progression was created. A survival acceleration factor (AF) was calculated for this patient group using a parametric accelerated failure time model with Tx as a binary explanatory variable to distinguish between pts who crossed over and those who did not. Second, the AF was applied to the survival times of all pts in the HiDEX group who crossed over. This method allows reconstruction of the Kaplan-Meier curve as though crossover had not occurred and estimation of OS from the start of Tx until the last observation. The best fitting parametric model was identified using the new Kaplan-Meier curve and standard model selection methods. Mean OS was extracted from the model, as required for use in health-economic evaluation.

Results: After adjusting for crossover, the difference in median OS in the POM + LoDEX and HiDEX groups was 7.0 months (12.7 *vs*. 5.7 months; Table 1; Figure 1). Extrapolation using a log-normal model fitted to the adjusted OS data produced an estimated difference in mean survival time of 14.6 months (28.0 *vs*. 13.4 months; Figure 1). Predicted survival at 3 years was 21% for pts treated with POM + LoDEX compared with 8% for those receiving HiDEX.

Table 1. ROC analysis based on progression to dialysis at 2 years

	Overall survival, months		
Analysis	**POM + LoDEX (n=302)**	**HiDEX (n=153)**	**Difference**
Intent-to-treat, median (95% CI)	12.7 (10.4-15.5)	8.1 (6.9-10.8)	4.6
Crossover adjustment, median (95% CI)	12.7 (10.4-15.5)	5.7 (4.2-7.5)	7.0

POM, pomalidomide; LoDEX, low-dose dexamethasone; HiDEX, high-dose dexamethasone.

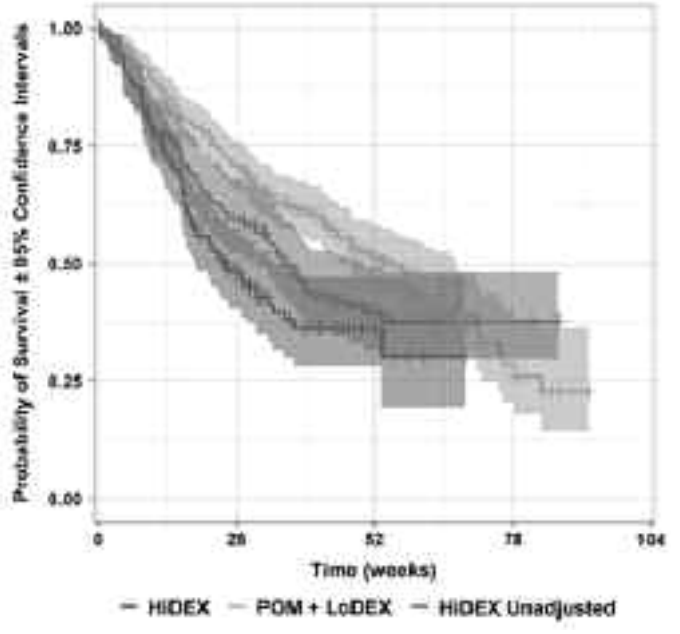

Figure 1.

Summary and Conclusion: The MM-003 ITT analysis showed a significant increase in median PFS and OS for POM + LoDEX *vs*. HiDEX. Survival adjusted for crossover in the HiDEX arm is comparable to 2 observational studies of RRMM pts refractory to current Tx, which reported median OS of 3.9 months (Tarant et al, 2013) and 5.3 months (Gooding et al, 2013). Our analysis suggests that median OS with POM + LoDEX is double that with HiDEX after accounting for crossover. Extrapolation over a lifetime horizon predicts a mean survival difference between POM + LoDEX and HiDEX of more than 14 months. The study provides important evidence for understanding the clinical efficacy and evaluating the overall economic value of POM in the Tx of RRMM pts.

P968

MICRORNA (MIRNA) EXPRESSION PROFILING IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) TREATED WITH CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (CRD)

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Background: Circulating miRNAs are emerging as new diagnostic and predictive markers in hematologic malignancies, including multiple myeloma and may add additional prognostic information to cytogenetics. In our recent update of the phase 1/2 CRd trial in NDMM (NCT01029054) we reported a trend towards lower 3-year progression-free survival (PFS) in patients with high-risk cytogenetics and a trend towards inferior outcome in those with a high-risk SKY92 signature (Jasielec et al, ASH 2013). Here we report serum miRNA profiling results in a cohort of patients from our study.

Aims: The aim of this study was to identify circulating miRNAs that may serve as prognostic markers in patients with NDMM treated with CRd.

Methods: Patients (pts) received 28-day (d) cycles of carfilzomib (CFZ) 20–36 mg/m^2 IV (d1, 2, 8, 9, 15, 16), lenalidomide (LEN) 25 mg PO (d1–21), and dexamethasone 40/20 mg PO once weekly (cycles 1–4/5–8). For cycles 8–24, CRd was given with a modified CFZ schedule (d1, 2, 15, 16), followed by LEN alone after cycle 24 (Jakubowiak *et al.* Blood 2012). All pts provided written informed consent. Serum samples from 30 pts were collected prior to starting therapy and retrospectively analyzed for miRNA expression by real-time (RT) PCR using Exiqon serum/plasma LNA Universal RT miRNA PCR panels. Detectable miRNAs in all samples were mean-normalized. Univariate Cox regression of PFS was performed to derive a nominal *P*-value. Differences in mean miRNA expression in cytogenetic and international staging system (ISS) subgroups were explored using a two-sided *t* test and ANOVA, respectively.

Results: In the 30 pts with available serum samples, median age was 62 y (range, 39–81), 20 (67%) had ISS stage II/III disease, and 8 (27%) had high-risk cytogenetics, including 5 (17%) pts with del17p. All 30 serum samples were successfully analyzed for miRNA expression with 79 miRNAs identified in ≥80% of samples and an average of 104 miRNAs per sample. Four miRNAs of interest were associated with PFS in univariate Cox regression. Two of the four miRNAs were associated with increased risk for progression: miR-378 (hazard ratio [HR]=2.87; p=0.03) and miR-99 (HR=17.24; *P*=0.02); the other two were associated with decreased risk: miR-103a (HR=0.0025; *P*=0.005) and miR-199 (HR=0.41; *P*=0.04). Further analysis revealed a statistically significant difference in PFS based on miR-99/miR-199 expression ratio (p=0.008) (Figure). There was no difference in mean expression of the four miRNAs between pts with high-risk and standard-risk cytogenetics and pts with and without del17p. MiR-99 expression based on ISS staging exhibited a trend for significance by ANOVA (*P*=0.07) with higher miR-99 expression in ISS stages II and III. Subsequent *t* test analyses revealed significant differences between ISS stage I and III (*P*=0.04) and II and III (*P*=0.04).

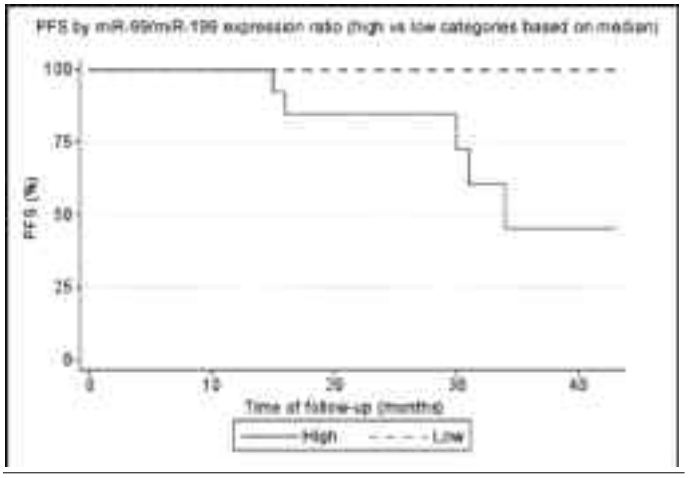

Figure 1.

Summary and Conclusion: In our cohort, miRs-99, -199, 103a, and 378 were associated with different PFS in pts treated with CRd. The expression profile of these biomarkers, especially their combinations, may serve as powerful prognostic markers in NDMM and merit further validation.

P969

IMPACT OF CONSOLIDATION WITH BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE (VTD) UPFRONT IN MULTIPLE MYELOMA (MM) WITH PARTIAL RESPONSE (PR) AT COMPLETION OF INDUCTION WITH VTD

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[1]Service des maladies du sang, Hôpital Huriez, CHRU, Lille, [2]Hôpital Purpan, CHU, Toulouse, [3]Institut Paoli Calmette, Marseille, [4]Hôpital du Bocage, CHU, Dijon, [5]Hôpital St Louis, [6]Hôpital St Antoine, Paris, [7]Hôpital Michallon, CHU, Grenoble, [8]Hôpital Sud, CHU, Amiens, [9]CHRU Hôtel Dieu, Nantes, France

Background: The impact of consolidation on response rates and PFS has recently been demonstrated after induction and autotransplantation upfront in Multiple Myeloma (MM). We further showed that patients in ≥ VGPR following the intensification procedure benefited most from consolidation.
Aims: Question remains as to the benefit of consolidation for patients in PR at completion of induction – feature of partial resistance to the induction regimen.
Methods: This study has included a group of 121 newly diagnosed MM patients that underwent auto from 2009 to 2011 across 9 IFM centers. Patients were to be eligible for auto in first-line of treatment, aged less than 65 years old and treated with VTd-auto-VTd regimen. We then collected data from the 54 patients that reached only PR at completion of the induction procedure.
Results: The median age was 57 years, the sex ratio was 1,25, 58% had ISS 2 and 3, 25% had adverse FISH, including t(4;14) and/or del17p (similar in the 2 groups). Overall, 37 patients (n=37/54, 68%) improved depth of response (at least VGPR) at completion of consolidation (in relation to transplantation and consolidation) in this series, including 33% (18/54) and 35% (19/54) that reached VGPR and CR, respectively. Respectively 57% (31/54) and 30% (16/54) of patients improved the depth of response after ASCT (at least VGPR) and after consolidation (at least VGPR if PR after ASCT, or CR if VGPR after ASCT). With a median follow-up of 40 months, improved depth of response translated into lower relapse rate compared with patients remaining in PR, 19% *vs.* 36%. This difference was more striking in patients that reached CR *vs.* other, 8% and 38%, respectively (p=0.039). The median TTP was prolonged in patients that improved depth of response after consolidation (p=0.012), with a 3-year TTP of 87% *vs.* 18% otherwise. In multivariate analysis, lack of improved depth of response to consolidation independently predicted shorten median TTP [OR=4.4, 95%CI=1-21; p=0.039], with elevated LDH and beta2m, and adverse FISH. The safety profile of VTd consolidation in this population was similar to that reported.
Summary and Conclusion: This study showed an improved response rate in relation to the VTd consolidation phase in patients that partially responded to the induction VTd regimen. This improved quality of responses translated into a lower relapse rate with a prolonged TTP and PFS. This study shows that VTd consolidation should be recommended to patients solely on PR at completion of induction with VTd, feature of lower sensitivity to VTd.

P970

FACTORS INFLUENCING EXTRA-MEDULLARY RELAPSE AFTER ALLOGENEIC TRANSPLANTATION FOR MULTIPLE MYELOMA

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Background: Despite a graft *versus* myeloma (MM) effect observed in several studies, relapse remains a major concern after allogeneic hematopoietic stem cell transplantation (allo-SCT).
Aims: We analysed risk factors, incidence and prognosis of MM extra-medullary relapses (EMR) after allo-SCT.
Methods: A retrospective, continuous, single-center cohort, including 79 patients who received an allo-SCT between 2000 and 2010 for MM (n=70) or plasma cell leukemia (n=9), was analysed. Median age at transplantation was 56 years (34-65). Allo-SCT was performed either in first line (14%) (14% including 6% of tandem auto allo-SCT), or in second line (46%) including 6% of patients with primary refractory disease and 40% of patients with chemo-sensitive post auto-SCT relapse, relapse having occurred at median 2,1 years post auto-SCT), or in third line or later (41%). Reduced-intensity conditionings were used in most cases (97%). Donors were siblings in 42%, matched unrelated in 29% (including 7% HLA 9/10 mismatches) and 29% of grafts were cord blood units. Other grafts were mainly peripheral blood stem cells (PBSC) (68%) or bone marrow (3%). Median follow-up after transplant was 4,8 years for living patients (3,0-12,8).
Results: Pathological examination was performed in 8 cases (42%). Other cases were diagnosed by MRI (47%), CT-scan (42%) and/or ultrasound (10%). In one patient, EMR was in soft tissues, not adjacent to the bone (EMS) and in 17 patients, bone plasmocytomas were observed (EMB). One patient had both types of EMR. EMS was pleural or hepatic. EMB involved mainly long bones (7/18) or skull/orbits (4/18). EMR occurred at a median of 1.3 year post allo-SCT (0-2.1 years), most of the time at first relapse (84%). Factors associated with a significantly increased incidence of EMR in a multivariate logistic regression were CD4 lymphocytes count before allo-SCT superior to 0.212 G/L and absence of pre-allo-SCT exposure to lenalidomide. Rescue treatments allowed a 41% response rate (CR : 21%, PR : 20%). Median survival after relapse was 1.57 year [0.26 ; 3.01] in EMR group not significantly different from that of patients with post allo-SCT relapse without EMR (1.56 year [0.63 ; 2.58]).
Summary and Conclusion: EMR is a frequent event after allo-SCT for multiple myeloma. Although, prognosis is poor, it is not different from non-EMR post allo relapses. Higher CD4+ T-Lymphocyte count before allo-SCT and no pre-allo-SCT lenalidomide exposure were associated with increased incidence of EMR.

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IMPACT OF 18 FDG-PET/CT ON THE OUTCOME OF MULTIPLE MYELOMA

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Background: Several studies have reported the interest of PET/CT for the clinical and prognostic evaluation of multiple myeloma patients (MM). Although its routine use is not recommended, it is a common practice for the management of MM patients. However, few data are available on the clinical impact of such a practice.
Aims: The aim of this study is to identify the indications for PET/CT in MM patients in daily practice and its impact on the therapeutic management of these patients.
Methods: We retrospectively reviewed all PET/CTs performed in our center from January 2005 to December 2013 for MM patients. We analyzed the clinical indications justifying the PET/CT request as well as the clinical signs and the biological status at the time of the prescription. The results and their therapeutic implications were also analyzed.
Results: 267 PET/CTs were performed for 74 MM patients during the period studied. The clinical indications were the evaluation of the disease at diagnosis (13.1%, n=35), the assessment of treatment response (33.7%, n=90), the assessment of disease progression (44.8%, n=120), the assessment of progression of MGUS or SMM to symptomatic myeloma (6%, n=16). At diagnosis, 67% of the patients had a positive PET/CT. Concerning the treatment response assessment, 48% of the reviewed PET/CTs showed complete metabolic responses, 33% showed partial remissions, 3% gave equivocal results and 16% showed progressive disease while undergoing treatment. All patients undergoing first-line treatment had a PET/CT showing complete or partial metabolic response; these results did not change the therapeutic approach. Concerning the search for relapse, 41 % (n=49) of PET/CTs were performed in asymptomatic patients, 21 % (n=25) in patients with pain reported as nonspecific, and 29 % (n=35) in patients with symptoms suggestive of relapse. In the group of asymptomatic patients, the PET/CT results lead to a change in treatment in 18 % (n=8) of the cases; 6 of these 8 patients had a progression of their monoclonal peak and two had multi-treated disease with extramedullary relapse. All asymptomatic patients with no other signs of progression had a negative PET/CT. In patients with nonspecific symptoms, 88% of the PET/CTs did not provide useful information and didn't result in a change of treatment, whereas 12% of the PET/CTs were positive and corresponded to patients with biological progression of their disease. For patients with symptoms suggestive of relapse, 88% of their PET/CTs were positive and led to a change in treatment.
Summary and Conclusion: Our retrospective study on the position of PET/CT for the management of MM patients suggests that: 1) PET/CT is already routinely used in the management of MM patients and in some cases, replaces other radiological investigations. 2) The use of PET/CT for the evaluation of response to first-line treatment does not lead to any change in the therapeutic attitude. 3) In the context of the search for an eventual relapse, PET/CT is of little use in patients with no characteristic clinical signs of progression and should not be used in the follow up of patients unless there is a high suspicion of progression.

Myeloma and other monoclonal gammopathies - Clinical 4

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AN INTERNATIONAL, MULTICENTER, PROSPECTIVE, OBSERVATIONAL STUDY OF NEUTROPENIA IN PATIENTS BEING TREATED WITH LENALIDOMIDE + DEXAMETHASONE FOR RELAPSED OR RELAPSED/REFRACTORY MULTIPLE MYELOMA (RR-MM)

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Background: Neutropenia is a common dose-limiting toxicity in patients with multiple myeloma receiving lenalidomide regimens. At the present time, little information is available regarding the characterization of neutropenia events and their optimal management in this setting.
Aims: This prospective observational study was designed to characterize neutropenia, its management, and its impact on delivery of lenalidomide regimens in patients with RR-MM in a clinical practice setting.
Methods: Eligible patients were ≥ age 18, had been diagnosed with relapsed or relapsed and refractory MM, and provided informed consent where required by local regulations. Patients were either starting treatment with lenalidomide plus dexamethasone or were still in their first cycle at the time of enrollment. Patients were observed from the start of lenalidomide treatment for up to 12 months. Data collected at each routine office visit included: lenalidomide administration, including any dose interruptions or reductions; absolute neutrophil count and body temperature if noted; G-CSF use; and hospitalizations related to MM. The primary outcome was the incidence of grade 3/4 neutropenia.
Results: A total of 198 eligible patients were enrolled between February 2011 and July 2012 at 34 centers in 9 countries (Australia, Austria, Czech Republic, France, Germany, Greece, Ireland, Spain, and UK). All patients were included in the primary analysis set and 69 (35%) completed the 12-month observational period. The most common reasons for early withdrawal were disease progression (21%), tolerability issues with lenalidomide (15%), and death (12%). Fifty-four percent of patients were men and the median age was 70. Most patients (83%) had a current diagnosis of relapsed MM, and the median time from the initial to current diagnosis was 36 months. At baseline, 21% of patients were stage III per the International Staging System. Most (60%) had 1-2 prior therapies and comorbidities were present in 84% of patients. Most patients (68%) received 25 mg/d lenalidomide in the standard schedule (days 1-21), 61% of patients received the dexamethasone dose and schedule included in the lenalidomide prescribing information. For the primary outcome, 62 patients (31%; 95% CI: 25 - 38%) had at least one grade 3/4 neutropenia event and the median time to first grade 3/4 neutropenia was 8.8 weeks (Q1, Q3: 5.9 – 17.3 weeks); half the patients with grade 3/4 neutropenia had 3 or more events. Six patients (3%) experienced febrile neutropenia. G-CSF was used in 23% of patients; filgrastim was administered for a mean of 6.9 days and a median of 1.0 day (range: 1-141). More patients with than without grade 3/4 neutropenia experienced lenalidomide dose reduction (37% *vs* 18%), received G-CSF (40% *vs* 15%), and experienced unplanned hospitalizations (50% *vs* 42%).
Summary and Conclusion: Incidence of grade 3/4 neutropenia in patients with RR-MM receiving lenalidomide and dexamethasone in clinical practice was similar to that reported in phase 3 studies. Grade 3/4 neutropenia events occurred in later cycles than the cytotoxic chemotherapy setting; therefore, different neutropenia monitoring and management may be required. G-CSF use was reactive rather than prophylactic. While lenalidomide toxicity is usually first managed with dose modifications per consensus panel guidelines (Dimopoulos et al, 2011), G-CSF support may be important for patients to maintain optimal dosing for continued response.

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LONGITUDINAL COMPARISON OF PATIENTS' AND PHYSICIANS' PERCEPTIONS OF PATIENTS' HEALTH-RELATED QUALITY OF LIFE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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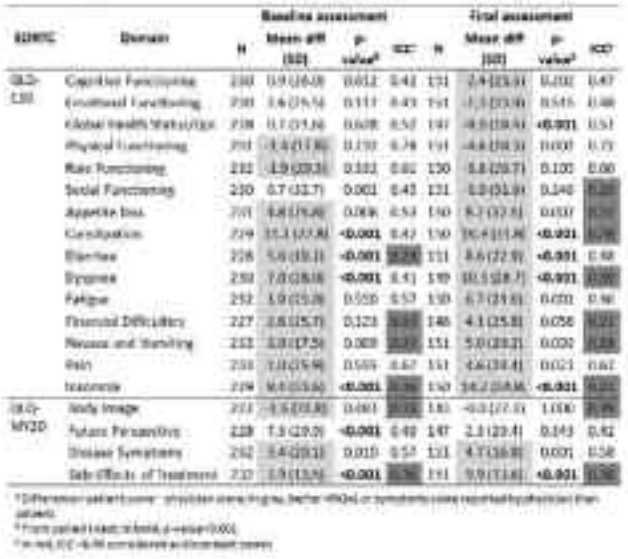
Background: The use of novel therapeutic agents has significantly improved both progression-free and overall survival in multiple myeloma (MM) patients. In this context, it is important to evaluate health-related quality of life (HRQoL). Particularly, exploring the level of concordance between physicians' and patients' perceptions of patients' HRQoL could improve physicians' understanding of patients' feelings and may help in the management of a patient's routine care.
Aims: To compare, on several occasions, physicians' and patients' perceptions of patients' HRQoL in relapsed/refractory MM (RRMM) context.
Methods: A multicenter, observational study is being conducted in Italy, Germany, France, UK, Ireland and Belgium in RRMM patients starting 2nd or 3rd line treatment. Both physicians and patients completed the following EORTC questionnaires at baseline, month 3, and month 6 or discontinuation visit: 1) the Quality-of-Life Questionnaire Core (QLQ-C30), including 15 domains: Global Health Status/QOL, Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, Fatigue, Nausea and Vomiting, Pain, Dyspnea, Insomnia, Appetite Loss, Constipation, Diarrhea and Financial Difficulties; and 2) the QLQ-Multiple Myeloma (QLQ-MY20), including four domains: Disease Symptoms, Side-Effects of Treatment, Body Image and Future Perspective. Paired t-tests and Intra-Class Correlation Coefficients (ICC) were calculated to compare patient- and physician-reported scores at the baseline assessment and then at final assessment (month 6 or discontinuation). Discordant scores were defined as those with an ICC<0.40.
Results: As of November 2013, this interim analysis included 32 physicians who had enrolled 244 patients in the study (mean age=70; 53% male). At baseline, the average time since diagnosis was 3 years, 16% of patients were ECOG performance status ≥2 and 94% of patients were starting 2nd line treatment. At baseline assessment, 236 (97%) EORTC questionnaires were completed by physicians and 235 (96%) by patients. At final assessment, 154 (63%) EORTC questionnaires were completed by physicians and 153 (63%) by patients. According to ICC (Table 1), discordance was observed at baseline assessment between patients and physicians for five symptom domains (Diarrhea, Financial Difficulties, Nausea and Vomiting, Insomnia, Side-Effects of Treatment) and one HRQoL domain (Body Image). Discordance was still observed at final assessment for all these domains except for Diarrhea. Discordance was also observed at final assessment for three additional symptom domains (Appetite Loss, Constipation, Dyspnea) and one additional HRQoL domain (Social Functioning). For all these domains, physicians reported better scores for their patients, indicating better perception of HRQoL and symptoms than reported by the patients themselves.

Table 1. Comparison of EORTC scores at baseline and final assessments



Summary and Conclusion: While no major discordance was observed between patients' and physicians' ratings of functioning and HRQoL domains, there was substantial discordance between the perceptions of physicians and patients for the symptoms and side effect domains. In this interim analysis, physicians underestimated the detriment of RRMM disease and treatment on patients' HRQoL as well as possible negative effects of prior treatment as compared to the individual patient's experience. This discordance persisted when ratings were repeated over time.

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TRANSIENT ELEVATION OF AST OR LDH AS AN EARLY PHARMACODYNAMIC BIOMARKER OF RECOMBINANT CIRCULARLY PERMUTED TRAIL (CPT) IN TREATING PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: Circularly permuted TRAIL (CPT), a recombinant mutant of human Apo2L/TRAIL, selectively induces tumor cell apoptosis by activating pro-apoptotic receptors DR4 and DR5. CPT was found to be effective and well tolerated for patients with relapsed or refractory multiple myeloma (RRMM) in Phase1/2 clinical trials. Transient elevations of serum AST and LDH were observed early after CPT treatment in most response patients, but not in the non-respondent, which could not be simply ascribed to liver injuries. It was assumed that the increased AST and LDH might be associated with CPT therapy responses.
Aims: To determine the relationship between the elevation of AST or LDH and the clinical responses to CPT in RRMM patients, and to evaluate the predictive value of AST or LDH elevation as a pharmacodynamic biomarker of CPT.
Methods: We retrospectively analyzed the data from three Phase1/2 studies of CPT. The relationship between $AST_{D2/3}$, $LDH_{D2/3}$ or $ALT_{D2/3}$ (AST, LDH or ALT level assayed on day 2 or day 3 after initial CPT dosing) and the best clinical responses was evaluated. Statistical analyses were performed to evaluate whether ΔAST/ΔALT ratio or ΔLDH/ΔALT ratio (ΔAST, ΔLDH or ΔALT: ratio of $AST_{D2/3}$, $LDH_{D2/3}$ or $ALT_{D2/3}$ to baseline value) was a potential biomarker in predicting therapy responses, and to determine the cut-off point. Two myeloma cell lines sensitive to CPT (RPMI8226, NCI-H929) and one resistant (U266B1) were cultured, and the concentrations of AST, ALT and LDH in the cytoplasma or the medium of CPT-treated cells were detected to determine whether CPT-induced myeloma cell death could result in an elevation of AST or LDH.
Results: Of 93 CPT-treated RRMM patients, 24.7% achieved a partial response (PR) or better and 36.4% achieved a minimal response (MR) or better with an average serum $AST_{D2/3}$ of 222.03±332.10 U/L and 164.23±269.60 U/L, respectively. Both $AST_{D2/3}$ levels were much higher than that in non-respondent patients (*P*=0.025, *P*=0.013, respectively). It was the same with $LDH_{D2/3}$ (*P*=0.033, *P*=0.011, respectively), but not with $ALT_{D2/3}$ (*P*=0.358, *P*=0.556, respectively) (Table 1). The elevation of AST or LDH was transient with a peak on day 2 or day 3 after CPT treatment, and usually disappeared within one week for AST or two for LDH, which was uniquely observed in the first therapy cycle. The average ΔAST in patients with ≥PR or in those with ≥MR was higher than that in non-respondent ones (*P*=0.031, *P*=0.025, respectively). So it was with ΔLDH (*P*=0.008, *P*=0.004, respectively), but not with ΔALT (*P*=0.062, *P*=0.202, respectively) (Table 1). The average ΔAST/ΔALT or ΔLDH/ΔALT in response patients was significantly higher than that in the non-respondent (*P*=0.001, *P*=0.007, respectively) (Table 1). ΔAST/ΔALT was found to be an applicable biomarker for predicting responses of ≥MR by logistic regression (*P*=0.005), while ΔLDH/ΔALT was not (*P*=0.771) since there were some missing data of LDH. Receiver operating characteristic curve analysis showed that ΔAST/ΔALT with 1.64 as the cut-off point could differentiate between response and non-response with the sensitivity of 63.6% and the specificity of 80.00%. All the three cell lines were verified to contain abundant AST (633.33±290.66 U/L) and LDH (1618.67±571.36 U/L) but only detectable level of ALT (19.33±6.43 U/L). In CPT-sensitive cells, the AST or LDH released into the medium of CPT-treated cells was about three times that of vehicle control, much higher than in resistant cells. However, there was no notable change of ALT in any cell line. It was suggested that the transient elevations of AST and LDH in RRMM patients were most likely resulted from CPT-induced myeloma cell death.

Table 1. Serum AST, ALT and LDH in patients treated with CPT

	Response		Non-response	*P* value
	≥ PR	≥ MR		
$AST_{D2/3}$ U/L	222.03±332.10	164.23±269.60	40.50±43.31	0.025, 0.013
$LDH_{D2/3}$ U/L	926.23±1082.74	718.65±882.27	198.87±240.55	0.033, 0.011
$ALT_{D2/3}$ U/L	34.04±40.25	30.19±32.69	26.20±28.18	0.358, 0.556
ΔAST	13.23±21.64	9.13±17.81	1.94±1.72	0.031, 0.025
ΔLDH	7.29±7.00	5.15±5.55	1.13±0.87	0.008, 0.004
ΔALT	2.41±2.50	1.97±1.45	1.44±1.62	0.062, 0.202
ΔAST/ΔALT	-	4.06±3.70	1.65±1.35	0.001
ΔLDH/ΔALT	-	3.71±3.83	1.30±0.76	0.007

$AST_{D2/3}$, $ALT_{D2/3}$ or $LDH_{D2/3}$: serum AST, ALT or LDH detected on day 2 or day 3 after initial CPT dosing. ΔAST, ΔLDH or ΔALT: ratio of $AST_{D2/3}$, $LDH_{D2/3}$ or $ALT_{D2/3}$ to baseline value. Data are expressed as mean±SD. Independent *t* tests were conducted to compare the differences between response (≥ PR or ≥ MR) and non-response.

Summary and Conclusion: Our results demonstrated for the first time that the early transient elevations of serum AST and LDH after CPT treatment were probably due to the cell apoptosis induced by CPT, and were positively related to the therapy responses of CPT. ΔAST/ΔALT ratio could be a useful pharmacodynamic biomarker for CPT therapy in RRMM patients.

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LONG TERM CRYOPRESERVED AUTOLOGOUS BLOOD STEM CELLS FOR SALVAGE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN RELAPSED MULTIPLE MYELOMA PATIENTS

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Background: Second ASCT (ASCT2) has been considered to be a safe and feasible treatment option for relapsed Multiple Myeloma (MM) patients (pts), especially those who have had durable remissions after first ASCT (ASCT1). The long-term stability of cryopreserved stem cells (SCs) collected prior to ASCT1 has therefore become an important issue in pts who require ASCT2 after a prolonged period of disease remission. There is limited data with small numbers of pts that describe the viability of long-term stored SCs, especially in novel therapy era that immunodulators and proteasome inhibitors have been increasingly utilized as induction or re-induction before ASCT.
Aims: Our study aims to evaluate the stability and engraftment of long-term cryopreserved SCs for salvage ASCTs in a larger number of pts and to assess the effect of novel agent therpy on engraftment parameters after ASCT2.
Methods: We retrospectively reviewed 102 relapsed MM pts who underwent salvage ASCT2 using SCs collected before ASCT1 at PMCC between January 2003 to June 2013. Patient demographics, disease characteristics, chemotherapy treatment and ASCT details were obtained from pts medical charts and transplant database. Neutrophil engraftment was defined as the first of 3 consecutive days that neutrophil counts were ≥0.5x10^9/L. Platelet engraftment was defined as day of platelet counts ≥20 x10^9/L without supportive transfusion. Paired simple T-test was used to compare time to engraftment between transplantations.
Results: One hundred and two pts (65 males, 64%) underwent ASCT2 as salvage treatment for relapsed MM. Median age at diagnosis, ASCT1 and ASCT2 were 56, 57 and 61 years, respectively. MM subtypes included IgG 47%, IgA 23%, light chain disease 26% and others 4%. Majority (98%) of pts received high dose cyclophosphamide followed by G-CSF for SC mobilization and melphalan 200mg/m^2 for conditioning. All collected SCs were cryopreserved in 10% DMSO. SC storage times were 1.9 (0.4-24.7) and 47.2 (15-131) months for ASCT1 and ASCT2. Median time duration between the two transplants was 45 (14-128) months. Median numbers of CD34+cells infused at ASCT1 were 6.43x10^6/kg (2.6-33.2) and 5.7x10^6/kg (1.2-16.9) at ASCT2. There was no significant difference in median time to engraftment between ASCT1 and ASCT2; 12 (6-16) *vs* 11 (10-15) days for neutrophil engraftment (p=0.33) and 11 (10-20) *vs* 11 (10-20) days for platelet engraftment (p=0.26). There was no delayed engraftment of greater than 21 days in any pts. We further investigated the stability of SCs based on years of cryopreservation at ASCT2. There was no difference in time to neutrophil (p=0.06) and platelet engraftment (p=0.74) between groups of pts who were given short (<6years) and long (≥6years) term cryopreserved SCs. We also compared 26 pts who received novel agent-based induction (bortezomib=10 pts, thalidomide=18 pts and lenalidomide=1 pt) prior to SC collection with 76 pts who were never exposed to novel agent treatment; no significant differences in time to engraftment were noted. After median time to relapse of 38 (19-119)months, 68 pts (67%) were treated with novel agents (bortezomib=34 pts, thalidomide=21 pts and lenalidomide=18 pts) for re-induction before proceeding to ASCT2. Similarly, there was no significant difference in engraftment between pts who had novel agents at relapse when compared to those who were not treated with novel agents. Although we found no time to engraftment differences between two ASCTs, we did observe pts who received higher number of CD34+ SCs (≥5x10^6/kg) engrafted faster in ASCT1 (neutrophil p=0.005; platelet p=0.03). For ASCT2, neutrophils engrafted significantly faster (p=0.006) but platelet recovery did not reach significance (p=0.17) for ≥5x10^6/kg SCs infused.
Summary and Conclusion: Long-term cryopreserved SCs can be safely used in salvage ASCT in relapsed MM pts even in the era of novel agents. Cryopreservation of SCs for >6 years results in no loss of viability as determined by comparable times to engraftment in both upfront and salvage ASCT. Additionally, treatment with the novel agents does not interfere with stem cell quality.

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MULTIPLE MYELOMA OF ELDERLY: COMORBIDITY INDEXES, SCALES, AND PERSONALIZED TREATMENT

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Background: The necessity of personalized treatment approach has been emerged after the introduction of the various new treatment modalities for multiple myeloma (MM). Consideration of comorbidity, disability and frailty represents significant part of the treatment in the elderly population of patients (pts).
Aims: The aim of study was to analyse the impact of the Charlson Comorbidity Index (CCI) and scale of Instrumental Activities of Daily Living (IADL) on the course of disease, and propose a model of personalized approach based on those parameters.
Methods: The study included 110 newly diagnosed MM pts (age of ≥ 65yrs: 55 male/55 female, mean age 71 yrs, range 65-81). IgG myeloma had 68pts (61,8%), IgA 25pts (22,7%), light chains 15pts (13,6%), IgD 1pts (0,9%), and non-secretory 1pts (0,9%). According to the clinical stage, distribution was: I 5pts (4,7%), II 21pts (19,6%); III 81pts (75,7%). Regarding ISS score, 12,4%pts had ISS1 score; 31,5% ISS2; and 56,2% had ISS3. Renal impairment existed in 36pts (34%). According to the CCI, median score was 1 (range 0-5). Median age adjusted CCI (aaCCI) score was 5 (range 3-9). Most of the pts had CCI 0-1 (82pts, 74,5%); 2-3 had 21pts (19,1%); 4-5 had 7pts (6,4%). In a view of aaCCI, 49pts (44,5%) had aaCCI 3-4; 5-6 had 51pts (46,4%); aaCCI ≥7 had 10pts (9,1%). Median IADL score was 6 (range 0-8), with IADL ≥6 in 70pts (64,2%); 3-5 in 26pts (23,9%); and 0-2 in 13pts (11,9%). Thalidomide combinations were applied in 63pts (57,3%); 5pts (4,6%) were treated with bortezomib; and 42pts (38,1%) with conventional chemotherapy.
Results: ISS3 correlated with high scores of CCI or aaCCI (R=0,314, p<0,003; R=0,317, p<0,002), and lower IADL (R=0,259, p<0,007). The probability of adverse events (AE) was 70% higher for CCI ≥2 score (OR=1,72); and 28% for aaCCI (OR=1,28). There was 2x higher probability of the AE for IADL<3 (OR=2,25). Treatment response (CR/VGPR/PR/MR) was achieved in 81pts (73,6%) with median duration 14m (range 3-85m). The median overall survival (OS) for the group was 36m (range 6-98m). Patients with CCI 0-1 had significantly longer duration of remission (Breslow 4,37; p<0,037), and OS (Log Rank 1,738; p 0,187). Patients with aaCCI ≥5 had significantly shorter OS (Log Rank 4,209; p<0,040). The OS was found significantly longer in pts with IADL >3 (Log Rank 6,62; p<0,001). Furthermore, aaCCI ≥5 and IADL >3 scores were indicated as major variables in the proposed model for the personalized treatment approach with clear impact on the OS of elderly MM pts (Omnibus test of Model Coefficients, Chi-square 8,46, p=0,037; Hazard rate: IADL ≥3 0,46; 95% CI 0,24-0,9; aaCCI ≥5 1,43; 95% CI 0,76-2,71).
Summary and Conclusion: Age adjusted Comorbidity Index and scale of Instrumental Activities of Daily Living represents important parameters for the treatment and course of disease in elderly myeloma patients, indicating variables as aaCCI ≥5 and IADL >3 of importance for the appropriate treatment choice and possible dose adjustment.

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SMOULDERING MULTIPLE MYELOMA RISK FACTORS FOR PROGRESSION. AN ANALYSIS OF 289 CASES IN THE DANISH MULTIPLE MYELOMA REGISTRY

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Background: There is a need for models of how to stratify Smouldering Multiple Myeloma (SMM) patients at risk of progression to Multiple Myeloma as new clinical trails show that some patients may benefit from early treatment. Major risk models for progression includes presence of immunoparesis (reduction in one or more of the non M-protein immunoglobulin) and abbarent plasma cell percentage (PC)% >95% or bone marrow PC% >10% together with abnormal free light chain ratio (FLCr) and M-protein ≥ 3g/dL and also recently including chromosomal aberrations[1–5]. However, currently there is no consensus on how to classify low, intermediate or high risk disease[6].
Aims: To analyze risk factors for progression and overall survival (OS) for SMM in a population based cohort of patients and to develop a new proposal for a scoring system for time to progression (TTP) to symptomatic multiple myeloma.
Methods: We conducted a study of the 289 newly diagnosed SMM patients registered within the Danish Multiple Myeloma Registry for OS between 2005 and 2013. The median follow-up for TTP and OS was 25,6 mo. and 31.9 mo., respectively. First we made a univariate cox regression analysis to estimate risk factors for TTP and OS. Secondly we calculated a multivariable cox regression including univariat significant risk factors.
Results: Of the registered data a M-protein ≥ 3g/dL resulted in a markedly shortening TTP (HR 3,8 P<0.0001). Furthermore the presence of immunoparesis of 1 or more of the non M-protein immunoglobulins and high level of Clonal Plasma Cell percentage (PC%) were associated with a significantly shorter TTP (P=0.0006 and P=0.01 respectively). The multivariable analysis showed that both an M-protein ≥ 3g/dL and immunoparesis were associated with significantly shorter TTP. Neither M-protein size nor immunoparesis or PC% had an effect on overall survival (OS) (table 1). Using immunoparesis, M-protein ≥ 3g/dL and PC% ≥ 10%, we created a scoring system of 1, 2 or all 3 of the variables and identified a low-risk (1), intermediate (2) and high-risk (3) population. Applying this model we predicted a median TTP of 32 months for the highrisk group *versus* not reached for the intermediate and low risk groups.

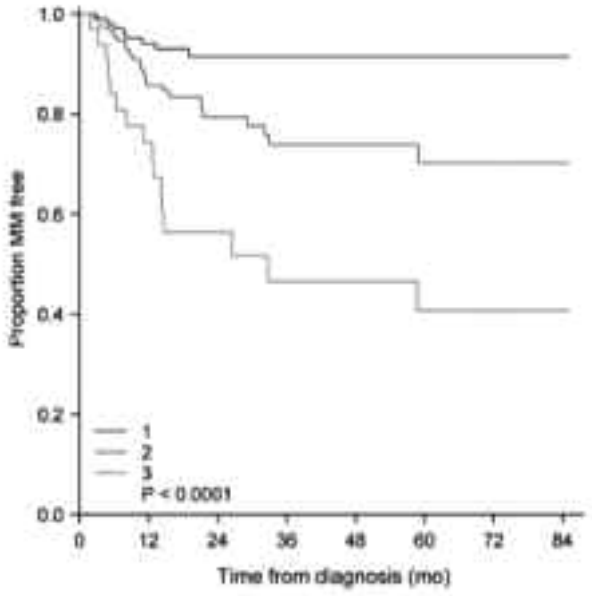

Figure 1. Time to progression (TTP) to symptomatic disease having either 1, 2 or 3 of the risk factors; immunoparesis, M-protein≥ 3g/dL and PC% ≥ 10%. Low-risk; 1, intermediate risk; 2, and high-risk; 3. The model predicted a median TTP of 32 mo. for the high-risk group.

Summary and Conclusion: Our study concludes that the presence of an M-protein ≥ 3g/dL and immunoparesis in SMM patients remains important risk factors for progression to Multiple Myeloma. We found that none of the risk factors for TTP influenced OS. To our knowledge this is the largest retrospective population based cohort study of risk factors for SMM patients. The model did not include a FLC ratio nor presence of abbarant %PC ≥ 95%.
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P978

THE UK AND IRELAND PERSPECTIVE: EUROPEAN POST-APPROVAL SAFETY STUDY (PASS) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH LENALIDOMIDE, BORTEZOMIB, AND THALIDOMIDE

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Background: EU PASS is an observational non-interventional study designed to investigate the safety of lenalidomide (LEN) and other approved treatments (Txs) for relapsed/refractory multiple myeloma (RRMM) patients (pts) in a true-to-life setting.
Aims: To compare the incidence of adverse events (AEs) and second primary malignancies (SPMs) in RRMM pts treated with LEN + dexamethasone (DEX) or other anti-myeloma Txs in routine clinical practice in the UK and Ireland.
Methods: This multicentre, post-authorisation safety study included RRMM pts who had received ≥1 prior Tx. Pts were enrolled at investigator's discretion into a LEN cohort (LEN + DEX: approved combination for RRMM Tx) or a background cohort (all other Txs, including novel agents). AEs were graded according to NCI-CTCAE (v3) and SPMs were defined using MedDRA terms under Neoplasms SOC. Assessments for SPMs were conducted up to 36 months (mos) after Tx discontinuation.
Results: A total of 388 pts across institutions in the UK (n=23) and Ireland (n=2) were enrolled. Of these, 35% (n=136) received LEN, 58% (n=225) received bortezomib (BORT), 5% (n=19) received thalidomide (THAL) and 2% (n=8) received other Txs. Baseline demographics were similar with the exception that more pts in the LEN cohort received ≥2 prior lines of Tx (81%) *versus* BORT (38%) and THAL (47%) (a reflection of standard UK pathways). Median age was 69 yrs (range 30–91) and 59% (n=229) were male. Overall, 58% (n=223) of pts had a good performance status (ECOG score 0–1), 14% (n=56) had an ECOG score 2–4 and 28% of pts had missing or unknown ECOG scores. In the LEN cohort 38% of pts (n=52) were treated for >12 mos and 14% (n=19) for >24 mos. Median Tx durations were 8 mos (range 0–34), 4 mos (range 0–22), and 5 mos (range 1–9) for the LEN, BORT and THAL cohorts, respectively. Median overall follow-up was 5.4 mos (range 0.1–35). The proportion of pts having at least one grade (Gr) 3–4 AE was 60% (n=82), 44% (n=99) and 47% (n=9) in the LEN, BORT, and THAL cohorts, respectively. Gr 3–4 neutropenia was reported in 13% (n=18), 1% (n=2) and 5% (n=1) of pts, and Gr 3-4 thrombocytopenia occurred in 4% (n=5), 3% (n=6) and 0% of pts in the LEN, BORT and THAL cohorts, respectively. The incidences of Gr 3-4 peripheral neuropathy (PN) during the study was the lowest in the LEN cohort (1.5%, n=2), the highest in the BORT cohort (9.3%, n=21), and 5.3 % (n=1) in the THAL cohort. The percentage of pts with all grades peripheral neuropathy (PN) at baseline was 38% (n=52), 24% (n=55) and 11% (n=2) for the LEN, BORT and THAL cohorts respectively. Subsequently, 1.5% (n=2), 4% (n=9) and 0% (n=0) of pts developed Gr 3-4 PN in the LEN, BORT and THAL cohorts, respectively. Study discontinuation rates due to AEs were 14% (n=19), 26% (n=58) and 21% (n=4) in the LEN, BORT and THAL cohorts, respectively. The percentage of pts with SPMs was low with 3% (n=4) in the LEN, 1% (n=2) in the BORT and none in the THAL cohort.
Summary and Conclusion: Results of this study in RRMM show that the incidences of Gr 3–4 AEs are the highest in the LEN cohort, with the exception of PN which was higher in the BORT cohort. The rates of study discontinuation due to AEs were the lowest in the LEN cohort. In general, the incidences of SPMs were low in all cohorts with similar incidences of invasive and non-invasive SPMs observed in both the LEN (1.5%, n=2) and BORT (0.4%, n=1) cohorts.

P979

IMPACT OF RENAL IMPAIRMENT ON THE EFFICACY AND SAFETY OF LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (RD) IN CHINESE PATIENTS (PTS) WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM): MM-021 TRIAL

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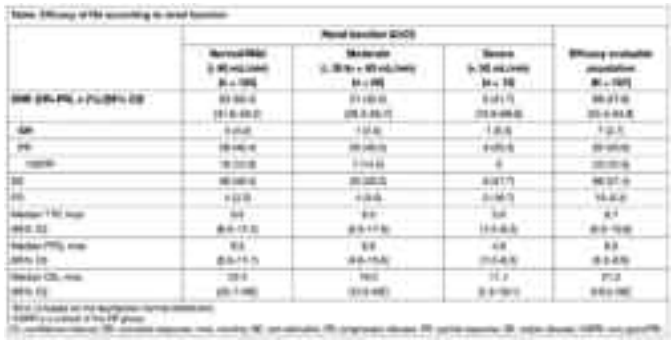
Background: Renal impairment (RI) is a common complication of multiple myeloma. In China, based on the pivotal MM-021 trial, the Rd regimen is approved for the treatment (Tx) of RRMM pts who have received ≥ 1 prior Tx (Hou, J Hematol Oncol 2013). As lenalidomide (LEN) is mainly excreted by the kidney, its plasma concentration and half-life are longer in pts with RI; therefore, dose adjustment is recommended in these pts.
Aims: To evaluate the efficacy and safety of Rd in RRMM pts with RI from the MM-021 trial.
Methods: MM-021 was a phase 2, multicenter, single-arm, open-label study. RRMM pts received LEN (25 mg on D1–21/28 day cycle) plus low-dose dexamethasone (40 mg/day on D1, 8, 15, and 22) until disease progression or discontinuation. The starting dose of LEN was adjusted according to baseline renal function: 25 mg/day for pts with normal/mild renal function (creatinine clearance [CrCl] ≥ 60 mL/min); 10 mg/day for moderate RI (CrCl 30–60 mL/min); and 15 mg every other day for those with severe RI (CrCl<30 mL/min, not requiring dialysis). Thromboprophylaxis was mandatory during the study. The primary endpoint was overall response rate (ORR) defined as the percentage of pts who achieved a best response of partial response or better. Secondary endpoints included time to progression (TTP), progression-free survival (PFS), overall survival (OS), and safety.
Results: A total of 199 pts were enrolled (intent-to-treat population) and 187 pts were evaluable for efficacy. At baseline, 66% of pts (n=131) had normal/mild renal function, 27% (n=54) had moderate RI, and 7% (n=14) had severe RI. The ORR was 50% in pts with normal/mild renal function compared with 42% in those with moderate or severe RI (Table). With a median follow-up of 17.6 months, 42 pts completed their Tx and 157 discontinued. Compared with pts with normal/mild RI, impaired renal function was associated with shorter TTP, PFS, and OS (Table). Pts with normal/mild renal function and moderate RI had similar rates of treatment-emergent adverse events (TEAEs), including grade 3–4 neutropenia (26% and 20%, respectively), anemia (21% and 30%), and thrombocytopenia (11% and 13.0%). The incidences of grade 3–4 neutropenia, anemia, and thrombocytopenia were higher in pts with severe RI (36%, 57%, and 57%, respectively). Few pts (n=2) experienced grade 3–4 peripheral neuropathy. TEAEs led to Tx discontinuation in 5% of pts (n=7) with normal/mild renal function, 13% of those with moderate RI (n=7), and 29% of pts with severe RI (n=4).

Table 1. Efficacy of Rd according to renal function



Summary and Conclusion: This subgroup analysis from the MM-021 study showed that in heavily pretreated RRMM pts with RI, Rd improved clinical outcomes and was generally well tolerated regardless of renal function. For Chinese pts with normal/mild RI, LEN 25 mg/day is the appropriate approved starting dose. Dose adjustments should be made based on CrCl at the start of Tx and renal function should be monitored on a regular basis.

P980

SUPPRESSION OF THE NON-INVOLVED HLC PAIR CORRELATES WITH SURVIVAL IN NEWLY DIAGNOSED AND RELAPSED/REFRACTORY PATIENTS WITH MYELOMA

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Background: Immune suppression is linked to short survival in multiple myeloma (MM) patients (Bryant, C *et al.* Blood Cancer Journal, 2013). A novel immunoparesis was recently identified as a risk marker for MGUS transformation to MM which relied upon the assessment of isotype matched immunoglobulin concentrations *i.e.* measurement of IgGκ concentration in patients producing monoclonal IgGλ (heavy/light (HLC) pair suppression.
Aims: Here we evaluate the role of HLC pair suppression in MM patients and comment on the potential of a disease specific risk stratification model.
Methods: 203 patients with multiple myeloma and measurable disease were enrolled. 156 patients were newly diagnosed: median age: 66 (32-94) years, male/female: 82/74, ISS stage I: 59, II: 63, and III: 34; 63 IgGκ, 37 IgGλ and 33 IgAκ, 23 IgAλ. 47 were relapsed/refractory (median age: 63 (40-86) years, male/female: 24/23, ISS stage I: 20, II: 17, III: 10; 19 IgGκ, 14 IgGλ, 8 IgAκ, 6 IgAλ. Patients in the first cohort received different treatment regimens and those in the second cohort were treated with Bendamustine-Bortezomib-Dexamethason. Median follow-up: 46 months in the first and 21 months in the latter group. HLC pair suppression was described as 50% reduction below the published normal range (IgGκ<1.67, IgGλ<0.951, IgAκ<0.285, IgAλ<0.22) and

systemic immunoparesis described as 50% below published normal ranges (IgG<3g/L, IgA<0.2g/L, IgM<0.1g/L). Results were compared to ISS stage, bone marrow plasma cell infiltration and high risk cytogenetics t(4;14), t(4;16) del17p, ampl 1q21) and correlated with survival. Kaplan Meier survival curves were compared using log-rank test, univariate and multivariate analysis was performed using Cox proportional regression analysis (SPSS, version 18).
Results: 100/100 IgG and 56/56 IgA MM patients had an abnormal Ig'κ/Ig'λ ratio at presentation, with 33/33 IgG and 14/14 IgA MM patients having an abnormal Ig'κ/Ig'λ ratio at relapse. HLC pair suppression was identified in 59/100 IgG and 23/56 IgA patients at presentation and 21/32 IgG and 6/14 IgA patients in the relapsed refractory setting. HLC pair immunosuppression correlated significantly with OS both in patients with newly diagnosed (median: 53 months *vs*. not reached, p=0.04; figure 1a) and relapsed/refractory disease (median OS: 22.8 months *vs*. not reached, p=0.006, figure 1b). HLC pair suppression showed a stronger correlation in patients with IgG (p=0.042) as compared to those with IgA-components, where only a tendency of shorter survival was noted (p=0.091). Immunosuppression of the HLC pair was not correlated with suppression of the non-involved isotypes both in the newly diagnosed and in the pre-treated patients. Univariate analysis showed a significant correlation between HLC pair suppression and high risk cytogenetics (p=0.038) but not with plasma cell bone marrow infiltration (p=0.144). A three factor tiered model employing HLC pair suppression and ß2microglobulin (≥5.5mg/dl) revealed significantly different survival estimates both in newly diagnosed (0 factor. 75% OS: 40 months, 1 factor, 75% OS: 25 months, 2 factors, 75% OS: 12 months, p<0.001) and in pre-treated patients. (0 factor, 75% OS not reached, 1 factor, 75% OS: 21 months, 2 factors, 75% OS: 8 months, p<0.0001). As previously reported, survival was significantly lower in patients with highly abnormal HLC ratio (<0.022;>45) compared to those with less abnormal HLC ratios (17.6 *vs*. 56.6 months, p<0.006) in newly diagnosed and also in previously treated patients (21.7 months *vs*. not reached, p<0.001).

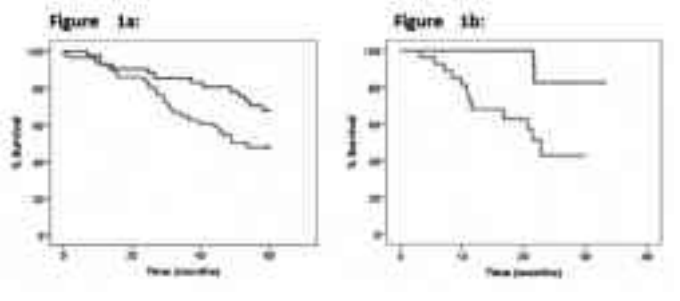

Figure 1. (a) OS in HLC pair suppression in previously untreated patients (n=100); (b) OS in HLC pair suppression in patients with relapsed/refractory MM (n=47)

Summary and Conclusion: Severe suppression of the HLC pair of the involved isotype of the M-component is a significant risk factor for shorter survival, both in newly diagnosed and previously treated patients while for non-involved isotypes no correlation was noted.

P981

PHASE 1 PHARMACOKINETIC (PK) STUDY OF IXAZOMIB CITRATE (MLN9708) PLUS LENALIDOMIDE AND DEXAMETHASONE IN ASIAN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Despite progress in the treatment of MM, the prognosis for pts with RRMM remains poor, with variable, often diminishing responses to subsequent therapies following first-line treatment. Ixazomib citrate is an oral proteasome inhibitor in phase 3 development for the treatment of pts with MM or amyloidosis. However, all clinical experience thus far has been in Western populations.
Aims: This open-label phase 1 PK study (NCT01645930) was conducted to determine the plasma PK parameters of ixazomib when administered in combination with lenalidomide and low-dose dexamethasone in Asian pts with RRMM. The study also evaluated the safety profile of ixazomib citrate, recommended phase 2 dose, and response rates.
Methods: East Asian pts, from Singapore, South Korea, and Hong Kong, aged ≥18 years, with RRMM following 1–3 prior therapies and an ECOG PS of 0–2 were enrolled. Pts received ixazomib citrate 4 mg PO on days 1, 8, and 15 of 28-day cycles; lenalidomide 25 mg PO on days 1–21; and dexamethasone 40 mg PO on days 1, 8, 15, and 22. The maximum tolerated dose (MTD) of ixazomib citrate (defined as the highest dose at which ≤33.3% of pts per dose group experienced a DLT) was evaluated following a standard 3×3 de-escalation schedule during cycle 1, starting at 4 mg. Treatment continued until disease progression or unacceptable toxicity. Blood samples for PK analysis were collected throughout cycle 1 and before dosing on day 1 of cycle 2. AEs were graded according to NCI-CTCAE v4.03.
Results: Twenty-nine pts were enrolled from centers in East Asia (ethnicity: Chinese, 45%; Korean, 31%; Other, 24%) and received ixazomib citrate. Median age was 62 years (range 38–75), 52% were male, and 21%/48%/24% had ISS stage I/II/III MM (7% unknown). Cycle 1 DLTs occurred in two pts: elevated ALT (grades 2 and 3), ALP (grade 2), and GGT (grade 2) in one pt; and diarrhea (grade 3) in the second pt. All enrolled pts received 4 mg and the MTD of ixazomib citrate in combination with lenalidomide and dexamethasone was 4 mg. Maximum plasma concentration (C_{max}) of ixazomib was 7.07 and 11.8 ng/mL/mg on days 1 and 15, respectively. The area under the concentration-time curve (AUC_{0-168}) increased from 188 to 429 h· ng/mL/mg between days 1 and 15. Median time to maximum plasma concentration (T_{max}) was 1.5 and 2.0 hours on days 1 and 15, respectively. PK parameters were consistent across all Asian ethnicities evaluated. Best confirmed response rate (≥PR) was 35% (10% ≥VGPR, including 1 pt with a CR), after a median of 2 cycles (range 1–12). AEs occurred in 26 pts (90%), resulting in dose reductions in 13 pts. The most common AEs (any grade) included diarrhea (45%), anemia (31%), and dizziness (31%). Nineteen pts (66%) experienced grade 3/4 AEs; the most common were diarrhea (28%), anemia (24%), and neutropenia (17%). Serious AEs occurred in 11 pts (38%; treatment-related in 7 pts), with one treatment-related death (pneumonia).
Summary and Conclusion: Ixazomib citrate in combination with lenalidomide and low-dose dexamethasone demonstrated clinical activity in Asian RRMM pts with a manageable AE profile. The study is ongoing for further evaluation of response. The recommended dose of ixazomib citrate in Asian pts is 4 mg, the same as in Caucasian pts, with a similar toxicity and tolerability profile. Asian pts are currently being enrolled in ongoing phase 3 studies (NCT01850524, NCT01564537, NCT01659658).

P982

A MULTINATIONAL OBSERVATIONAL STUDY IN MULTIPLE MYELOMA (PREAMBLE): INITIAL RESULTS OF TREATMENT PATTERNS

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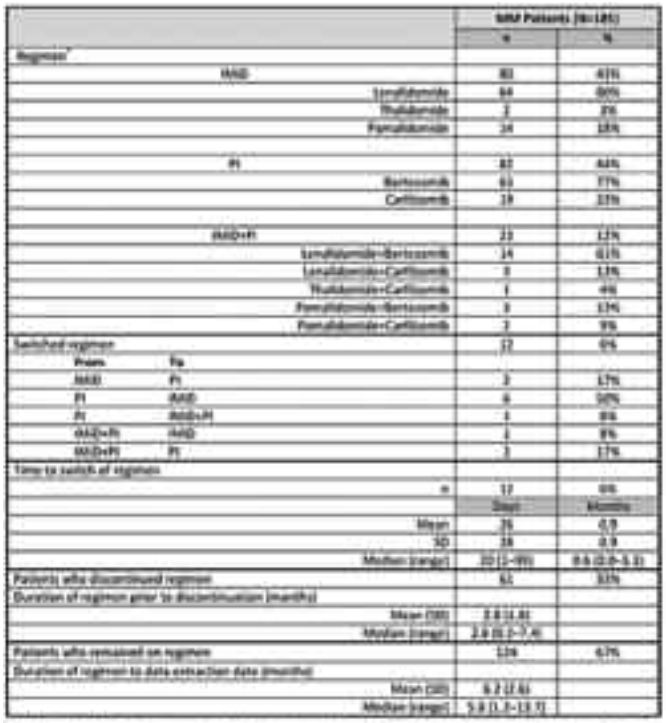
Background: Therapeutic options have improved for patients with multiple myeloma (MM) in the past decade, with regulatory approvals of several agents from two therapeutic classes, immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs). However, there are limited data on the real-world effectiveness of these agents in the setting of relapsed and/or refractory (R/R) MM.
Aims: PREAMBLE (Prospective Research Assessment in Multiple Myeloma: an Observational Evaluation) is a multinational, observational cohort study undertaken to prospectively evaluate the real-world effectiveness, healthcare resource utilization, and patient-reported outcomes associated with IMiD, PI, and IMiD+PI use in patients with R/R MM. Here, we report initial data on treatment patterns for patients enrolled in PREAMBLE.
Methods: This study includes patients with R/R MM who received ≥1 prior therapy and were treated with IMiD, PI, or IMiD+PI within 90 days prior to or 30 days after study enrollment. Treatment was administered according to the standard practice of participating physicians. Data are collected every 6 months during a 3-year observational period. At the time of the last data cut-off (November 22, 2013), 185 enrolled patients were on treatment and were eligible for evaluation. Median follow-up time was 6.2 months.
Results: Median number of prior therapies received among the 185 patients was 1 (range: 1–10), with 34 (18.4%) having received ≥3 prior therapies. On PREAMBLE, 43% of patients were treated with an IMiD (80% received

lenalidomide), 44% with a PI (77% received bortezomib), and 12% with IMiD+PI (61% received lenalidomide plus bortezomib) [Table]. Sixty-one patients (33%) had discontinued the regimen (for reasons other than death) after a median of 2.6 months. Twelve patients (6%) switched regimens within the first month.

Table 1. Treatment patterns for R/R MM.



Summary and Conclusion: The PREAMBLE study provides valuable real-world information regarding treatment patterns for R/R MM. This report shows that 1/3 of patients were not able to remain on their treatment for R/R MM. Additional analyses will address the reasons for treatment switch and discontinuation.

P983

A COMBINATION THERAPY WITH BORTEZOMIB (BOR) AND THALIDOMIDE IN NEWLY DIAGNOSED MYELOMA PATIENTS IS ASSOCIATED WITH A LOW INCIDENCE OF SECOND PRIMARY MALIGNANCIES (SPMS)

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Background: The availability of novel drugs (immunomodulatory drugs, IMiDs, and proteasome inhibitors, PI) for the front line treatment of Multiple Myeloma (MM) has dramatically changed patients' outcomes translating into extended progression free (PFS) and overall survival (OS). Despite these impressive results, concerns have been raised regarding the increased risk of SPMs among patients receiving IMiDs (lenalidomide and thalidomide), especially when associated with alkylating agents such as melphalan (MEL). It has long been known that the use of MEL can result in an increased incidence of SPMs, whilst the use of PI in newly diagnosed MM (NDMM) patients (pts) was not associated with an increased risk of SPMs in a phase III study aimed at comparing BOR, MEL and prednisone (MP) *vs* MP. However the impact of an upfront treatment containing both a PI and an IMiD in association with high dose MEL still has to be investigated.
Aims: To answer the question whether the association of an IMiD and a PI can result in an increase risk of developing SPMs in transplant eligible NDMM we have evaluated the incidence of SPMs in pts enrolled in the GIMEMA 26866138-MMY-3006 multicentre phase III study aimed at comparing BOR, thalidomide and dexamethasone (VTD) *versus* TD as induction before and consolidation after 2 sequential courses of high dose MEL.
Methods: The trial enrolled 480 transplant eligible NDMM, of which 474 received assigned treatment. For the purpose of the present analysis, data on the incidence of SPMs were specifically chased and were available for 63% of the overall population (148 VTD and 151 TD).
Results: With median follow up of 73 months, 25/299 pts (8%) developed a SPM: 7 (2%) SPMs were hematologic and 18 (6%) were non hematologic. SPMs are detailed in table 1. The median time from trial entry to development of the SPM was 36 months (range 8.4-69.0). The number of pts developing a SPM was lower in VTD arm (5%) compared to TD arm (11%, p=0.068). Among pts developing a SPM, the proportion of solid and hematologic SPMs was similar between treatment arms, with 75% and 25% of VTD pts *vs* 71% and 29% of TD pts developing a solid or a hematologic SPM, respectively. On the overall population the incidence rate (IR) of developing a SPM was 1% at 1 year and 9.9% at 6 years (yrs). This incidence was lower in patients randomised to VTD compared with patients randomised to TD (6% *vs* 13% at 6 yrs, p=0.037); when looking at the IR of solid tumours a trend was seen for a lower incidence of developing a second malignancy in those pts that received BOR based treatment (5% *vs* 9.6% at 6 yrs for VTD and TD, respectively); similarly, a trend towards a lower incidence of hematologic SPMs in the BOR arm was observed (1% *vs* 4% at 6 yrs for VTD and TD, respectively).

Table 1. SPMs details

SPM	Number	Time to SPM development (mo) in the 2 arms	
Solid			
		TD	VTD
Colon	3	44.4/53.5/69.0	
Melanoma	3	10.2	8.4/27.9
Prostate	2	29.6/35.4	
Breast	2		31.6/41.5
Colangiocarcinoma	1	14.5	
Pancreatic adenocarcinoma	1	38.3	
Head and neck	2	25.0	61.1
Astrocytoma	1	11.9	
Lung	1	26.5	
Mesothelioma	1	59.0	
Tumour nos	1		61.3
Hematologic			
		TD	VTD
LH+LNH	1	54.6	
LNH	2	31.1/37.2	
LAM	4	33.4/40.7	68.8/UNK

mo: months; NOS: not other specified; UNK: unknown

Summary and Conclusion: Our data compare favourably with those previously reported on the incidence of developing SPMs in NDMM treated with BOR frontline in association with MEL and provide demonstration that, with a follow up of 6 years, VTD combined with two sequential courses of high-dose MEL was associated with a low risk of developing SPM. Interestingly our data support the possibility that an IMiD-based treatment incorporating BORT might decrease the risk of developing a second malignancy compared to thalidomide. Randomised trials with PI and IMiDs treatment should prospectively address this issue. More extensive data will be presented at the meeting.

P984

DIFFERENT OUTCOMES AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION ACCORDING TO INVOLVED SITES IN MYELOMA PATIENTS WITH EXTRAMEDULLARY PLASMACYTOMA: BONE VERSUS SOFT TISSUE PLASMACYTOMA

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Background: Few reports have published the outcomes of autologous stem cell transplantation (ASCT) in multiple myeloma (MM) patients with extramedullary plasmacytoma (EP). Furthermore, no published studies have analyzed the results of ASCT in MM patients with EP based on whether the patients had plasmacytomas (PCs) located in the bone *versus* the soft tissue.
Aims: The aims of this study were to compare the characteristics of bone and soft tissue PCs, and to analyze the relapse pattern, clinical outcomes, and prognostic factors for ASCT in MM patients with EP.
Methods: We retrospectively reviewed 93 MM patients with EP detected at

diagnosis or during the course of the disease who underwent ASCT between October 2000 and July 2013 at 5 hospitals in Korea.
Results: This study included 51 men and 42 women, with a median age of 53 years (range, 22–67 years) at the time of diagnosis. Soft tissue PC (*n*=29) was found to be slightly more frequent in male patients, more commonly had an IgD monoclonal component, and occurred more often as light chain disease with increased lambda chain expression compared to the bone PC group (*n*=64). A greater percentage of patients with bone PC achieved either stringent complete remission (sCR) or CR after ASCT than patients with soft tissue PC (51.5% *vs.* 31.0%). After a median follow-up of 26 months following ASCT, progression-free survival (PFS) and overall survival (OS) were better in the bone PC group than in the soft tissue group (median PFS, 27.7 months *vs.* 12.3 months [*P*=0.001]; median OS, 66.9 months *vs.* 37.1 months [*P*=0.035]). Patients who attained sCR or CR after ASCT had improved PFS compared to those who did not achieve CR, while OS times were not significantly different between the groups. We divided the patients into 4 different groups based on whether they had soft tissue PC *versus* bone PC, and whether the patients achieved sCR or CR after ASCT. In this analysis, patients with bone PC who achieved sCR or CR had improved PFS and OS compared to those without CR after ASCT (estimated 3-year PFS rate, 45.3% ± 9.6% *vs.* 30.2% ± 9.0%; estimated 3-year OS rate, 85.3% ± 7.0% *vs.* 69.9% ± 9.0%). Patients with soft tissue PC who attained sCR or CR also had marginally improved PFS and OS than patients without a CR (estimated 3-year PFS rate, 22.1% ± 11.0% *vs.* 0%; estimated 3-year OS rate, 49.7% ± 15.7% *vs.* 29.2% ± 22.9%). Interestingly, patients with soft tissue PC who achieved sCR or CR after ASCT did not have improved survival compared to patients with bone PC who did not achieve CR after ASCT. These results reflect the fact that, even though ASCT led to a greater response in MM patients with soft tissue PC, the presence of EP contributed to poor prognosis after ASCT, and this could not be overcome by ASCT. Multivariate analysis identified abnormal cytogenetics, soft tissue PC, no CR after ASCT, and advanced stage on the International Staging System at diagnosis as factors that predicted poor PFS and OS after ASCT.
Summary and Conclusion: These results indicate that additional treatments such as consolidation therapy, maintenance therapy, or tandem ASCT might be needed to overcome the poor outcome of ASCT in MM patients with soft tissue PC identified at diagnosis or during the disease course.

P985

EARLY MORTALITY TREND IN MULTIPLE MYELOMA: A THIRTY-YEAR POPULATION-BASED STUDY

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Background: Overall survival (OS) is progressively increasing in multiple myeloma (MM) in the era of new agents. However, early mortality (within the first two months from diagnosis) remains a serious problem. Early mortality occurred in 10 % of patients registered onto MRC trials (Augustson) and may reach 12 % in patients with severe renal impairment (Dimopoulos)
Aims: In order to highlight the impact and pattern of early death in real-life MM patients, we analyzed our registry over the past 30 years.
Methods: All patients with symptomatic MM were consecutively included in the Granada population-based registry since 1985. Patients with smoldering MM, plasma cell leukemia or those with only palliative care were excluded. The patients were divided into three 10-year groups according to the date of diagnosis: 1985-1994, 1995-2004 and 2005-2014.
Results: 518 patients were included, 247 men and 271 women (52,3%), median age 66 years (12-91). 507 patients were valuables, and 67 of them (13,21 %) had early death. OS was 20, 25 and 36,6 months respectively in the three periods of time. Percentage of early deaths was 16,4%, 15,3% and 9% respectively in the three groups. Early death was associated in univariate analysis with age (median 69 years, p=0,002), body mass index (median 25,7 Kg/m^2; p=0,031), ISS III (73,7 %, p<0,001) and renal impairment (median creatinine 3,86 mg/dl; p<0,001). In the multivariate regression analysis, only renal impairment (p=0,013) behaves as an independent predictor of early death, whereas age shows only a marginal effect (p=0,09). An alternative analysis of six 5-year periods demonstrated similar outcomes. Patients diagnosed over the last five years showed 6,9 % of early death and their median OS was not reached.
Summary and Conclusion: As expected, early mortality in real-life MM patients could be higher that reflected in clinical trials. Early death remains a serious problem in real-life MM patients. The clinical results over the three periods of time are encouraging, with OS being progressively improved whereas early death is trending downward. Early death is frequently related to older age and ISS 3, but is only significantly associated with severe renal impairment. Renal failure in MM is a medical emergency which requires immediate therapy.

P986

INCREMENTAL PROGNOSTIC VALUE OF BONE MARROW ABNORMALITIES IN APPENDICULAR SKELETONS DETECTED BY WHOLE-BODY MULTIDETECTOR CT IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Imaging by multidetector CT (MDCT) and/or MRI have been utilized for detecting lytic bone lesion or myelomatous lesions, mainly axial skeletons, in the evaluation of multiple myeloma, however, bone marrow involvement in appendicular skeletons (AS) can be directly visualized by MDCT with shorter acquisition time. The presences of these bone marrow abnormalities in AS could correlate clinical variables which thought to relevant to prognosis in myeloma.
Aims: In this study, we report a clinical and prognostic implication of abnormal bone marrow lesions (ABLs) in AS detected by MDCT in patients with newly diagnosed multiple myeloma (NDMM).
Methods: We included 98 newly diagnosed multiple myeloma from February 2008 to December 2013. All the patients were received MDCT as an initial workup for lytic bone lesion of MM and CT value of ABLs in AS were measured using circular lesion of interest and expressed by Hounsfield Units (HU). The highest CT value (CTv), expressed in HU, was recorded in each patient. Receiver operating characteristics (ROC) curves of ABL for prediction of death event was constructed and appropriate cut-off point was determined. From the analysis, we set the cut-off point as 5.3 HU as a most relevant CT value for survival. Patients with ABLs in AS with higher than 5.3 HU considered as a high-CTv group and lower were considered Low-CTv group. Survival analysis was also performed using this cut-off point.
Results: There were 50 male and 48 female patients with median age of 72 years. The median follow-up duration was 19.2 months (range: 0.5 to 72 months). The number of patients with Durie-Salmon (D-S) stage 3 and international staging system (ISS) stage 3 was 62 (64%) and 43 (55%), respectively. Patients with myeloma subtype of IgG, IgA, light chain only and non-secretary subtypes were 55, 31, 11 and 1, respectively. Using the cut-off value 5.3 HU, 50 (51%) patients were defined as High-CTv group. There were significantly more patients with D-S and ISS III, and with high-risk features (del13q, del13 or del17p in cytogenetics and t(4;14), t(14;16) and del17p in interphase FISH) in High-CTv group compared with Low-CTv group. High-CTv group showed significantly shorter survival (median OS, not reached in both groups, p=0.006) by Kaplan-Meyer analysis compare to those with Low-CTv group. ABLs with CTv ≥5.3 HU was an independent prognostic factor for OS in both univariate (HR: 10.3; 95% CI: 1.3-81.1; p=0.027) and multivariate (HR 10.1; 95% CI: 1.3-82.2; p=0.030 after adjusting for ISS, high-risk features and age) analysis. ROC analysis to predict the death event was performed using four logistic regression models of ISS only (Model 1), ISS + high-risk features (Model 2), ISS + high-risk features + age (Model 3), and ISS + high-risk features + age + High-CTv group (Model 4). In Model 4, significant incremental predictive ability was shown compared to other three models by net reclassification improvement and integrated discrimination improvement (**Table**).

Table 1. NRI and IDI analysis for four prognostic models

Models	Model 1	Model 2	Model 3
Model 1			
Model 2			
Model 3			
Model 4			

Abbreviations: AUC: area under curve, NRI: net reclassification improvement, IDI: integrated discrimination improvement

Summary and Conclusion: Our study showed that ABLs with High-CTv associate with myeloma tumor burden and high-risk features. Patients with Hig-CTv had poorer prognosis compared to those with Low-CTv. By adding to other pre-existing risk factors including ISS, cytogenetically high-risk features, and age, the findings of MDCT can provide the incremental predictive ability in patients with NDMM.

P987

IMPACT OF PRIOR THERAPY (TX) ON THE EFFICACY AND SAFETY OF LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (RD) IN CHINESE PATIENTS (PTS) WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM): MM-021 TRIAL

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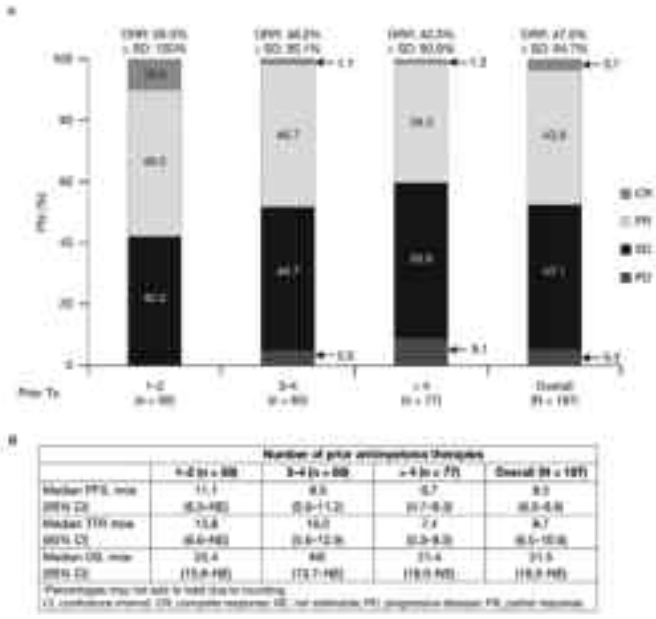
Background: The MM-021 registration trial demonstrated the efficacy and safety of the Rd regimen in Chinese RRMM pts (Hou, J Hematol Oncol 2013). Following the trial, Rd was approved in China for the Tx of RRMM pts who have received ≥ 1 prior Tx. Pts in the MM-021 trial had received between 1 and 15 prior regimens (median: 4). Evidence suggests LEN + DEX is more effective when used at first relapse (Stadtmauer, Eur J Haematol 2009).
Aims: To evaluate the impact of the number of prior antimyeloma Tx on outcomes in the MM-021 study.
Methods: MM-021 was a phase 2, multicenter, single-arm, open-label registration study in which RRMM pts received lenalidomide (LEN; 25 mg/day on D1–21/28 day cycle) and low-dose dexamethasone (40 mg/day on D1, 8, 15, and 22) cycles until disease progression or discontinuation. All pts received thromboprophylaxis during the study. The primary endpoint was overall response rate (ORR), defined as a partial response or better. Secondary endpoints included progression-free survival (PFS), time to progression (TTP), overall survival (OS), and safety. Data were analyzed according to the number of Tx that pts received prior to study screening: 1–2, 3–4, or >4.
Results: A total of 199 pts were enrolled; all were included in the safety analysis and 187 were included in the efficacy-evaluable (EE) population. The median age was 59 years (range 35–81), 63% were male, and most pts (86%) had Durie-Salmon stage III disease. Overall, 41% of pts had received >4 prior antimyeloma Tx, 33% had received 3–4, and 26% had received 1–2. Most pts had received prior Tx with thalidomide (THAL; 69%) or bortezomib (BORT; 64%) or both (45%; separately or in combination); only 13% of pts had had no prior exposure to THAL or BORT. With a median follow-up of 17.6 months (mos), the median Tx duration was 8.3 mos (range 0.9–24.8). The ORR was 48% in the EE population and highest in pts who had received 1–2 prior Tx; however, pts responded in each category (Figure). Median PFS, TTP, and OS were longer in pts who had received 1–2 prior Tx *vs*. those who had received 3–4 and >4 prior Tx (Figure). The most common grade 3–4 treatment-emergent adverse events (TEAEs) were anemia (26%), neutropenia (25%), thrombocytopenia (15%), pneumonia (13%), and leukopenia (10%). The incidence of grade 3–4 TEAEs was lowest in pts with 1–2 prior Tx (60%); the incidences in pts who had received 3–4 (71%) and >4 (75%) prior Tx were comparable with that in the overall safety population (70%). TEAEs led to discontinuation of LEN in 6%, 11%, and 10% of pts who had received 1–2, 3–4, and >4 prior Tx, respectively.

Table 1. Efficacy of Rd according to number of prior therapies (efficacy evaluable population)



Summary and Conclusion: This sub-analysis showed that Rd is an effective Tx option for Chinese RRMM pts who have relapsed after ≥ 1 prior Tx, including THAL and/or BORT. Rd efficacy was greatest in pts who had received 1–2 prior Tx. Tolerability was similar in heavily and less heavily pretreated pts, with anemia and neutropenia the most common TEAEs. Discontinuations were infrequent (≤ 11%) even in heavily pretreated pts who had received >4 prior Tx.

P988

AN ONGOING OPEN-LABEL PHASE 1/2A STUDY OF THE SAFETY AND EFFICACY OF MELFLUFEN AND DEXAMETHASONE COMBINATION FOR PATIENTS WITH RELAPSED AND RELAPSED-REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Melflufen (L-melphalanyl-p-L-fluorophenylalanine ethyl ester hydrochloride (MF)), is a targeted analogue of melphalan. The mechanism of action is targeted alkylation of tumor cell DNA: transport of MF into cells is rapid and once inside the cytoplasm, peptidases, often overexpressed in malignant cells, will rapidly cleave MF releasing melphalan. Treatment with melflufen results in efficient intracellular trapping of melphalan preferentially in tumor cells, due to the higher enzymatic activity and the differential rate of transport of MF into cells (rapid) and melphalan out of cells (slow). It has been demonstrated that treatment with MF results in at least a 10-20 fold higher intracellular concentration of melphalan compared with direct treatment with equimolar doses of melphalan. When studied in cultures of human tumor cells representing approximately 20 different human cancers, including MM, MF showed 50 to 100 fold higher cytotoxicity and tumor growth suppression compared with that of melphalan. In efficacy studies conducted in mice and rats, superior antitumor activity of MF over melphalan was observed with comparable toxicity (Wickström 2010, Chauhan 2013). In a first-in-man study, a total of 45 patients with advanced solid tumors received a total of 141 cycles of MF at doses of 25-130 mg, with a safety profile which was similar to that of melphalan in equimolar doses.
Aims: To study the safety and efficacy of melflufen and dexamethasone (dex) combination for patients with RRMM.
Methods: This is an ongoing open-label, phase I/IIa, multicenter trial of MF plus dex currently enrolling patients with RRMM (NCT01897714). Phase I follows the standard 3 + 3 modified Fibonacci design with 3 to 6 patients in each cohort, depending on dose limiting toxicity (DLT) observed. Up to 4 dose levels will be tested; IV MF at 15 mg, 25 mg, 40 mg and 55 mg, given on day 1, with a fixed dose of dex 40 mg PO or IV on days 1, 8 and 15 of each 21 day cycle. An additional 20 patients will be treated at the MTD in the Phase IIa part of the study. The primary objectives are to determine the MTD in Phase I and the Objective Response Rate in Phase IIa. Adult patients with measureable disease, ≥ 2 lines of prior therapy, life expectancy ≥ 6 months, ECOG ≤2,

absolute neutrophil count ≥ 1.0 x 10^9/L, platelet count ≥ 75 x 10^9/L, hemoglobin ≥ 8.0 g/dl, total bilirubin ≤ 1.5 x upper limit of normal (ULN), estimated CrCL ≥ 45 ml/min, serum Cr ≤ 2.5 mg/dL and AST/ALT ≤ 3.0 x ULN are eligible for the study. All patients are required to provide signed informed consent.
Results: To date (Feb 20), 9 patients have received a total of 24 cycles of MF in doses up to 25 mg. The median number of cycles administered is 2.7 (range 1-7) with 4 patients remaining on treatment. The patient population has a median age of 68, and has received a median of 5.2 (range 3-10) previous lines of treatment, with 100% of patients being exposed to both an IMiD and bortezomib. The first two dose levels have been administered without DLT's. 3 patients have reported 5 Grade 3/4 treatment related adverse events (TRAE); G3 thrombocytopenia, G3 pneumonia, G4 febrile neutropenia, G3 nausea and G3 neutropenia. No patient has discontinued due to TRAE. Initial PK data support the expected pattern with an increased rate of distribution of MF to peripheral tissues during the infusion, and a delayed redistribution of melphalan formed peripherally.
Summary and Conclusion: Updated information will be presented at the time of the conference.

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A PHASE 1 STUDY OF THE EFFECT OF A HIGH-FAT MEAL ON THE PHARMACOKINETICS OF IXAZOMIB CITRATE (MLN9708), AN INVESTIGATIONAL ORAL PROTEASOME INHIBITOR, IN PATIENTS WITH ADVANCED SOLID TUMORS OR LYMPHOMA

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Background: Ixazomib citrate is an oral proteasome inhibitor under phase 3 investigation in patients (pts) with multiple myeloma (MM) and amyloidosis. Ixazomib citrate immediately hydrolyzes to the active moiety ixazomib in aqueous solutions or plasma. In ongoing clinical studies, ixazomib citrate is taken on an empty stomach.
Aims: To characterize the effect of a high-fat meal on the single-dose pharmacokinetics (PK) of ixazomib.
Methods: This is a phase 1, single-dose, open-label, randomized, multicenter, two-period, two-sequence, cross-over study (NCT01454076). Pts were randomized to receive a single 4 mg dose of ixazomib citrate with or without a standard high-fat breakfast on Day 1, followed by administration under the respective alternate food intake condition (fasted to fed, or fed to fasted) on Day 15 of Cycle 1. Serial blood samples were collected over 0–216 hr after ixazomib citrate dosing on Days 1 and 15 for PK characterization. From Cycle 2, ixazomib citrate 4 mg was administered on an empty stomach on Days 1, 8, and 15 of a 28-day cycle. Plasma PK parameters were estimated by non-compartmental methods using Phoenix WinNonlin version 6.2. Effects of the high fat-meal on ixazomib AUC_{0-216} and C_{max} were evaluated by analyses of variance on log-transformed values. Safety and response data after multiple dosing were also collected.
Results: 24 pts (19 Caucasian, 4 African American, 1 Hispanic; 13 M, 11 F; 22 solid tumor, 2 lymphoma) were enrolled. Mean age was 62 years (range 46–85), mean weight 74 kg (range 43–112), and mean body surface area 1.9 m^2 (range 1.4–2.4). Final data from 15 PK-evaluable pts were used to assess the impact of food on ixazomib PK. Following a single dose of ixazomib citrate, median T_{max} was 1 and 4 hr for fasted and fed treatment, respectively. Administration of ixazomib citrate with food resulted in an approximately 28% decrease in AUC and 69% decrease in C_{max} (Table). Although relatively modest, the observed decrease in AUC is inferred to be of potential clinical significance as, when dosed at 4 mg with a high-fat meal, ixazomib exposures would be expected to approximate those achieved at 3 mg (the first dose level reduction for pts experiencing drug-related toxicity). As of January 2014, 19 (79%) pts experienced drug-related adverse events (AEs) in Cycle 2 and beyond following multiple dosing with ixazomib citrate on a weekly schedule, including 6 drug-related grade 3 AEs in 5 pts (anemia, n=2; dehydration, fatigue, peripheral edema, and vomiting, each n=1); no drug-related grade 4 AEs were reported.

Table 1.

N=15	Fasted	Fed	Fed vs. fasted, least square geometric mean ratio (90% CI)
C_{max}, ng/mL*	77.8 (57.4)	22.8 (54.4)	0.31 (0.21–0.45)
AUC_{0-216}, ng*hr/mL*	1488 (48.6)	887 (79.8)	0.72 (0.58–0.89)
T_{max}, hr*	1 (0.5–4)	4 (2–8)	–

*Geometric mean (%CV); †Median (range); CI, confidence interval

Summary and Conclusion: Administration of ixazomib citrate 4 mg after a high-fat meal resulted in a decrease in the rate and extent of oral absorption, with an approximately 30% reduction in total systemic exposure. These results support the continued administration of ixazomib citrate on an empty stomach (no food for 2 hr before and 1 hr post dose) in ongoing phase 3 studies in relapsed/refractory MM, newly diagnosed MM, and amyloidosis.

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ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF SYSTEMIC IMMUNOGLOBULIN LIGHT-CHAIN AMYLOIDOSIS: RESULTS IN A SERIES FROM A SINGLE INSTITUTION

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Background: Amyloidosis constitutes a heterogeneous group of diseases characterized by the deposition of amyloid fibrils in organs and tissues. Accurate identification of precursor protein is essential, given the diverse prognosis and therapeutic approach. The diagnosis and classification are based on histologic demonstration of amyloid deposits and characterization of the amyloid precursor. Immunohistochemistry is the classical method for amyloid typing. However, reported technical limitations of immunohistochemistry gave rise to the introduction of strategies such as immunoelectron microscopy and proteomic analysis.
Aims: To describe the role of immunohistochemistry in the diagnosis of 82 patients with immunoglobulin light chain (AL) amyloidosis diagnosed and treated at a single institution between 1995 and 2013.
Methods: At the time of diagnosis, all patients were systematically investigated by clinical and laboratory evaluation. Diagnosis of AL amyloidosis required amyloid demonstration by Congo red stain in a biopsy specimen. All Congo-red positive biopsies were tested for the four most common amyloid proteins (kappa, lambda, AA and TTR), using commercial antibodies
Results: Eighty-two patients were included in the study (42M/40F). The involved light-chain isotype was lambda in the majority of patients (76.8%). Immunohistochemistry allowed a definite diagnosis of AL amyloidosis in 70 patients (85.4%). Positive tissue was more frequently kidney (29 patients), followed by abdominal fat aspiration (9 patients), liver (7), tongue (5), gastrointestinal tract (5), heart (5), skin (2), lung (2), salivary glands (2), with bone marrow, lymphadenopathy, muscle and bladder in one case each. More than one tissue was biopsied in 33 patients. All immunohistochemistry results were concordant with serum and/or urine monoclonal protein and among diferent samples from the same patient when available. In 51 patients with subcutaneous fat aspiration available, only 32 showed Congo red positivity by optical microscopy, allowing amyloid proten by immunohistochemistry in 14 cases (27.4%). In those patients without effective amyloid typing (12), diagnosis of AL amyloidosis was firmly established based on the presence of typical clinical signs in combination with a monoclonal gammopathy and a Congo red positive biopsy.
Summary and Conclusion: Immunohistochemistry performed by an experienced pathologist, combined with clinical and laboratory information and genotyping, allows AL amyloidosis diagnosis in the majority of daily practice cases. Nowadays, this is the standard in our institution, saving more complex diagnostic techniques for patients in whom the diagnosis is not clear.

Myeloma and other monoclonal gammopathies - Clinical 5

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SAFETY PROFILE OF BORTEZOMIB IMPACTS SURVIVAL OF CARDIACAL

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Background: Light chain amyloidosis (AL) is characterized by a median overall survival (OS) from diagnosis of approximately 3 years, but with clinically overt cardiac involvement this is reduced to 1 year. Bortezomib (B) has shown great promise in the treatment of AL, especially of cardiac involvement. However, efficacy might be hampered by severe safety issues with use of B, primarily of neuropathy type.
Aims: We sought to study the prognostic impact of the safety profile of B in AL with attention to cardiac involvement.
Methods: This study has included 27 patients with AL, of these 18 had cardiac and 9 kidney but no cardiac involvement. AL was diagnosed as outline in international consensus criteria, as to the hematologic and organ responses. B was given IV twice weekly at the starting dose of 1.3 mg/m², in combination to weekly cyclophosphamide in 13 patients (52%).
Results: The median age was 63, sex ratio was 18/9, all cardiac AL had Mayo Cardiac Stage III but 3. 70% were at diagnostic. Seven patients died during the first month, all of them but one had cardiac AL. The overall hematologic response rate was 56% and at least VGPR 41%. The responses were 56% and 39% in cardiac AL, similar to patients with no cardiac involvement (56% and 44%), respectively. The median duration of response was 13 months overall, 10 and 20 months in cardiac and in no cardiac AL, respectively (p=NS). With a median follow-up of 41 months from start of B, 70% relapsed and 59% died in the study as a whole, and 67% and 67% in the cardiac group, respectively. The median time to progression was 20 (95CI 4;35.5) months as a whole, and 13 (9;17) months and 20 (0;43) months in cardiac and in no cardiac AL (p=NS). The estimated 4-year OS was 55%, 62% and 50% as a whole, in patients with no cardiac involvement, and with cardiac AL, respectively. Interestingly, all cardiac AL that survived beyond 6 months remain alive at F-up date. We then looked at the safety profile of B in the studied population, and found that 25% and 75% experienced some degree of hematotoxicity in cardiac and in no cardiac AL (p=NS). The non hematology toxicity rate was 62% and 38%, respectively (p=NS). 26% of patients needed dose reduction of B and 33% dose interruption of B in the study before cycle 4, all related to non hematological toxicity of neuropathy, fatigue and GI AEs; and was similar in cardiac as compared to no cardiac AL. The safety profile did not impact the response rate or the duration of response. Several variables negatively impacted survival in univariate analysis in the group with cardiac AL, including decreased *LVEF (p=0.025), NYHA greater than 2 (p=0.007),* Mayo Cardiac Stage III (p=0.028), no hematological (p=0.002) and no organ responses (p=0.05), occurrence of non hematological toxicity (p=0.002) with the consequence of dose reduction of B (p=0.09) and dose interruption of B (p=0.04). In multivariate analysis, independent variables that impacted survival were hematological response (OR=5.1, 95%CI=1.5-18; p=0.011) and non hematological toxicity (OR=3.6, 95%CI=0.8-14; p=0.05).
Summary and Conclusion: Bortezomib is a very rapid and effective therapy for AL particularly of cardiac involvement. However, patients may develop severe side effects with Bortezomib that preclude efficacy of Bortezomib given IV, and consequently impact negatively survival. This data favours use of sub cutaneous Bortezomib in AL although not validated in this indication yet.

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IMPACT OF INITIAL FGD-PET CT AND SERUM FREE LIGHT CHAIN ON TRANSFORMATION OF CONVENTIONALLY DEFINED SOLITARY PLASMACYTOMA TO MULTIPLE MYELOMA

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Background: Solitary plasmacytoma (SP) is a localized proliferation of monoclonal plasma cells resulting in a mass in either bone (SBP: Solitary Bone Plasmacytoma) or soft tissue (EMP: Extra-Medullary Plasmacytoma), without evidence of Multiple Myeloma (MM) but with high risk of transformation to MM. Presence of focal lesions on magnetic resonance imaging (MRI) has been demonstrated to favor progression to MM in patients (pts) with plasmacytoma. FGD-PET CT (PET) provides additional valuable information to assess plasmacytoma in the context of MM, compared with MRI, but its exact role remains debatable.
Aims: We aimed to determine the impact of PET pre and post therapy on the risk of transformation of SP to overt MM.
Methods: This retrospective study included 43 pts diagnosed with one SP clinically, confirmed with histology, either EMP (10 pts) or SBP (33 pts). All pts were treated locally with surgery and/or radiotherapy and had PET and MRI performed at diagnosis prior to (initial) and at the end of therapy. SP was diagnosed as outlined in international consensus criteria.
Results: The median age was 57.5 years with 33% pts older than 65, the sex ratio was 1.8, 48% pts had abnormal involved serum free light chain (isFLC) value and 64% had abnormal sFLC ratio (K/L). 33% had ≥2 hypermetabolic lesions on initial PET, and 20% had ≥2 focal lesions on initial MRI. With a median follow-up of 50 months, the median overall survival (OS) was not reached for the whole cohort, with a 6-year OS at 79.4%. 14 patients transformed to MM with a median time to MM progression (TTMM) of 71 months (95%CI : 59;101). The TTMM for the ≥2 hypermetabolic lesions on initial PET group was 23 months (9;37) *vs* not reached otherwise (p=0.003). Conversely, the median TTMM for the ≥2 focal lesions on initial MRI group was 30 months (9;51) *vs* not reached otherwise, without significant difference. Abnormal initial K/L ratio (p=0.022) and abnormal initial isFLC (p=0.002) impacted TTMM, 36 months (14;58) and 21 months (0;42) *vs* not reached otherwise, respectively. A normalized PET at completion of treatment did not reach significance, as to normalized MRI, but the absence of normalized isFLC value also negatively impacted TTMM, 21 months (10;32) *vs* not reached otherwise (p=0.016). Using multivariate analysis, independent variables that shortened TTMM were initial PET (OR=5, 95%CI=0-9; p=0.032) and abnormal initial isFLC (OR=10, 95%CI=1-87; p=0.008). Interestingly, initial PET did not influenced OS, median 71 months for the ≥2 hypermetabolic lesions on initial PET group *vs* not reached otherwise, respectively (p=ns).
Summary and Conclusion: An abnormal involved sFLC value and the presence of at least 2 hypermetabolic lesions on PET/CT at diagnosis of SP were the 2 predictors of early evolution to myeloma in our series. This data analysis will need confirmation in a larger study, and the study of these 2 risk factors may lead to a different management of patients with SP in the future.

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NEGATIVE FLOW CYTOMETRY IS ASSOCIATED WITH A BETTER PROGRESSION-FREE SURVIVAL IN TREATED MULTIPLE MYELOMA PATIENTS: THE SINGLE-CENTER EXPERIENCE OF CHU DE CAEN IN A DAILY PRACTICE

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Background: Multiple Myeloma (MM) is an hematologic malignancy characterized by bone marrow plasma cell infiltration and a serum monoclonal component. It is usually preceded by a non-active stage (smoldering myeloma, SM) based on international CRAB criteria. The response depth under treatment, defined in International Myeloma Working Group (IMWG) guidelines as partial response (PR), very good partial response (VGPR), complete response (CR) and stringent CR (sCR) is associated with a better outcome in overall and progression-free survival. Flow cytometry is a useful tool for the diagnosis, prognostic and high sensitivity minimal residual disease (MRD) evaluation in plasma cell diseases. Furthermore, it can define a better-outcome group of patients with CR and negative MRD (immunophenotyping CR, iCR).
Aims: For more than two years, we prospectively characterized in our center (CHU de Caen, France) 208 bone marrow samples of 127 patients in 4-color flow cytometry (127 at diagnosis, 23 at relapse and 58 MRD or protocol post-treatment samples).
Methods: We used a CD38/138/SSC/45 primary gating and CD19, CD20, CD56, CD117, CD33, CD28 and intra Kappa/lambda antibodies panel. Samples retained for analysis must have more than 20,000 nucleated cells per panel (mean 150,000 nucleated cells per tube approximately), and antibody positivity in a cell population was noted if more than 20% of these cells expressed the antibody.
Results: Disease status at diagnosis was active MM (61%), SM (13%) and MGUS (23%). We found in a daily practice that the most frequent phenotype was CD19-/CD20-/CD56+/CD117+/CD33-/CD28- (22% of cases), with high heterogeneity. With a median follow-up of 20,4 months in active MM (n=89), no single-marker prognostic value was found. More than 85% of these active MM patients received a multiple-agent therapy including Bortezomib (V): V-Thalidomide-Dexamethasone (16,8%), V-Lenalidomide-Dexamethasone (23,5%), V-Cyclophosphamide-Dexamethasone (9%) and V-Melphalan-Dexamethasone or V-Dexamethasone (32,8%). Autologous stem-cell transplantation was performed in 36 patients (40,4%) with a very good overall survival (98% at 100 months). We evaluated post-treatment MRD by cytology and flow cytometry in 21 patients, including 11 patients with only VGPR: 2 VGPR patients had a negative MRD, and 9 MRD-negative patients (41%) had

plasma cells in cytology (1-4%). We found that negative MRD in at least VGPR patients was associated with a largely better PFS (p=0,003, median PFS not reached *vs*. 27,8 months).

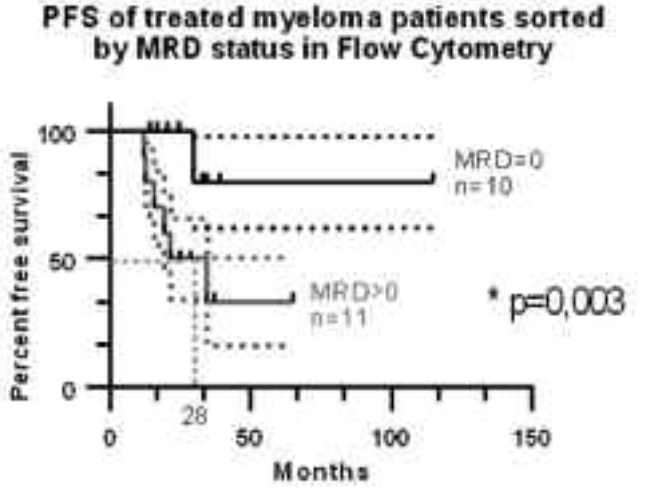

Figure 1.

Summary and Conclusion: In this prospective real-life study we confirm the important role of flow cytometry in multiple myeloma for the MRD evaluation of treated patients.

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HIGH INCIDENCE OF INTACT IMMUNOGLOBULIN OR FRAGMENTS THEREOF IN URINE OF PATIENTS WITH MULTIPLE MYELOMA

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Background: Intact immunoglobulin or fragments thereof (intact / fragmented Ig) can be found in the urine due to nephrotic injury or the preferential scavenging of albumin by the renal FcRn receptor leading to immunoglobulin catabolism (Sarav *et al.* JASN 2009; 20: 1941). Intact/fragmented Ig can be detected in urine in a proportion of patients with multiple myeloma (MM) at presentation and are associated with reduced survival (Ludwig *et al.* ASH 2012 abstract 1828).
Aims: To determine the incidence of intact /fragmented Ig and Bence Jones protein (BJP) in urine and compare to concentrations of serum free light chains (sFLC) in MM patients at diagnosis.
Methods: 215 patients with MM, median age 66 years old (range 47-82) with a male/female ratio 100/115, 163 IgG (104 IgGκ/59 IgGλ), 48 IgA (25 IgAκ/23 IgAλ), 3 IgDλ and 1 IgEκ were evaluated. Immunofixation was performed using Hydrasys 2 apparatus (Sebia, France) and antisera from the same company. sFLC were measured using Siemens BN™ II nephelometer and immunoassays (Freelite™, The Binding Site, Birmingham, UK).
Results: In 45/163 (27%) IgG MM patients urine immunofixation (uIFE) revealed presence of intact /fragmented IgG (including 25 IgG + BJP, 17 IgG + albumins, 3 only IgG). Overall monoclonal protein was detected in uIFE of 100/163 (60%) IgG MM patients (including 41 BJP, 25 BJP + IgG, 17 IgG + albumins, 3 IgG, 14 BJP + albumins). Abnormal sFLCκ/λ ratio was found in 100/130 (77%) IgG MM patients (increased monoclonal sFLC in 104 (80%) patients and decreased alternate sFLC in 30 (23%) patients. In 12/48 (25%) IgA MM patients uIFE revealed presence of intact/fragmented IgA (including 8 IgA + BJP, 11 IgA + albumins). Overall monoclonal protein was detected in uIFE of 37/48 (77%) IgA MM patients (including 13 BJP, 8 BJP + IgA, 12 BJP + albumins, 4 IgA). Abnormal sFLC ratio was found in 39/43 (90%) IgA MM patients (increased monoclonal sFLC in 38 (88%) patients and decreased alternate sFLC in 22 (51%)) patients. In 1/3 IgDλ MM patients uIFE revealed presence of IgD (1 IgD + BJP + albumins, 2 BJP). In 3 IgD MM patients sFLC ratio was abnormal, monoclonal sFLC was increased and alternate sFLC was decreased. In the only 1 IgEκ MM patient uIFE revealed presence of BJP and albumins; sFLC ratio was abnormal, monoclonal sFLC was increased and alternate sFLC was decreased. There was no difference in serum creatinine concentrations between patients with or without intact/fragmented Ig in urine.
Summary and Conclusion: In one fourth of IgG, IgA and IgD MM patients intact/fragmented Ig can be detected in urine at diagnosis. Urine immunofixation detects monoclonal protein (FLC and intact/fragmented Ig) in 60% to 77% while serum free light chain assay in 80% to 90% of IgG and IgA MM patients, however the last one is inadequate for detection of intact/fragmented Ig in urine. Prognostic significance of the presence of intact/fragmented Ig in urine is under investigation.

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VALIDATION OF THE SPECIFIC HEMATOPOIETIC-CELL-TRANSPLANTATION COMORBIDITY INDEX IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Major step forward in the treatment of multiple myeloma (MM) has been achieved with intensification of treatment in terms of high-doses therapy followed by the autologous stem cell transplantation (ASCT). Introduction of various new drugs for multiple myeloma (MM) has appointed the necessity of personalized approach.
Aims: The goal of study was to compare and validate the impact of the Hematopoietic-Cell-Transplantation Comorbidity Index (HCT-CI), and Charlson Comorbidity Index (CCI) on the course of disease in MM pts.
Methods: The study included 146 newly diagnosed MM pts (age of ≤65yrs: 73 male/73 female, mean age 57yrs, range 35-65). IgG myeloma had 93pts (63,7%), IgA 25pts (17,1%), light chains 23pts (15,7%), IgD 3pts (2,1%), and non-secretory 2pts (1,4%). According to the clinical stage, distribution was: I 5pts (3,4%), II 28pts (19,2%); III 113pts (77,4%). Renal impairment existed in 35pts (24%). Regarding ISS score, 28,2%pts had ISS1 score; 30% ISS2; and 41,8% had ISS3. Most of the patients had HCT-CI 0-1, 114pts (78 %, range 0-6), while score ≥2 had 32pts (22%). Charlson comorbidity index (CCI) score 0-1 had 116pts (79,5%), CCI≥2 was found in 30pts (20,5%). Age adjusted CCI (aaCCI) score 0-1 had 22pts (15,1%), aaCCI 2 had 55pts (37,7%), and 69pts (47,3%) had aaCCI≥3 (range 0-7). In the induction treatment, thalidomide combinations were applied in 70pts (47,9%). Five patients (3,4%) were treated with bortezomib; and 66pts (45,2%) with conventional chemotherapy. High-dose therapy (HDT) with Melphalan 200mg/m² followed by the ASCT was applied in 58pts (39,7%).
Results: Treatment response (CR/VGPR/PR/MR) was achieved in 54pts (93%) treated with HDT+ASCT with median duration 24m (range 6-92m), comparing to the 88pts (60,3%) that were not included in the programme of the HDT+ASCT (56 pts, 63,6%, median duration 15m, range 1-88m). The mean overall survival (OS) of patient treated with HDT+ASCT was 71,4m (range 10-92m) with probability of 5-years survival in 63% pts, while pts treated out of ASCT program had median OS 38m (range 7-98) with probability of 5-years survival in 35% pts. Patients with HCT-CI 0-2 had significantly longer OS in comparison to the patients with HCT-CI ≥3 (median 48m *vs*. 30m; Log Rank 5,45; p=0,02). Similarly, patients with CCI 0-2 had significantly longer OS in comparison to the patients with CCI ≥3 (median 48m *vs*. 30m; Log Rank 4,41; p=0,036). Furthermore, in the proposed model for personalized treatment approach among variables HCT-CI≥2, CCI≥2, and ISS≥2, only ISS≥2 was indicated as major factor with significant impact on the OS of younger MM pts (χ^2 7,89; p=0,048; HR 2,067; 95% CI 0,99-4,29).
Summary and Conclusion: High values of Hematopoietic-Cell-Transplantation Comorbidity Index and Charlson Comorbidity Index (HCT-CI/CCI≥2) are of the similar importance for the course of disease in younger myeloma patients, while the treatment outcome and overall survival of these patients in proposed model is mainly influenced by the level of disease aggressiveness based on ISS system.

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RELATIONSHIP BETWEEN CHROMOSOMAL ABERRATIONS AND CLINICAL OR LABORATORY VARIABLES IN MULTIPLE MYELOMA

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Background: Clinical significance of some chromosomal aberrations (CA) detected at diagnosis in multiple myeloma (MM) is well established. However, the relationship between different types of CA and some clinical or laboratory variables is less known and sometimes controversial.
Aims: The aim of our study was to assess the type and frequency of CA detected by FISH combined with cytoplasmic light chain detection (cIg-FISH) in MM patients, and their associations with clinical and laboratory parameters. In addition, significance of abnormal karyotype detected by chromosome banding analysis (CBA) was analyzed.
Methods: A total of 65 patients with MM at diagnosis were included into the study. Probes for cIg-FISH were chosen to detect t(4;14), t(14;16), t(11;14), deletion of 1p32, 13q14, 16q23, gain of 1q21, trisomy of 5/9/15. All cases were divided into hyperdiploid MM (H-MM) or non-hyperdiploid MM (NH-MM). The laboratory and clinical assessments included: age, gender, bone marrow plasmocytes, hemoglobin, albumin, β2microglobulin, creatinine, LDH, type of secreted heavy and light chains, serum free light chains (sFLC), sFLC ratio (sFLCR), bone lesions. Additionally, in 56 patients CBA was performed.
Results: The median age of 65 patients (29 males, 36 females) was 60 (range

37-79). The frequency of CA detected by clg-FISH was as follows: t(4;14) in 16/65 (24.6%), t(14;16) in 4/65 (6.1%), t(11;14) in 6/35 (17.1%), del(13q) in 36/65 (55.4%), del(16q) in 12/65 (18.5%), del(17p) in 8/65 (12.3%), del(1p) in 6/60 (10.0%), and amp(1q) in 31/65 (47.7%) of patients. H-MM by clg-FISH was established in 30/61 (49.2%) patients. Abnormal karyotype by CBA was detected in 18/56 (32%) of patients, 9 cases with hyperdiploidy and 9 with hypo/pseudodiploidy. In all but one abnormal karyotype was complex. The H-MM and NH-MM status evaluated by clg-FISH and by CBA were consistent in all cases. Translocation t(4;14) was detected in 37.9% of males and 13.9% of females (p=0.030), and amp(1q) in 69.0% of males and 13.9% females (p=0.001), respectively. All together, the high-risk aberrations, *i.e.* t(4;14) or t(14;16) or del(17p) were observed in 52% of males and 21% of females (p=0.010). The frequency of t(4;14) and amp(1q) was also dependent on the age (p=0.023 and p=0.043, respectively). Regarding group of patients with t(4;14) or t(14;16) together, 42% of patients younger than 60 years had one of two translocations as compared with 18% for older ones (p=0.037). Translocation t(4;14), amp(1q), del(16q) and NH-MM were significantly more often observed in cases producing λ than κ chains, and the frequencies in λ-MM *vs*. κ-MM were as follows: 37.5% *vs*. 13.5% (p=0.029), 64.0% *vs*. 27.9% (p=0.007), 33.3% *vs*. 10.8% (p=0.031) and 73.9% *vs*. 40.0% (p=0.013), respectively. Among many laboratory variables only sFLC level was associated with CA detected by clg-FISH. High sFLC level was related to amp(1q) (p=0.039). However abnormal karyotype detected by CBA was associated with Hb<10 mg/dl (p=0.014) and β2microglobulin >5.5 mg/l (p=0.002) as well as ISS stage 3 (p=0.002). Abnormal metaphases were observed in 2/24 (8%) patients in ISS stage 1-2 and in 14/26 (56%) patients in ISS stage 3 (p=0.001). Advanced bone lesions were present in 6/7 (86%) patients with del(17p) and 21/54 (39%) patients without this anomaly (p=0.052).

Summary and Conclusion: We found gender- and age-dependent differences in prevalence of high-risk aberrations in newly diagnosed MM patients, with t(4;14) and amp(1q) being more common in men and younger patients. Additionally, MM with λ chains was related to high-risk aberrations.

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COST-EFFECTIVENESS IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM): LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE (RD) VERSUS BORTEZOMIB PLUS MELPHALAN AND PREDNISONE (VMP)

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Background: Treatment paradigms in multiple myeloma are evolving, with new evidence indicating that health gains are maintained through continuous treatment. The FIRST trial showed that continuous Rd significantly improved progression-free survival (PFS) and provided an overall survival (OS) benefit at interim analysis, compared to a fixed duration of either 18 months of Rd or the combination of melphalan- prednisone - thalidomide (MPT) in transplant-ineligible NDMM patients (Facon, *et al.* Blood 2013). Fixed treatment duration of VMP, approved by the Food and Drug Administration in the United States (US) for this population, also demonstrated superior efficacy to MP (San Miguel, *et al.* N. Engl. J. Med 2008) (San Miguel, *et al.* J Clin Oncol 2013) and similar to MPT through indirect comparison (Kumar, *et al.* Am J Hematol 2001) (NICE 2011). Given the different available treatment options, questions arise regarding the balance between the costs and benefits of continuous *versus* fixed duration therapy.

Aims: To conduct a cost-effectiveness analysis in order to evaluate the costs and benefits of continuous Rd *versus* fixed duration VMP in NDMM patients.

Methods: A partitioned survival model was developed to perform this analysis from a US payer perspective over a lifetime horizon. The model projected average lifetime PFS and OS associated with each treatment using observed trial data and estimated quality-adjusted life-years (QALY) based on treatment-specific utility weights for each health state. Continuous Rd outcomes were taken from the FIRST trial, while VMP outcomes came from the VISTA study. In order to establish relative efficacy between Rd and VMP, the OS for VMP was set to be the same as that observed in the MPT arm of the FIRST Trial, based on the NICE guidance of VMP similarity *versus* MPT in first-line multiple myeloma (NICE 2011). Costs, including for drugs, administration, medical care, second- and third-line anti-myeloma regimens, and management of toxicity, were calculated in 2014 US dollars based on resource utilization within the respective trials, and published literature. Both clinical and cost outcomes were discounted at 3% per annum.

Results: Continuous treatment with Rd was estimated to result in 6.81 life-years (LY; discounted), relative to 5.53 for fixed treatment duration of VMP. QALYs were estimated to be 4.33 and 3.42 (discounted), respectively. Discounted lifetime costs were $349,331 for patients starting with Rd *versus* $270,991 for patients starting with VMP. Incremental cost-effectiveness ratios for continuous Rd relative to VMP were $86,126 per QALY gained and $61,509 per LY gained.

Summary and Conclusion: Continuous Rd was estimated to result in 23% to 27% more LYs and QALYs relative to VMP in transplant-ineligible NDMM patients. Despite the additional costs incurred with continuous treatment, Rd was cost-effective relative to fixed duration therapy with VMP, with cost-effectiveness ratios well within levels published for other recent advances in front-line oncology setting.

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SYSTEMIC MASTOCYTOSIS WITH MONOCLONAL GAMMOPATHY

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Background: Mastocytosis is due to a clonal neoplastic proliferation of mast cells that accumulate in one or more organ systems. It is a rare disease associated to a somatic mutation of the proto-oncogene c-kit(KIT). Systemic mastocytosis with associated clonal hematologica non-mast celle lineage disease is a unique subtype of mast cell neoplasm. While associated myeloid neoplasm is frequent, associated with lymphoproliferative disorders are rare. Some cases of systemic aggressive with plasma cell myeloma are reported in the littérature.

Aims: We aimed to determine the frequency of monoclonal gammopathy in patients with systemic mastocytosis and compare the mast cell infiltration in mastocytosis disease with Waldenstrom macroglobulinemia (percentage of mast cell, morphologic and immunohistochemical in bone marrow biopsy)

Methods: We retrosepctly analyzed the data of 1500 patients included in french protocol CEREMAST (Blood Protein Electrophoresis,Immunoelectrophoresis, bone marrow biopsy and aspiration).

Results: All the date are not analysed (late breakng abstract).

Summary and Conclusion: Plasma cell dyscrasia is not rare in patients with systemic mastocytosis (about 5%). Some patients died du to clonal evolution with multiple myeloma. The immunoglobulin isotype is frequently IgA or IgM suggesting a hyperexpression of TGFb. Some studies comparing systemic mastocytosis with monoclonal gammopathy and lymphoplasmacytic lymphoma (Waldenström's macroglobulinemia) may be interesting to see the difference in the mast cell infiltration.

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MANAGEMENT OF RENAL IMPAIRMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: PRELIMINARY DATA OF A LARGE OBSERVATIONAL, PROSPECTIVE STUDY

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Background: Impaired renal function (IR) is commonly observed in patients (pts) with relapsed refractory multiple myeloma (RRMM) and it is associated with a shorter survival. No prospective description of the renal response to new anti-myeloma drugs within the clinical practice has been performed so far in the RRMM setting.

Aims: To describe renal responses in pts with RRMM presenting with moderate or severe IR (creatinine clearance (CrCl)<50 ml/min). Secondary objectives include myeloma response, overall survival, safety and health resource utilization. We present a descriptive analysis with baseline data of the first 100 pts included.

Methods: An observational, prospective, multicentre study is currently ongoing with 300 pts with RRMM and IR planned to be included, 225 with moderate (30 ≤ CrCl<50 ml/min) and 75 with severe (CrCl<30 ml/min) renal insufficiency, measured in 24h urine. Signed informed consent is required. A follow up of 36 months after end of treatment is planned for every pt. Renal and myeloma responses will be evaluated according to the International Myeloma Working Group (IMWG) criteria.

Results: Of the 100 pts included, 50% had moderate and 50% severe IR. Comorbidities affecting renal function were arterial hypertension (47%) and diabetes mellitus (23%), and other comorbidities were: 21% motor or sensory neuropathy, 17% primary malignancy, 10% heart failure. The mean (standard deviation) CrCl (Cockroft-Gault) was 31.4 (11.9) and the mean eGFR was 32.7 (15.1) and 32.6 (15.3) ml/min according to the MDRD-4 and CKD-Epi formulas, respectively. Kidney disease classification according to MDRD-4 was: 3.3% of pts at stage 2 (eGFR 60-90); 16.5% at stage 3a (45-59); 39.6% at stage 3b (30-44); 26.4% at stage 4 (15-29) and 14.3% at stage 5 (<15). Mean age was 73.6

(8.5) y and 55% were men. Myeloma subtypes were: 57% IgG, 20% IgA, 17% Bence-Jones, 3% non-secretory, 1% IgD, and 1% IgM (1% not available (NA)). At relapse 40% of pts had detectable urine M-protein, 51% κ and 49% λ. At relapse, 47% of patients were ISS 3, 27% ISS 2, and 8% ISS 1 (NA in 15%). 13 (13%) patients had high-risk cytogenetic findings at time of relapse. At study entry, 50% of the pts were in first relapse, 34% in second relapse, and 16% in third or subsequent relapse. Main findings at relapse were: anemia (64%), bone lesions (37%), hypercalcemia (19%), and extramedullary plasmacytomas in 11% of pts. Main first-line anti-MM therapies were bortezomib in 80% of the pts, alkylating agents in 40% of the pts, autologous hematopoietic stem-cell transplantation in 12% and lenalidomide in 8%. In pts with a first relapse, a bortezomib or a lenalidomide-based therapy was administered in 38% and 46% of the patients, respectively.

Summary and Conclusion: Pts with RRMM and renal impairment are usually elderly with high ISS and present concomitant hypertension or diabetes. Evaluation of renal function by 24h urine is feasible in this population. The most frequent therapies in these pts are lenalidomide or bortezomib plus dexamethasone. Further analysis will be available as more pts are included in the study.

P1000

NUTRITIONAL COUNSELING IN SYSTEMIC IMMUNOGLOBULIN LIGHT-CHAIN (AL) AMYLOIDOSIS: A PROSPECTIVE RANDOMIZED, CONTROLLED TRIAL

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Background: Poor nutritional status is common in patients with systemic immunoglobulin light-chain (AL) amyloidosis and has been associated with mortality and impaired quality of life (QoL).

Aims: We investigated whether nutritional counseling is beneficial to the maintenance of nutritional status and QoL in AL outpatients.

Methods: We performed a single-center, randomized, controlled trial (July 2009 - October 2013). Adult (≥18 year-old) AL treatment-naïve outpatients (N=144) at diagnosis were randomized (1:1) to ad-libitum food intake (usual care; N=72) and nutritional counseling (personalized dietary prescription, regular dietetic advise by a registered dietician; N=72). All the patients gave written informed consent. The primary endpoint was the change in body weight at 12 months. Secondary endpoints included change in QoL evaluated by the Medical Outcomes Study 36-item Short Form General Health Survey (SF-36) and overall survival.

Results: Forty-one patients (N=25 usual care, N=16 nutritional counseling) died before the first follow-up visit. In the modified intention-to-treat population (patients who underwent at least one control visit during the study [month 3]; N=103), body weight remained stable in patients assigned to nutritional counseling ([N=56]; WL=0.8±4.9 kg; P=0.214), whereas in the usual care group a significant decrease in body weight was observed ([N=47]; WL=2.4±5.5 kg; P=0.003). Moreover, we observed a significant increase in the mental component summary of QoL assessment in patients randomized to nutritional counselling (8.1 [95%CI: 2.3, 13.9]; P=0.007). In all randomized patients, nutritional counselling significantly improved survival (HR=0.57 [95%CI: 0.35, 0.94]; P=0.028). In the multivariable model, after adjusting for baseline body mass index, hematologic response to treatment and cardiac stage, a trend in reduced mortality was still observed for patients undergoing nutritional counseling (HR=0.62 [95%CI: 0.37, 1.05]; P=0.074).

Summary and Conclusion: Nutritional counseling is helpful in maintaining nutritional status and effective in improving mental quality of life in AL outpatients.

P1001

ISOTYPE MATCHED IMMUNOGLOBULIN SUPPRESSION IN COMBINATION WITH CYTOGENETIC MARKERS PREDICTS OUTCOME IN MULTIPLE MYELOMA PATIENTS

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Background: The IMWG-consensus report on risk stratification in multiple myeloma (MM) recommends the definition of low risk patients (pat.) using cytogenetic markers in combination with serum albumin and β2M concentrations (Chng et al, 2014). However, using the proposed model, there are still a considerable number of low risk pat. progressing after autologous stem cell transplantation (autoPBSCT). Immunosuppression has been associated with poorer outcome in MM patients (Bryant et al, 2013) and recent evidence shows that isotype matched immunosuppression may provide additional prognostic power.

Aims: To assess whether uninvolved immunoglobulin suppression can improve stratification of MM patients.

Methods: Presentation samples from 83 MM pat. treated uniformly receiving at least one autoPBSCT were analysed for serum heavy-/light-chain (HLC) levels using commercially available immunoassays (The Binding Site, UK). HLC measurements were compared to published normal ranges. Median age was 59 (28-72) years and median follow up 26 (2-153) months (mo). 61 pat. presented with an IgG isotype (IgG kappa 69%, 42/61 pat.; IgG lambda 31%, 19/61 pat.) and 22 pat. with an IgA isotype (IgA kappa 73%, 16/22 pat.; IgA lambda 27%, 6/22 pat.). Cytogenetic aberrations included: t(4;14) (15%; 13/83 pat.); del17p13 (7%; 6/83 pat.); 1q21+ (18%; 15/83 pat.) and t(14;16) (2%; 2/83 pat.). Risk models combining ISS stage and cytogenetic markers were those from IMWG (Avet-Loiseau, 2013), IMWG-consensus (Chng 2013) and MRC (Boyd, 2012); two-tier (low-risk v intermediate / high-risk patients) models were tested for progression free survival (PFS). Kaplan-Meier survival curves were compared using the log-rank test; univariate analyses were performed using Cox proportional regression. ROC analysis was used to identify isotype matched immunoglobulin suppression (uHLC) cut-offs for survival studies.

Results: All pat. presented with an abnormal HLC ratio with the majority of pat. (84%; 70/83 pat.) also presenting with an uHLC. There was no association between uHLC levels and the detection of a t(4;14) (p=0.155), del17p13 (p=0.927) or 1q21+ (0.775). uHLC levels associated with PFS, both when tested as a continuous (hazard ratio (HR): 1.06; p=0.086), or as a categorical (HR: 1.84; p=0.062) variable using uHLC levels<0.26 (for IgG) and<0.36 (for IgA) g/L as cut-offs; with results approaching significance. Different models combining ISS and cytogenetic markers showed a trend towards better PFS for low-risk compared to intermediate / high-risk patients: IMWG (not reached (n=50) v 30.3 (n=33) mo; p=0.139); IMWG-consensus (not reached (n=19) v 31.9 (n=64) mo; p=0.165); MRC (not reached (n=42) v 30.3 (n=41) mo; p=0.056). In contrast, the addition of uHLC as a risk factor identified an increased number of intermediate / high-risk patients previously deemed as low-risk and improved the stratification of patients: IMWG + uHLC (not reached (n=38) v 28.4 (n=45) mo; p=0.015); IMWG-consensus + uHLC (not reached (n=16) v 31.4 (n=67) mo; p=0.105); MRC + uHLC (not reached (n=31) v 29.2 (n=52) mo; p=0.009).

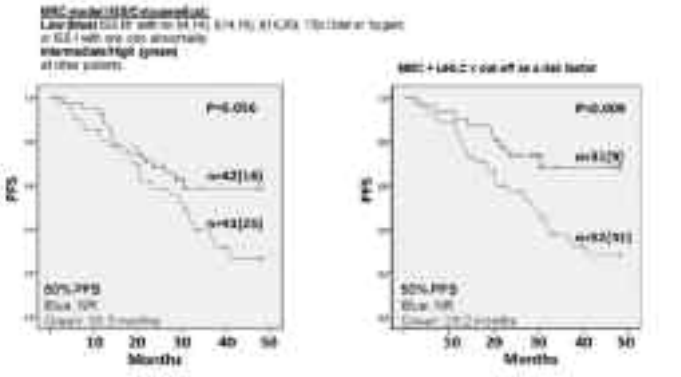

Figure 1.

Summary and Conclusion: Incorporation of the isotype matched immunoglobulin suppression refines current risk models in MM after autoPBSCT and identifies pat. with a particular good prognosis.

P1002

BONE MARROW ASPIRATION SIGNIFICANTLY UNDERESTIMATES THE BURDEN OF DISEASE IN MULTIPLE MYELOMA AT DIAGNOSIS AND FOLLOW UP COMPARED WITH THE BONE MARROW TREPHINE

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Background: The estimation of plasma cell percentage (PC%) on bone marrow biopsies (BM) remains a key feature in the laboratory diagnosis and monitoring of multiple myeloma (MM). BM aspirates (BMA) and BM trephines (BMT) are often reported independently. There is a perception that both techniques give different estimates of disease burden. Not only do BM represent a significant workload to laboratories but lack of standardisation and integrated reporting risks misdiagnosis and mis-classification of response.

Aims: To evaluate the difference in estimation of PC% on BMA and BMT and the potential clinical significance of these differences in the diagnosis and management of patients with MM.

Methods: Patients attending the myeloma clinic at the Royal Free Hospital who had BM biopsies performed from 1999-2014 were identified. Results were retrieved from the pathology results system to identify patients who had had both a BMA and BMT performed. The absolute difference in reported PC% between BMA and BMT, performed at different time points during diagnosis and

management was calculated and the ratio of PC% in the BMT to BMA calculated. The bone marrow criteria of the International Working Group on Myeloma for diagnosis (≥10% PC) and response (<5% PC) were used to assess the potential clinical significance of differences in PC%.
Results: 528 paired BMA and BMT were performed. 68 (12.9%) BMA were dilute/aparticulate and 12 (2.3%) trephines were inadequate for diagnosis; qualitative rather than quantitative reports were issued for 41 samples. PC% was reported on 136 diagnostic samples. The median PC% on BMA and BMT was 18.5% (range 0.5-98%) and 40% (range 5-95%) respectively with a mean difference in PC% between BMA and BMT of 22.6% (range -15% to 75%, p<0.0001); reported PC% was on average 4.03 times higher in BMT than BMA (range 0.67-70). In 40/136 (29.3%) cases the PC% was<10% on the BMA but ≥10% on the BMT. PC% was reported for 142 BM performed to assess treatment response. The median PC% on BMA and BMT was 3% (range<0.5-79%) and 10% (range 4-95%) respectively with a mean difference in PC% between BMT and BMA of 12.6% (range -15% to 78%, p<0.0001); reported PC% was on average 8.04 times higher in BMT than BMA (range 0.25-60). In 44/142 (31%) cases, the BMA was consistent with a CR, but this was not supported by the BMT. In only 1 case was the BMT consistent with a CR but the aspirate was not. PC% was reported for 113 BM performed for possible relapse. The median PC% on BMA and BMT was 14% (range 0.5-77%) and 30% (range 1-100%) respectively with a mean difference in PC% between BMA and BMT of 18.2% (range -28% to 81%, p<0.0001); PC% was on average 3.5 times higher in BMT than BMA (range 0.33-35). 18 BM were performed for cytopenias in 9 patients. Two patients had developed acute myeloid leukaemia, with circulating blasts on the peripheral blood film and one had developed MDS. In other patients cytopenias were attributed to either disease infiltration (3) or treatment effects (3).
Summary and Conclusion: There is a clinically significant discordance between PC% reported on BMA or BMT, manifesting either as under-estimation of disease burden by BMA or over-estimation by BMT, both at diagnosis and in assessment of treatment response. International consensus is needed to develop guidance on estimation of PC% in the BM in MM to ensure uniformity of reporting and to rationalise laboratory workloads.

P1003

AUTOIMMUNE DISEASES IN COURSE OF IMIDS: SELECTIVE OCCURRENCE AFTER LENALIDOMIDE

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Background: Immunomodulatory drugs (IMiDs) are active drug for the treatment of multiple myeloma (MM), and have important immunomodulatory properties. This activity may contribute to autoimmune diseases (ADs) occurrence. Despite some case reports, a systematic evaluation on ADs after IMiDs is still lacking.
Aims: To evaluate the occurrence of ADs and describe their characteristics in a cohort of patients affected by MM treated with IMIDs.
Methods: We conducted a retrospective study on consecutive IMID-treated MM patients at two Italian hematologic referral centers. Patients were grouped into three classes depending on the type of IMID used. The first group was composed of patients treated only with thalidomide (Thal) (n=474), the second group with lenalidomide (Len) (n=140), and the patients in the third group were first treated with Thal and then with Len (Thal-Len) (n=94).
Results: A previous AD before MM diagnosis was documented in 7 out of 474 patients (1.5%) in the Thal group, in 8 out of 140 patients (6%) in the Len group, and in one (1%) in the Thal-Len. The median line of therapy in which the IMID was used was 1 (range 1-4) in the Thal group, 1 (range 1-4) in the Len group, while in the third group the median line of therapy for Thal was 1 (range 1-3), and 3 for Len (range 1-6). Median therapy duration of IMIDs in the Thal group was 6 months (range 1-161), and 9 months (range 1-89) in the Len group. In the third group Thal treatment had a median duration of 6 months (range 1-94), and of 5 months (range 1-62) for Len. Two (0.4%) ADs cases have been observed in the Thal group: one autoimmune anemia (AHA) at 6 months and one vasculitis at 5 months. In the Len group 6 (4%) patients developed an AD, in particular: one AHA at 3 months, one idiopatic thrombocytopenic purpura (ITP) at 2 months, one Evans syndrome (combination of AHA and ITP) at 3 months, one optic neuritis at one month, one Grave's disease at one month, and one polymiositis at 42 months. In the Thal-Len group no ADs were observed after Thal, while one case of ITP occurred after Len at one month. All ADs were managed with Len discontinuation and steroid treatment, except for Grave's disease, which required thyroidectomy, and the polymiositis, which was fatal. Absolute risk of developing an AD was 0.4% for patients treated with Thal, 4.3% for Len, and 1.1% for the Thal-Len group. In univariate analysis ADs occurrence was more common after autologous transplantation (p=0.003), while history of ADs, previous number of therapy lines, disease status at AD occurrence, previous allogeneic transplantation, and type of drugs in combination with the IMiD had not impact. Also in multivariate analysis a previous autologous transplantation was the only factor associated with an increased risk of ADs (p=0.01). Interestingly, the timing of AD occurrence revealed a double pattern, with a clustering in the first 3 months in 6 out 9 cases, and a later occurrence in the remaining patients.
Summary and Conclusion: This is the first report to describe the occurrence of ADs in a large cohort of IMiD treated patient. Despite low, the incidence was not negligible in Len-treated patients, and a previous autologous transplant was shown to be a significant risk factor. ADs consisted mainly of autoimmune cytopenias, but other organs can be involved. In most cases the AD resolved after IMiD discontinuation and steroid treatment, however, a case of polymiositis was fatal. Our data may be helpful to the clinician in the interpretation of specific clinical conditions, such as cytopenias that occur in the early phase of treatment, and, more generally, in the clinical management of MM Len-treated patients.

P1004

IMPACT OF NFKB1-94INS/DEL ATTG POLYMORPHISM ON DISEASE CHARACTERISTICS AND TREATMENT OUTCOME ON BORTEZOMIB BASED THERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: NFκB1 complex plays important role in pathogenesis of multiple myeloma (MM), as it is associated with the immune response, inflammatory, apoptosis as well as with growth and survival of malignant plasma cells. NFκB1 -94ins/delATTG polymorphism affects promoter activity of the NFκB1 gene.
Aims: To find association between NFκB1 -94ins/delATTG and the MM susceptibility, age at diagnosis, the presence of bone lesion, the type of monoclonal protein, cytogenetics and prognostic stratification according to the International Staging System (ISS) and to investigate the treatment outcome in symptomatic myeloma patients with upfront bortezomib based therapy.
Methods: NFκB1 -94ins/del ATTG were tested applying PCR restriction fragment length polymorphism (RFLP) technique in a group of 315 patients diagnosed with multiple myeloma and 148 healthy blood donors. Treatment outcome was investigated in a selected subcohort, consisting of 153 patients, receiving upfront bortezomib based therapy.
Results: Allele frequency (±95%CI) of 94ins/delATTG was 40.5±3.9% in MM patients and 38.6±5.6% in healthy controls (p=0.5). No association has been found between NFκB1 polymorphism and age at diagnosis, the type of the monoclonal protein (non secretory MM, light chain only, light and heavy chain complex) or cytogenetics (hyperdiploid *vs*. immunoglobulin heavy chain translocations). Patients with homozygous insertion (I/I genotype) tended to be diagnosed in more advanced stages (stage 2 and 3 according to the ISS) compared to deletion carriers (I/D and D/D genotypes) [73% (66/91) *vs*. 61% (107/176), p=0.06]. In case of upfront bortezomib therapy I/I patients (n=45) had significantly better progression free survival (PFS) comparing to deletion carriers (n=66) [64.6±7.7% *vs*. 47.3±7.3% at 2 years, p=0.035]. ISS before bortezomib therapy was also proved to be a significant prognostic factor [ISS1 65.7±8.2% (n=40) *vs*. ISS2 62.7±9.0% (n=37) *vs*. ISS3 44.2±6.2% (n=76) at 2 years, p=0.007]. NFκB1 polymorphism influenced survival in non-advanced stages (ISS1; p=0.024 and ISS2; p=0.033), but not in ISS3 (p=0.551). In multivariate analysis NFκB1 polymorphism was proven to be an independent prognostic factor regarding PFS in patients with upfront bortezomib based therapy, in addition to ISS and age [HR: 0.564 (0.328-0969); p=0.038]. Similar therapeutic impact of NFκB1 polymorphism could not be observed in patients who received bortezomib at relapse or therapy resistance.
Summary and Conclusion: NFκB1 -94ins/delATTG is not a susceptibility factor for the development of MM, and it does not influence the clinical characteristics at diagnosis. NFκB1 -94ins/delATTG insertion homozygous patients experience a progression-free survival benefit on bortezomib based upfront therapy.

P1005

THIRD AUTOLOGOUS SALVAGE STEM CELL TRANSPLANT IN SUBJECTS WITH ADVANCED MULTIPLE MYELOMA

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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) has remained a standard of care in first-line treatment of multiple myeloma (MM) since the late 1990s. Virtually all patients (pts), however, will experience disease progression requiring active salvage treatments. As thalidomide, bortezomib, and lenalidomide alone or in combination are liberally used, pts will eventually present with resistant relapse. In addition to the long interval since the initial administration of melphalan,

exhausted bone marrow function may be a good reason to consider a further autotransplant. A second ASCT has proven feasible and effective in several retrospective series.
Aims: Whether a third salvage transplant is of comparable value to patients who have initially received tandem ASCT (still frequently used in Germany as initial treatment approach) is unclear. Therefore, we assessed the outcomes of pts receiving a third salvage ASCT (ASCT3) from two large German myeloma centres.
Methods: We queried the databases of Wuerzburg and Freiburg University Medical Centres for cases that were offered a third autotransplant after unique melphalan-based tandem ASCT as their first line therapies.
Results: We identified 37 pts with a median age of 63 (range, 48 – 77) years at their third salvage ASCT which occurred at a median of 58 (range, 72 – 349) mos. from myeloma primary diagnosis and a median interval from initial tandem autotransplant of 47 (range, 5 – 115) mos.. Median progression-free survival (PFS) from primary tandem ASCT had been 20 (range, 26 – 3) months. 31 pts (84%) had received IMiDs and/or bortezomib (27 had received both, 7 had been IMiD-naïve and 6 Bortezomib-naïve, respectively. 23 pts. (62%) had been refractory to lenalidomide, as well as 62% to bortezomib, 19% of the pts even had been double-refractory to both "novel compounds" prior to their 3rd transplant. At ASCT3, 32 pts received autografts which had been harvested at first-line treatment and 5 pts had undergone additional stem cell mobilisation. Median number of reinfused CD34+ cells was 3.15 (1 – 8.2) x10exp6/kg body weight with all patients achieving stable engraftment measured by improvement of platelet (PLT) and haemoglobine (Hb) count within 3 mos. of ASCT3 (PLT improvement in 65% and Hb improvement in 73%, respectively). Within 12 mos. from ASCT3 one patient died due to non-relapse associated cause (non-relapse mortality 2.7%). Overall response rate (partial response [PR] or better) was 54%; 4 pts achieved CR, 5 pts VGPR, respectively; 11 pts reached PR after ASCT3.
Summary and Conclusion: This retrospective analysis shows ASCT3 to be a very well tolerated salvage option in pts who have exhausted "novel compound"-based treatments in earlier relapse of MM. Interestingly, median duration of storage of their autografts of 46 (range, 1 – 152) mos. did not impair engraftment after salvage transplant. Median OS of 10 mos. from ASCT3 indicates that further salvage options still might be of some value in these "very late stage" MM pts.

P1006

TREATMENT WITH THE BILE-ACID SEQUESTRANT COLESEVELAM IMPROVES LENALIDOMIDE ASSOCIATED DIARRHOEA IN MYELOMA PATIENTS

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Background: There is increasing evidence for the long term use of lenalidomide for the treatment of multiple myeloma at induction, relapse and as maintenance following ASCT. In studies at all stages of disease maximum benefit has been gained by continuing treatment beyond best response until relapse, requiring patients to remain on treatment long term and making the management of side effects particularly important. The side effect profile of lenalidomide is predominantly early with skin rash and myelosuppression dominating, however later during treatment a proportion of patients develop diarrhoea that can be difficult to manage.
Aims: To identify the cause of diarrhoea associated with lenalidomide treatment for myeloma.
Methods: In our institution, all patients with gastrointestinal (GI) symptoms arising during and after chemotherapy treatment are seen and investigated in a specialist Gastroenterology/Oncology clinic using a peer-reviewed algorithm. All patients underwent investigations for the presence of coeliac disease, inflammatory bowel disease, colonic neoplasia, small bowel bacterial overgrowth and bile acid malabsorption using SeHCAT scanning. SeHCAT (^{75}selenium homocholic acid taurine) scanning is a simple non-invasive test for bile acid malabsorption with a sensitivity of 90-98% and specificity of 100%. We carried out a retrospective analysis of all myeloma patients referred to the clinic between April 2011 and April 2013 with diarrhoea whilst on lenalidomide, in order to audit their investigations and outcomes and identify the cause of their diarrhoea.
Results: 4 male and 7 female patients (median age 66 years, range 48-79) who developed diarrhoea while on lenalidomide treatment for myeloma were referred to the gastroenterologist. 45% had normal bowel function before starting lenalidomide, 55% had a degree of mild gastrointestinal dysfunction with a tendency to occasional loose stools before treatment but had not undergone previous GI investigations. Worsening GI symptoms started a median of 5 months (range 1-15) after lenalidomide initiation. These symptoms included diarrhoea (100%), urgency (91%), faecal incontinence (64%) and abdominal cramps (45%). A positive glucose hydrogen methane breath test raised the possibility of bacterial overgrowth in some cases but empirical antibiotic treatment did not resolve the diarrhoea. SeHCAT results confirmed bile acid malabsorption (BAM) in all cases, 8 had severe BAM (<5% SeHCAT seven day retention), 2 moderate (5-10%) and 1 mild (10-15%). To confirm BAM as the cause of the diarrhoea, patients were treated with advice to reduce dietary fat intake to 20% of total calories or colesevelam, a bile acid sequestrant or both. Nine patients were started on colesevelam. This was given at a dose of 625mg 3 times per day, building up to 6 times per day. All patients had a significant improvement in symptoms, often within just a few weeks. Two patients had resolution of diarrhoea on a low fat diet alone and did not require colesevelam. With these strategies no patient needed lenalidomide dose reduction or to stop treatment due to their diarrhoea.
Summary and Conclusion: Bile acids are reabsorbed predominantly via passive diffusion in the small intestine with additional active absorption at the terminal ileum. Normally, 95% are reabsorbed. When this process is disrupted and higher levels of bile acids reach the colon there is an increase in watery stool leading to diarrhoea. We describe a link between lenalidomide treatment and BAM that is easily identified and managed thereby improving the tolerance of lenalidomide and enabling patients to remain on treatment long term. Research to establish the molecular mechanism whereby lenalidomide induces BAM should be performed.

P1007

PHASE II TRIAL TO INVESTIGATE EFFICACY AND SAFETY OF BENDAMUSTINE, DEXAMETHASONE AND THALIDOMIDE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS AFTER TREATMENT WITH LENALIDOMIDE AND BORTEZOMIB

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Background: Despite the introduction of new and efficient drugs for multiple myeloma (MM) treatment, mainly bortezomib-based multidrug regimens, most patients relapse and lenalidomide based also provide only a temporary disease free state. After the third relapse the prognosis is usually dismal and new therapeutic approaches are needed.
Aims: The aim of this trial is to assess the tolerability and efficacy of bendamustine in association with dexamethasone and thalidomide (BDT) in relapsed/refractory patients after lenalidomide and bortezomib or who are ineligible to these drugs.
Methods: The primary endpoints of this multicenter phase II trial were the assessment of efficacy, in an intention to treat analysis, and toxicity. Treatment consisted of bendamustine (60mg/m^2, d 1, 8, 15), dexamethasone (20mg, d 1, 8, 15, 22) and thalidomide (100mg, d1-28) repeated every 28 days for 6 cycles.
Results: Up to now 18 of the planned 30 patients were enrolled and completed at least the first therapy cycle. They underwent a median of 2 previous treatment lines (range 1-6). Median age was 60 years and most patients presented with an ISS-1 (61%), a good performance status (50%) and had a IgG kappa monoclonal component (33%). 4 patients completed the planned treatment and another 4 underwent ≥4 treatment cycles. Treatment was usually well-tolerated and overall only 4 patients experienced grade 3/4 hematologic toxicity. During 53 cycles, 5 treatment related serious adverse events (9%) were recorded (3 cases of pneumonia, 1 cerebral hemorrhage, 1 deep vein thrombosis and 1 pulmonary embolism), leading to death in 2 patients (4%). Of note note, no grade 3/4 neurologic toxicity has been observed. Up to now the ORR was 5/9 (55%): 3 very good partial remissions and 2 partial remissions. Other 2 patients had stable disease and 2 progressed during treatment after 2 and 4 treatment cycles, respectively and died.

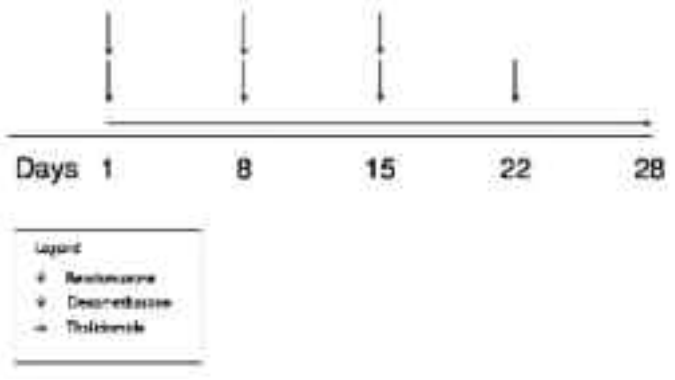

Figure 1.

Summary and Conclusion: In this interim analysis, BDT demonstrated to be an effective regimen for relapsed/refractory MM patients. The toxicity, as expected, in these heavily pretreated patients was not negligible, but manageable and no grade 3/4 neurologic side effect was observed.

P1008

PROGNOSTIC IMPACT OF IMMUNOPHENOTYPIC RESPONSE AND NORMALIZATION OF SERUM FREE LIGHT CHAIN AMONG PATIENTS WITH MULTIPLE MYELOMA

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Background: With the development of novel therapeutic agents, more than 30% of patients with multiple myeloma (MM) achieve complete response (CR) as defined by the International Myeloma Working Group (IMWG). However, most patients that achieve CR ultimately die due to relapse, suggesting the presence of minimal residual disease (MRD) in these patients. Multicolor flow cytometry (FCM) allows the detection of$<10^{-4}$ clonal plasma cells in normal bone marrow cells and has been used for detecting MRD after treatment. FCM-defined immunophenotypic response (IR) has emerged as a more relevant prognostic factor in patients with MM. However, the relevance of the prognostic impact of IR and the normalization of serum free light chain (sFLC) ratio remain unclear.
Aims: We retrospectively analyzed the impact of FCM-defined CR (iCR), conventional immunofixation negative CR (CR), and CR plus FLC κ/λ normal CR (sCR) on the prognosis of 86 patients with MM who obtained more than very good partial response (VGPR) at Kameda Medical Center, Kamogawa, Japan.
Methods: Among the 164 patients treated at our hospital between April 2005 and December 2013, 86 patients that achieved more than VGPR (50 CR, 36 VGPR) were included in this study. The study population consisted of 50 males and 36 females with a median age of 71 years. All patients received at least one course of novel agent-containing therapeutic regimen. Treatment responses were assessed using the IMWG criteria, and the best response to treatment during the course of disease was assessed by simultaneous analysis by serum immunofixation, sFLC measurements, and FCM analysis of bone marrow plasma cells. FCM-defined MRD was evaluated by single-tube 6-color FCM, CD45-CD38 gating strategy, and combination analysis by CD19, CD56, and cytoplasmic κ/λ. Negativity of clonal PCs was defined as less than 10^{-4} neoplastic PCs in bone marrow samples determined by FCM. Overall survival (OS) and progression-free survival (PFS) were analyzed by the Kaplan–Meier method, and differences between curves were calculated by two-sided log-rank test. Subjects were classified into four categories, *i.e.*, MFC positive or negative and κ-λ ratio normal or abnormal, and PFS and OS were compared between groups.
Results: At a median follow-up of 38.7 months, 3- and 5-year overall survival rates of all of the patients with more than VGPR were 94% and 79.2%, respectively. Achievement of normal sFLC ratio was observed among 64% of the CR patients and 55% of VGPR patients. Kaplan–Meier estimated 3- and 5-year OS in patients with CR were 100% and 76%, respectively. Among 50 patients with CR, normalization of sFLC κ/λ and MRD negativity were achieved in 31 (62%) and 19 (34%) patients, respectively. Among 31 CR patients with normal sFLC κ/λ, 12 were MFC-negative and 19 were MFC-positive. MFC was positive in 12 (63%) and negative in 7 patients (37%) among the 19 CR patients with abnormal sFLC κ/λ. Among 36 patients with VGPR, 20 patients (55%) obtained normal sFLC κ/λ and 5 (14%) obtained MFC negativity. Seventeen of 31 MFC-positive patients with VGPR had normal sFLC κ/λ. Patients who achieved FCM negativity did not relapse within a median observation period of 82.6 months, and they were superior PFS and OS compared to those who did not, especially in the group that achieved CR ($P = 0.016$ and $P = 0.003$, respectively). In the VGPR group, although achievement of normal sFLC κ/λ or MFC negativity did not show survival advantage in PFS, they tended to exhibit superior survival compared to those who did not.
Summary and Conclusion: Although both normalization of sFLC κ/λ and achievement of FCM negativity are important for longer PFS and OS, obtaining MFC negativity seems to have greater implications for longer survival.

P1009

EVALUATION OF VAD PROTOCOL IN MULTIPLE MYELOMA OVER A PERIOD OF 10 YEARS

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Background: In Algeria, multiple myeloma (MM) is ranged with non-Hodgkin's lymphoma at the 3rd place of hematological malignancies with an incidence of 0.63/100000 inhabitants. Treatment is necessary for the symptomatic myeloma. The treatment choice depends largely on the patient's age. VAD (vincristine, adriamycin, dexamethasone) has long been the reference induction treatment of young patients, in whom high dose chemotherapy (HDC) therapy with autologous stem cell transplantation (ASCT) is planned. We report in this study the results of VAD treatment in a series of patients under 66 years old of age over 11 years of follow up.
Aims: Over a period of 11 years (2000-2010), 410 pts with MM were diagnosed and156 pts aged less than 66 years treated with VAD: 83 male and 73 female (sex ratio: 1.1). The median age was 54 years (32-65). Thirteen pts (08%)<40 years old. The most frequent sign at diagnosis is pain syndrome 129 pts (83%), anemia syndrome: 12 pts (8%) and plasma cell mass: 5 pts.
Methods: The median time to diagnosis: 5 months (0-24 months). The median rate of Hb: 9g/dl (3-14), hyper calcemia >120 mg /l: 37 pts (24%), serum creatinine>20 mg /l: 52 pts (33%). Rate of albumin<35g /l in 71 pts (45%). Monoclonal peak: 128 pts (82%), with a peak ≥ 50 g /l: 55 pts (43%), which is represented by an IgG: 88 pts (56%), IgA: 29 pts (18%), IgD: 3, biclonal IgG / IgA: 1pt, 25 cases of light chains MM, non-secreting: 3 pts and unspecified type in 7 pts. On radiology, diffuse bone lesions: 139 pts (89%) and spinal cord compression: 19 pts (12%). According to Salmon-Durie staging system: stage III: 150 pts (96%) including 52 pts (33%) stage B, stage II: 4 pts and stage I: 2 pts, Stage A: 104 pts (67%). International Staging System(ISS) used in 59 pts (38%) I: 17 pts, II: 16 pts, III: 26 pts. At December 2012, the median follow-up is 68 months (26-144).
Results: Thirty-four pts are no evaluable because early death after 1-2 cycles. One hundred and twenty two pts are evaluable, the average of overall response (OR) after 4 cycles was 71% (87 pts) with 22% (27 pts) in complete remission (CR) and 49 % (60 pts) in partial remission (PR). Twenty-nine percent (35 pts) was in treatment failure. HDC with ASCT: 63 pts (51%), with status before autologous: 23 CR, 27P R, 13in treatment failure. After HDC with ASCT: 4 pts died during the procedure (3 infectious syndromes and one pulmonary embolism), 47in CR, 10 in PR and 2in procedure failure. At December 2012: 17 pts were still alive (10 CR, 6 PR, 1 failure) and 46 pts died. The overall actuarial survival and the event-free survival are res 22% and 18% pectively
Summary and Conclusion: Conclusion: In this study, it appears that VAD induce a good immediate response but not permanent over time, which implies the need for a maintenance treatment also for pts with or without HDC.

Bone marrow failure syndromes & PNH

P1010

A SIMPLE METHOD TO DETECT COPY NUMBER-NEUTRAL 6P-LOH IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA USING DUPLEX QUANTITATIVE PCR

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Background: The presence of HLA allele-lacking leukocytes (HLA-LLs) due to copy number neutral loss of heterozygosity (LOH) as a result of uniparental disomy in the short arm of chromosome 6 (CNN-6pLOH) is compelling evidence for the immune pathophysiology of acquired aplastic anemia (AA), and may serve as a predictor of the response to immunosuppressive therapy. However, the high expenses associated with the SNP array analysis hinder the popularization of detecting 6pLOH in the management of AA. The presence of 6pLOH can also be estimated by detecting HLA-LLs using flow cytometry with monoclonal antibodies specific for HLA-A alleles, but this assay cannot be applied for a substantial number of homozygotes for the HLA-A allele, or for those harboring HLA-B*40:02, an allele closely associated with the immune pathophysiology of AA, against which monoclonal antibodies are not available.
Aims: To establish a cost-effective and reliable method for detecting 6pLOH involving class I HLAs, especially the one involving HLA-B*40:02, in the leukocytes of AA patients, we examined the usefulness of duplex quantitative real-time PCR (2qPCR), and applied this method to detect 6pLOH involving HLA-B and -C loci.
Methods: 2qPCR was designed to compare the copy number of each allele in individuals heterozygous for the HLA-B or -C alleles. The 2qPCR allows the simultaneous amplification of each HLA-allele from genomic DNA in a single reaction. The PCR mixture contains two different TaqMan probes that are complementary to respective SNP sequences specific for individual HLA alleles, which are labeled with different fluorochromes (FAM and VIC), as well as one primer pair complementary to consensus sequences. Fluorescent intensity for each allele was plotted on the X-axis and Y-axis. The presence of 6pLOH led to deviation of tested samples from standard line generated from the heterozygotes, and 6pLOH clones were regarded to be significant if the distance of the sample dots from the standard line exceeded that of control dots that occurred as a result of nonspecific dispersion. The 6pLOH clone size could be estimated by the degree of deviation. The TaqMan probe mixtures (mixtures B and C) were designed to distinguish HLA-B*40:02 from the majority of the other HLA-B alleles, and HLA-C*03 from the other HLA-C alleles, respectively: HLA-C*03:03 and *03:04 are highly associated with HLA-B*40:02 due to linkage disequilibrium in Japanese population. Approximately 50% of Japanese individuals and 90% of those possessing at least one HLA-B*40:02 allele were expected be analyzable using the B or C reaction mixtures.
Results: Six 6pLOH (+) cases that had previously been identified by the SNP array were found to be positive for 6pLOH by the 2qPCR analysis. The estimated 6pLOH clone sizes were similar between the SNP array (range, 5.6% to 53.9%, median 19.6%) and the 2qPCR analysis (range, 11% to 48%, median 13%). When a new AA patient set was analyzed by 2qPCR, 6pLOH was found in 10 of 68 patients (15%), and the median clone size was 14% (range, 4%–50%). Six of the ten 6pLOH (+) cases were heterozygous for the HLA-A allele and were therefore analyzable with flow cytometry. HLA-LLs were detectable in all of the six patients. Consistent with our previous data, HLA loss due to 6pLOH was more frequently found in HLA-B*40:02 allele, compared to those with the other HLA-B alleles (20%, 6/30 *vs* 3.8%, 4/106; *P*=0.008). The 2qPCR assay was completed within three hours after DNA preparation.
Summary and Conclusion: A rapid and cost effective method for detecting 6pLOH with high sensitivity and specificity was established. We are conducting mass screening of AA patients' samples for the presence of 6pLOH. The 2qPCR method confirmed the preferential loss of HLA-B*40:02 due to 6pLOH in AA. Further studies to clarify the pathogenic antigens presented by HLA-B*40:02 are therefore warranted.

P1011

EPIGENETIC ALTERATIONS IN FANCONI ANAEMIA: ROLE IN DISEASE PROGRESSION AND THERAPEUTIC POTENTIAL

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Background: Fanconi anaemia (FA) is an inherited disorder characterized by chromosomal instability, progressive bone marrow failure and increased incidence of haematological and non-haematological malignancies. Mutations in 16 FA genes have been identified which disrupt a DNA repair complex, resulting in increased chromosomal fragility. However, the phenotype is variable even amongst patients from the same family and with the same mutation. This raises the possibility that other factors, such as epigenetic modifications, may affect the disease phenotype. Epigenetic changes play an important role in oncogenesis of several haematological malignancies and solid tumours. These changes may be pharmacologically manipulated with DNA hypomethylating agents and histone deacetylase inhibitors.
Aims: The aim of our project was to explore whether the epigenetic profiles in FA differ from non-FA individuals and whether these could be manipulated to alter the disease phenotype.
Methods: We assessed the expression level of Epigenetic genes by Q-PCR on twelve blood samples from FA patients and 15 healthy volunteers using an 84 epigenetic gene PCR array. The same samples were used in a methylation array to study the DNA methylation profile of tumour suppressor genes. To test the effect of Vorinostat on chromosomal instability in FA, we treated mononuclear blood cells from different patients with Vorinostat and quantified the percentage of DEB-induced chromosomal breakages by cytogenetic analysis.
Results: As compared to unaffected cells, FA patients had decreased expression levels of genes involved in DNA methylation (DNMT1, DNM3Tβ), genes encoding proteins that regulate the activity of histones by acetylation (CIITA), phosphorylation (PAK1), ubiquitination (RNF20), deacetylation (HDAC2, HDCA8, HDAC9, HDAC10, HDAC11) and methylation (SETD6). We also assessed methylation status of tumor suppressor gene promoters in one FA patient and found that the FA patient had global hypomethylation in the promoter regions of tumour suppressor genes as compared to non-FA individuals, which is a frequent early event in cancer, and correlates with severity of disease. Following incubation with Vorinostat, FA cells exhibited increased expression of DNM3Tβ, but reduced expression of CIITA, HDAC9, PAK1, and USP16, all involved in different aspects of epigenetic and immune regulation. Given the ability of Vorinostat to modulate epigenetic genes in FA patients, we investigated its functional effects on the FA phenotype. Treatment of FA cells with Vorinostat significantly reduced the number of DEB-induced breaks per cell and decreased by 80% the number of aberrant cells produced by this DNA cross-linking agent.
Summary and Conclusion: We identified different epigenetic patterns in FA cells relative to non-FA cells, indicating that epigenetic changes might play a role in the pathophysiology of FA. The reduction in chromosomal breaks by Vorinostat suggests it might have a therapeutic effect in FA. The use of Vorinostat is attractive in the context of FA since FA patients are extremely sensitive to chemotherapy and radiotherapy commonly used to treat tumors of these patients.

P1012

CHRONIC IDIOPATHIC NEUTROPENIA DOES NOT DISPLAY FREQUENT STAT3 MUTATIONS: IMPLICATIONS FOR DIFFERENTIAL DIAGNOSIS FROM OTHER CLINICAL SYNDROMES WITH T-CELL-MEDIATED SUPPRESSION OF HEMATOPOIESIS

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Background: Chronic Idiopathic Neutropenia (CIN) is a benign disorder of granulopoiesis characterized by prolonged and unexplained reduction in the number of circulating neutrophils. The pathogenetic cause of neutropenia in CIN is attributed to impaired BM granulopoiesis of multifactorial origin. That said, our recent studies strongly implicate expanded T-cell populations in both PB and BM in the development of CIN. Such expansions are more prominent in the BM CD8+ subset and can display pronounced repertoire skewing, often verging on monoclonality. This raises diagnostic uncertainties given that severe cytopenias can also develop in a range of clinical syndromes associated with chronic $CD8^+$ expansions, most notably T-large granular lymphocyte (T-LGL) leukemia. Recently, STAT3 mutations predominantly clustering in exon 21, encoding the Src homology 2 (SH2) domain which mediates the dimerization and activation of the STAT protein, were found to be frequent in chronic lymphoproliferative disorders of natural killer cells (CLPD-NKs) and T-LGL leukemias, helping to discriminate them from reactive expansions. The available information about the frequency of STAT3 mutations in CIN is very limited.
Aims: Taking all the above into consideration, here we aimed at obtaining deeper insight into the role of STAT3 dysregulation in CIN pathogenesis, with obvious implications for improved understanding of the true nature of cytotoxic T-cell expansions in CIN.
Methods: We screened for STAT3 mutations a large and well-annotated cohort

of 167 patients, including 19 males and 148 females with a median age of 54 years. All patients had an absolute neutrophil count below 1500/μL, normal karyotype, lack of anti-neutrophil antibodies and no clinical, serologic or ultrasonic evidence of any underlying disease that might be associated with neutropenia. Exon 21 sequences of the STAT3 gene were amplified on genomic DNA from mononuclear cells of (mostly) PB and BM samples. PCR products were subjected to bidirectional Sanger sequencing and sequence data were analyzed using the Vector NTI alignment tool. The sensitivity of our approach for detecting STAT3 mutations was 15%.
Results: Overall, only 4/167 cases (2.4%) were found to carry STAT3 exon 21 mutations. Two of 4 concerned single nucleotide insertions at position 963, leading to frameshift; the remaining two concerned: (i) c.1169G>A, p.K685K (silent) and (ii) c.1033A>T leading to the Y640F substitution, a recurrent lesion in malignant T/NK lymphoproliferations that is associated with STAT3 phosphorylation and, consequently, increased transcriptional activity of STAT3.
Summary and Conclusion: In conclusion, we demonstrate that STAT3 mutations are very infrequent in CIN despite its almost consistent association with cytotoxic T-cell expansions. From a pathogenetic perspective, this finding indicates that CIN may justifiably be considered as distinct from malignant T/NK lymphoproliferations, though sharing similar clinical presentation. From a diagnostic perspective, our results offer hints that STAT3 mutations may be a useful tool to discriminate malignant lymphoproliferations from reactive expansions, helping to establish a reliable diagnosis of CIN.

P1013

GENOTYPING STRATEGIES FOR DIAMOND BLACKFAN ANEMIA PATIENTS IN CANADA

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Background: Diamond Blackfan anemia (DBA) is characterized by a variable phenotype, which mainly includes anemia, craniofacial malformations, thumb and other limb abnormalities, cardiac defects, urogenital malformations, and a modestly increased risk of leukemia and other malignancies. Mutations in multiple genes can cause DBA; hence the genetic diagnostic process is often lengthy and costly.
Aims: The aim of the present study is to compare which methods of genetic testing are most effective in identifying mutations in DBA patients.
Methods: Patients were enrolled on the Canadian Inherited Marrow Failure Registry (CIMFR), which is a multicenter prospective cohort study of inherited bone marrow failure syndromes (IBMFS). The following methods for genetic testing were used: targeted gene sequencing (TGS), comparative genomic hybridization (CGH) array, single nucleotide polymorphism (SNP) array, and a next generation sequencing panel (NGSP) that was developed in our laboratory. Specific genotype data were extracted from the registry and categorized as causal, most-likely causal (novel), most-likely not causal and not causal based on published data and *in-silico* analysis A correlation between the method of testing and identification of a genotype was determined by Pearson test.
Results: The most common IBMFS among the 399 patients enrolled on the CIMFR is DBA (N=64). Diagnostic criteria were based on clinical features, blood counts, fetal hemoglobin, marrow findings, erythrocyte adenosine deaminase levels and genetic testing. Of the 64 patients, 42 had genetic testing using at least one of the methods: TGS (34), NGSP (12), CGH arrays (8 patients). NGSP was used in 4 patients without previous testing by other methods and in 8 patients who had already been tested for at least on DBA gene. As well, CGH/SNP arrays were used in 2 patients who had not been previously tested by other methods and in 6 patients who had already been tested for at least one DBA gene. Overall, previously published causal mutations or novel most likely causal mutations were identified in a total of 33 of the 42 patients tested (79%). These include mutations in RPS19 (10 patients), RPS26 (7), RPL11 (5), RPL5 (2), RPS24 (2), RPL35a (2), RPS7 (1). The mutations included frame shift (12 patients), missense (6), nonsense (4), splicing site (3), small indels (2), and large deletions (6). Among patients tested by NGS 75%, causal or most likely causal mutations were successfully identified in 75%, by TGS in 55%, and by SNP/CGH arrays in 50%, (R=0.1306, p>0.05).
Summary and Conclusion: The majority (80%) of Canadian patients can now be genotyped based on known genes and the methods described. The most commonly mutated DBA genes in Canada are *RPS19*, *RPS26* and *RPL11*. Our findings suggest that NGSP combined with methods to detect deletions are the most effective method to detect mutations in known DBA genes.

P1014

PARAMETERS OF CELL IMMUNITY IN PATIENTS WITH APLASTIC ANEMIA AND THEIR RELATION TO PNH-CLONE

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Background: It is known that immune-mediated suppression of hemopoiesis serves as a basis for pathogenesis of aplastic anemia (AA) in most cases. Therefore, the immunosuppressive therapy is the main method for AA treatment. According to present views, PNH-clone can be present in more than half of AA patients. It is also known that immunosuppressive treatment efficacy is higher in patients with PNH-clone associated AA, possibly due to specific features of immunopathogenesis in this category of patients.
Aims: Aim – to compare the main parameters of cell immunity in AA patients without PNH-clone or with PNH-clone of different size.
Methods: We have evaluated 41 patients with AA, different size PNH-clone has been revealed in 59,6% of cases. Detection of PNH-clone and immune parameters of peripheral blood was performed using multicolor flow cytometry. PNH diagnostics was performed using CD235a/CD59 for red blood cells, FLAER/CD24/CD15/CD45 for granulocytes and FLAER/CD14/CD64/CD45 for monocytes. Also we have measured the main peripheral blood lymphoid subsets: T-, B- and NK-cells.
Results: The results of comparative evaluation of lymphoid subsets in groups of AA patients without PNH-clone and with PNH-clone of different size (0-10% and >10%) have revealed similar tendencies. Nevertheless, typical for AA increase of CD3+ and CD8+- T-cells (87,4% and 39,2% *vs*. 69,04% and 23,07%, respectively, in norm, p<0,05) and decrease of NK-cells and CD19+ B-cells (7,31% and 4,7% *vs*. 13,12% *и* 13,22% in norm) was more pronounced in subjects with larger PNH-clone. As for minor lymphoid subsets, the level of T-regulatory cells (CD4+/CD25+) was decreased both in patients with PNH- and without PNH-clone, and NKT-cells were also increased similarly in both groups. While activated T-cells (HLA-DR+CD3+) were significantly increased in patients with large PNH-clone – 5,50% *vs*. 1,83% in norm (p<0,05).
Summary and Conclusion: Thus, along with similar tendencies, we have revealed some diversities in lymphoid subsets impairment in AA patients with or without PNH-clone. It can be supposed, that these diversities serve as conditioning factors for the different clinical features and therapeutic response in patients with aplastic anaemia.

P1015

ABNORMAL METABOLITES RELATED TO BONE MARROW FAILURE IN APLASTIC ANEMIA PATIENTS

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Background: Metabolomics is the identification and quantitation of the small molecules that are involved in metabolic reactions. As a stem cell disease, aplastic anemia(AA) is extremely instructive and provides insights into the function and quantity of normal hematopoietic stem cells (HSCs) and their ability to regenerate.In this study, metabonomic analyses were performed to investigate the mechanisms that might influence the renewal and proliferation of HSCs in AA patients.
Aims: We aimed to characterize themetabolic pathways associated with bone marrow failure of aplastic anemia patients.
Methods: From September 2010 to February 2013, 40 AA patients (23 males and 17 females, median age of 39.95 years; range 11–64 years) were compared with 40 healthy control subjects (17 males and 23 females, median age of 38.50 years; range 15–68 years). Serum metabolites of aplastic anemia (AA) patients and healthy controls were investigated by using LC-MS. A wavelet-based method was utilized to find and align LC-MS peaks. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and optimized potential for liquid simulations (OPLS) were used to identify differences in metabolite levels and to reveal useful biomarkers.
Results: We obtained 32 metabolites in all and 23 most significantly altered metabolites were identified. In AA patients, metabolites involved in the biosynthesis of amino acids (L-glutamine, L-methionine, L-ornithine, L-tryptophan, L-phenylalanine, L-tyrosine, L-leucine, L-proline, and L-valine), aminoacyl-tRNA biosynthesis (L-glutamine, L-methionine, L-tryptophan, L-phenylalanine, L-tyrosine, L-leucine, L-proline, and L-valine), and ATP-binding cassette transporters (ABC transporters) pathways(L-glutamine, L-ornithine, L-phenylalanine, L-leucine, L-proline, and L-valine) were higher than normal, while the levels of metabolites involved in TCA cycle pathways(citric acid, succinic acid, and isocitric acid) were lower. Representative chromatograms are shown in Fig.A(AA patient group) and Fig.B(Normal patient group).PCA score scatter plot (Fig.C), PLS-DA score scatter plot (Fig.D), and OPLS score scatter plot (Fig. E) for two components, indicating separation between the AA patient group (A) and the normal group (B).

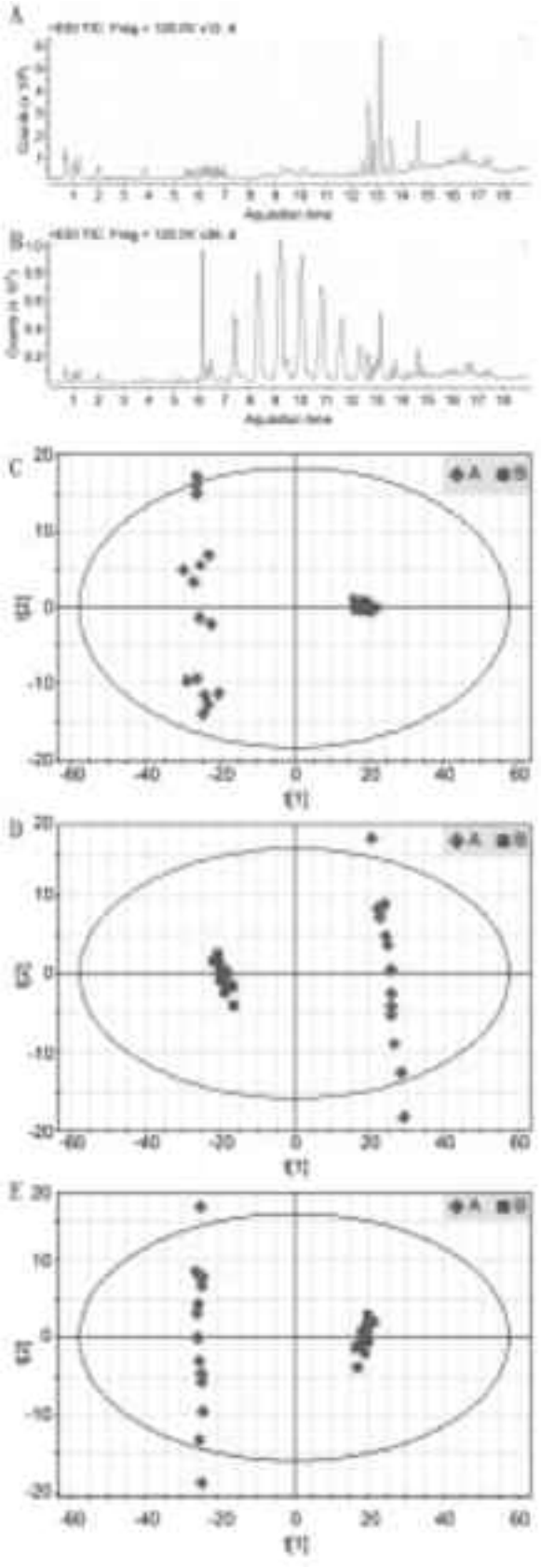

Figure 1.

Summary and Conclusion: In conclusion, our findings indicate that levels of amino acids involved in aminoacyl-tRNA, ABC transporter, and TCA cycle metabolism are aberrant in AA patients in comparison to the normal control. The underlying mechanism for these differences may be related to abnormal of mitochondria function and influence self-renewal and differentiation of HSC, so these changes may be the primary cause to result in bone marrow failure in AA.

P1016

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: CLINICAL EXPRESSION AND RESPONSE TO TREATMENT ARE MODIFIED BY A UNIQUE INTERACTION WITH CO-EXISTING GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired clonal hemolytic disorder that results from a somatic mutation of the X-linked *PIGA* gene, producing deficiency of complement (C)-regulatory proteins in the membrane with consequent hyper-susceptibility of the red cells to lysis by activated C. Glucose 6-phosphate dehydrogenase (G6PD) deficiency is an inherited abnormality of the X-linked *G6PD* gene that makes the red cell hyper-susceptible to oxidative damage with consequent risk of acute hemolysis. The clinical expression of PNH can be influenced by inherited factors: for instance, we have shown recently that a polymorphism of the complement receptor 1 (CR1) gene correlates with the blood trasfusion requirement of PNH patients on the anti-C5 moAB eculizumab (Rondelli *et al*, Haematologica 2014).
Aims: To illustrate, through the first report of a patient who has both PNH and G6PD deficiency, how the two abnormalities can interact.
Methods: Routine blood test, including flow cytometry for GPI-linked proteins. Quantitative enzyme analysis and genetic tests for G6PD deficiency.
Results: A 40yo woman from Sardinia (Italy) originally presented with mild anemia (Hb, 9.5-10.0 g/L), thrombocytopenia (40x10^9/L) and granulocytes at the low end of the normal range; at that time there were no signs of hemolysis and no GPI-negative cells were present. After relative stability for 2 years the patient developed signs of intravascular hemolysis (dark urine, LDH up to 5x upper normal level): the Hb level dropped and there was a high reticulocytosis: 59% of granulocytes were GPI-negative and a diagnosis of PNH was made. Over the following 6 months the size of the GPI-negative granulocyte population increased to 95%, with a parallel increase of intravascular hemolysis; meanwhile, the platelets returned to normal (320x10^9/L). The patient needed a first red cell transfusion, and on the same day she was started on eculizumab. LDH levels promptly returned to normal, but reticulocyte counts remained high (about 250x10^9/L) and transfusion requirement was not abrogated (6 units in the last 6 months). C3 fragment was present on 35% of GPI-negative red cells, despite the fact that the patient had a CR1 genotype associated with good hematological response to eculizumab. The peripheral blood smear revealed marked anisocytosis with spherocytes, macrocytes and hemighosts: a picture consistent with oxidative damage as seen in G6PD deficient patients during a hemolytic attack. In spite of high reticulocytes, the red cell G6PD activity was only about one-half of normal (5 IU/g Hb). DNA analysis revealed heterozygosity for the G6PD Mediterranean (Med) variant. However, mRNA sequence analysis showed that the GPI-negative clone expressed only the G6PD Med allele, suggesting that the PIG-A mutation took place in a stem cell in which the normal G6PD gene was inactive.
Summary and Conclusion: 1) In this patient we have witnessed the evolution from aplastic anemia to PNH – probably the rule rather than the exception; and we have further documented the gradual expansion of the GPI-negative populations, that entailed the correction of thrombocytopenia and neutropenia, but with the payload of increased intravascular hemolysis. 2) In this patient the poor response to eculizumab probably results from a unique interaction, whereby a large red cell population has both the PNH abnormality and G6PD deficiency. We hypothesize that complement activation on the surface of GPI-negative red cells results in oxidative damage, which these red cells, being G6PD deficient, are unable to cope.

P1017

INTERIM REPORT OF THE OPTIMA {OBSERVATION OF GPI-ANCHORED PROTEIN DEFICIENT (PNH-TYPE) CELLS IN JAPANESE PATIENTS WITH BONE MARROW FAILURE SYNDROME AND IN THOSE SUSPECTED OF HAVING PNH} STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) phenotype cells are derived from hematopoietic stem cells with an acquired mutation of the

phosphatidylinositol glycan class A (*PIGA*) gene. In patients with aplastic anemia (AA) or low-risk types of myelodysplastic syndromes (MDS), glycosylphosphatidylinositol-anchored protein deficient (PNH-type) cells are often detected at low frequencies with high-resolution flow cytometry-based methods. Because such bone marrow failure patients possessing increased PNH-type cells are reported to have a good prognosis and show a high response rate to immunosuppressive therapies as opposed to the other patient groups lacking PNH-type cells, detection of these cells may be potentially useful in determining an optimal treatment for patients with bone marrow failure.
Aims: To determine the prevalence and clinical significance of PNH-type cells in bone marrow failure patients, we conducted a nationwide multi-center prospective observational study named OPTIMA.
Methods: From July 2011, Japanese patients with AA, MDS and bone marrow failures suspected of having PNH were prospectively enrolled in OPTIMA. Six laboratories in different universities were assigned as a regional analyzing center. The percentage of PNH-type cells was measured by a high-resolution flow cytometry-based method that was originally established by Kanazawa University. A negative sample and a positive sample containing approximately 0.01% PNH-type cells were periodically sent to the six laboratories without any notice to check the quality of the individual flow cytometry assay. A liquid FLAER method (≥0.003%) and a cocktail method (≥0.005%) with anti-CD55 and anti-CD59 antibodies were used for the detection of PNH-type granulocytes and erythrocytes, respectively.
Results: As of July 2013, flow cytometry data on 1210 cases were collected for this interim analysis. Diagnoses were AA in 386, MDS in 339, PNH in 55, and 430 in undiagnosed bone marrow failure according to the case report forms. Periodic blind validation tests revealed inter-laboratory differences in the PNH-type cell percentage to be always within 0.02%. A total of 457 (37.8%) were found to be positive for PNH-type cells and 148 (12.2%) had ≥1% PNH-type cells. PNH-type cells were detected in 57%, 19.8% and 100% of patients with AA, MDS and PNH, respectively. In a half of patients having ≥1% PNH-type cells, lactate dehydrogenase levels exceeded the ≥1.5×upper limits of normal. Among different subsets of patients with MDS, increased PNH-type cells were detectable in 20 (21.3%) of 94 patients with RCUD, 33 (21.7%) of 152 with RCMD, and 5 (27%) of 22 with MDS-U, but not in any of 14 RARS and 32 RAEB patients.
Summary and Conclusion: A high-resolution flow cytometry-based method that enables the detection of minimal PNH-type cells below 0.01% was successfully transferred from Kanazawa University to other laboratories in Japan. Also, by implementing a uniform protocol to six individual laboratories across the country, a reliable system was established for bone marrow failure patients to undergo the flow cytometry test with equal accuracy in all of these laboratories. Our interim analysis confirmed previous findings that PNH-type cells were exclusively detectable in patients with benign types of bone marrow failure. Further accumulation of cases and prolonged observations are required to determine the clinical significance of the minimal PNH-type cells, especially in terms of their relation to response to immunosuppressive therapy.

P1018

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONES EMERGED AFTER IMMUNOSUPPRESSIVE THERAPY IN PATIENTS WITH SEVERE OR NON-SEVERE APLASTIC ANEMIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hematopoietic stem disorder caused by clonal expansion of a *PIG-A* mutated stem cell, and consequent the defective synthesis of glycosyl phosphatidyl-inositol-anchored proteins. Acquired aplastic anemia (AA) and PNH are closely related bone marrow failure disorders, and PNH often arises from AA. Retrospective studies have mostly investigated severe aplastic anemia (SAA) patients with PNH clones following immunosuppressive therapy (IST) such as antithymocyte globulin and ciclosporin (ATG/CsA) or high dose cyclophosphamide. However, the natural history of PNH clones emerged after IST, especially in non-SAA patients following CsA has not been documented.
Aims: The purpose of this study is to compare the fate and clinical relevance of PNH clones in patients with SAA and non-SAA (NSAA) after IST with ATG/CsA or only CsA.
Methods: A total of 174 patients diagnosed as AA were enrolled in this study from 2008 to 2012. 53 cases of SAA (age at diagnosis 10-65 years, median=39 yers, male 33, female 20) were treated with ATG/CsA, 121 cases of NSAA (age at diagnosis 19-70 years, median=36 years, male 64, female 57) were treated with CsA alone. There were no significant difference in age and gender. All patients were screened for PNH clone pro-treatment and followed-up for 18 to 76 months after treatment, and the median time was 22 months. Peripheral blood PNH cells were detected by lack of CD55 and CD59 expression on red cells and granulocytes by a two-color flow cytometry analysis from 2008 to 2009. Since 2010, PNH cells were defined as CD59 negative erythrocytes and FLAER negative granulocytes, monocytes (CD45/24/14/FLAER). PNH clone size was defined as percentage of GPI-AP deficient granulocytes in whole peripheral blood.
Results: After IST with ATG/CsA or CsA, positive PNH clones were detected in the ten SAA (18.2%) patients, significantly more than that of non-SAA group [nine patients (6.9%), *t=5.0408*, *P=0.025*]. PNH clones emerged in SAA group at 4 months to 65 months (median 7.5 months) after IST, while it was at 2 months to 124 months (median 12 months) in NSAA group (*t=1.118□P=0.279*). The number of AA patients developed with PNH clones in SAA group at 6, 12, 18, 24, and >24 months were 4, 7, 9, 9, 10 respectively, significantly higher than those of non-SAA patients (4, 5, 6, 7, 9, all values<0.05). PNH clone size of SAA group at 6, 12, 24, and >24 months were 13.38%, 14.88%, 20.0%, and 18.85% respectively, also significantly higher than those of non-SAA patients (14.88%, 5.31%, 5.47%, and 9.08%, all values<0.05). 2 patients of SAA group developed typical PNH with intravascular hemolysis during the observation periods 4.5 months and 65 months respectively. In NSAA group, 3 patients developed typical PNH at 12 months, 37 and 76 months respectively. None of the patients developed thrombosis over the period of observation. In SAA group, 80%of patients (8/10) with PNH colne responded to IST, and it was 74.4% (32/43 cases)in patients without PNH clone (*P=1.000*); while 55.6% (5/9) of NSAA patients with PNH clone responded to CsA, 38.9%(44/113) in NSAA patients without PNH clone (*P=0.482*).
Summary and Conclusion: PNH clone can emerge in SAA or NSAA patients after IST with combining with ATG and CsA, or only CsA. More and larger PNH clone developed in patients with SAA than in NSAA patients. PNH clone occurrence did not affect the therapeutic efficacy of IST for AA.

P1019

IDENTIFICATION OF MUTATIONS IN GENES OF THE TELOMERE COMPLEX IN PATIENTS WITH SHORT TELOMERES AND SUSPECTED TELOMEROPATHIES

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Background: Telomeres are involved in cellular replication and senescence. At any given time point, telomere length reflects the balance between the loss of telomere repeats with cell division and the addition of telomere sequences by telomerase. Pathological loss of telomere repeats due to telomerase insufficiency can lead to telomeropathies. The phenotype ranges from mild cytopenias to dyskeratosis congenita (DC), isolated or combined severe bone marrow failure (BMF), lung fibrosis, liver cirrhosis, vasculopathies, bone lesions and brain cysts. The identification of patients (pts) with telomeropathies is relevant to define an adequate therapeutic and monitoring strategy, since such pts are at a higher risk for stem cell deficiencies, malignancies, treatment-related mortality and immunosenescence. Mutations in genes of the telomerase or telomere-associated proteins DKC1, TERC, TERT, TINF2, NOP10, NHP2, TCAB1 and CTC1 have been described as pathogenic.
Aims: We aimed to 1) screen suspected individuals and pts for telomere length (TL), 2) search for mutations in genes of the telomere complex in pts with short telomeres and 3) identify short telomeres and mutations in relatives.
Methods: TL values were determined by automated multicolor flow-FISH (Baerlocher *et al.*, Nat Protoc. 2006). TL values were defined as very low (VL) and low (L) when below the 1st and 10th percentile of age-matched healthy individuals, respectively. Mutations in the genes of the telomere complex were analyzed by Sanger sequencing and/or next generation sequencing using the Ion PGM system with a custom Ion AmpliSeq panel.
Results: TL values were measured in blood samples from 100 pts and 63 relatives. TL values were VL or L in 87 pts and 40 affected or non-affected relatives. 42 pts and 22 relatives were screened for mutations. Three novel mutations were detected in the TERC gene in three pts with severe phenotypes (DC and/or BMF) and in two affected and two non-affected relatives; all of them had VL TL. The mutations were located in the functionally important pseudoknot/template and CR4/CR5 domains. In the TCAB1 gene, three novel mutations were detected in three pts with VL or L TL and BMF. A known pathogenic mutation in TINF2 was identified in a family with two brothers and their father all of whom had DC and VL TL. Fife pts with L and VL TL and various phenotypes carry mutations in the TERT, NHP2 and CTC1 genes, one of whom was compound heterozygous for two mutations in CTC1 and in addition carried a mutation in NHP2 that was also present in two asymptomatic siblings. 45% of the pts carry the G allele of the gene variant TERC n.514A>G (rs2293607) that has been shown to be associated with short telomeres. Furthermore, 25 pts carry at least one of three frequent gene variants in the TERT or TCAB1 gene that have been associated with various tumors.
Summary and Conclusion: We identified three known and nine novel putative pathogenic mutations in genes of the telomere complex in pts with L or VL TL, confirming the genetic basis of the suspected telomeropathy. In addition, we found a high frequency of gene variants associated with L TL values or various cancers. A careful diagnostic work-up including telomere length measurement and mutation analysis is useful to establish the diagnosis of hereditary telomeropathies, and close monitoring and adequate treatment strategies are highly recommended for such pts and their relatives.

P1020

INCREASED BONE MARROW (BM) PLASMA LEVELS OF SOLUBLE CD30 CORRELATE WITH BM PLASMA LEVELS OF INTERFERON (IFN)-GAMMA, CD4/CD8 T-CELL RATIO AND DISEASE SEVERITY IN APLASTIC ANEMIA

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Background: Immunologic abnormalities in patients with acquired aplastic anemia (AA) include decreased lymphocyte counts, inverted CD4/CD8 ratio and increased IFN-γ-producing T cells in peripheral blood (PB) and bone marrow (BM). CD30, a cell-surface molecule belonging to tumor necrosis factor receptor (TNFR) superfamily, was up-regulated on T cells which exposed to allogeneic antigens, and these $CD30^+$ T cells were the major sources of IFN-γ. Quickly after stimulation, surface CD30 was proteolytically cleaved by metallo proteinases and released into bloodstream as soluble CD30 (sCD30). Therefore, circulating sCD30 was thought to be reflective activation of the immune system.
Aims: The aim of this study was to evaluate the role of CD30 in the pathogenesis of AA.
Methods: Fifty-six AA patients (median age 28 years, 29 males), and twenty BM donors (median age 27 years, 10 males) were enrolled in the study. Of these patients, thirty-two patients were analyzed at diagnosis, and twenty-four patients in complete response (CR) after immunosuppressive therapy (IST) of the combination of antithymocyte globulin (ATG) plus CSA. Written informed consent was obtained from all the enrolled patients. The plasma and culture supernatants were stored at -80°C. The concentrations of sCD30 and IFN-γ were detected by commercially available ELISA kit, according to the manufacturer's instructions. The mRNA levels of CD30 in BMMNCs were detected by real-time PCR. The induction expression of CD30 expression on T-cell subsets from SAA patients and healthy controls were detected by Flow cytometry. Cell sorting was performed by using BD FACS Canto II (BD Biosciences).
Results: BM plasma sCD30 levels were found to be positively correlated with the severity of AA. The median plasma sCD30 levels in healthy controls, Non-SAA, SAA and VSAA patients were as of 30ng/mL, 32ng/mL, 46ng/mL and 62ng/mL, respectively. In addition, sCD30 level in BM plasma was higher than its corresponding PB level in each SAA patient, but healthy controls had comparable levels of sCD30 between BM and PB. We further analysed the mRNA levels of CD30 in BMMNCs by real-time PCR, which also revealed significantly increased CD30 expression in BMMNCs of SAA patients. In the group of SAA patients, BM plasma sCD30 levels were positively correlated with BM plasma IFN-γ levels, and negatively correlated with absolute lymphocyte counts (ALC), absolute neutrophil count (ANC) and CD4/CD8 T-cell ratio. Patients in CR had significantly lower levels of sCD30 than patients at diagnosis. BM samples pre- and post-IST were collected from 6 SAA patients who had responded to IST, and all patients had significant decrease in sCD30 levels after successful IST. *in vitro*, we stimulated T cells with alloantigen and found that T cells from AA patients could be induced to express higher level of CD30 on cell surface, and released more sCD30 and IFN-γ into culture supernatants. Allogeneic stimulated T cells were separated by FACS, and $CD30^+$ and $CD30^-$ T cells were separately cultured for 2 days. Culture supernatants of $CD30^+$ T cells contained significantly more IFN-γ than that of $CD30^-$ T cells both in healthy controls and SAA patients. When evaluated the cell-surface expression of CD30 on T-cell subpopulations, we found $CD4^+$ T cells from SAA patients and healthy controls expressed comparable level of cell-surface CD30, but $CD8^+$ T cells from SAA patients expressed significantly higher level of cell-surface CD30 than healthy controls. CsA significantly inhibited CD30 expression by $CD3^+CD8^-$ and $CD3^+CD8^+$ T cells from SAA patients and healthy controls.
Summary and Conclusion: Taken together, CD30 seems to be an important molecule that participates in mediating overproduction of IFN-γ in AA. The associations between sCD30 and ALC and CD4/CD8 T-cell ratio in AA warrant further study.

P1021

EVALUATION OF GENETIC TESTING FOR FANCONI ANAEMIA: FROM SANGER TO NEXT GENERATION SEQUENCE ANALYSIS

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Background: Fanconi anaemia (FA) is a rare inherited disorder that leads to bone marrow failure, physical abnormalities and increased malignancy risk. Physical abnormalities present in up to 75% cases include short stature, abnormal skin pigmentation, malformations of limbs and organs and developmental delay. In most populations FA affects ~1:360,000 births, with a ~1:300 carrier frequency and autosomal recessive inheritance for all genes except *FANCB* which is X-linked recessive. Sheffield Diagnostic Genetics Service has offered genetic analysis for FA since 2009. Analysis included Sanger sequencing of *FANCA, FANCC* and *FANCG* plus dosage analysis of *FANCA* using multiplex ligation-dependent probe amplification (MLPA). Next generation DNA sequencing (NGS) is now being introduced for all 16 known FA genes.
Aims: To evaluate the outcome of service provision to date to provide a comparator with the new NGS service.
Methods: All previously reported index cases (IC) and their relatives' records were examined for referral reason, genes analysed and mutations detected. Where requested, IC were sequentially analysed. *FANCA* was analysed by Sanger sequencing followed by dosage analysis to detect large deletions/duplications. This was followed by Sanger sequencing of *FANCC* and *FANCG*. For NGS validation purposes, in five previously analysed IC the FA-associated genes sequenced before were re-investigated. In a further 2 IC in whom no mutation had previously been identified, all 16 FA-associated genes in the TruSight Inherited Cancer Panel (Illumina) were analysed. In all IC investigated by NGS, sequence variants identified were compared with those previously documented by Sanger sequencing.
Results: Among 39 families referred, 35 IC required diagnostic confirmation of FA, 2 sought mutations where the IC was unavailable, 1 required only *FANCA* MLPA and 1 was referred to confirm research findings. Complementation suggested *FANCA* in 3 and *FANCC* in 1 IC, confirmed by mutation analysis. Pending bone marrow transplant was amongst referral reasons for 3 IC. In 15/34 IC, *FANCA* PCR/sequencing identified 2 mutated alleles but only 1 mutation in 2 IC. Subsequent *FANCA* MLPA in 14 IC confirmed 2 homozygous large deletions and identified a heterozygous deletion in a further IC. 8/16 IC were homozygous for *FANCA* mutations, 7 compound heterozygous and 1 simple heterozygous. 11 IC were subsequently analysed for *FANCC* mutations and 2 homozygous and 1 heterozygous mutations were identified. Lastly 6 IC were analysed for *FANCG* mutations and 1 homozygous mutation was found. Overall, of 35 IC, 18 had mutation(s) identified that confirmed their diagnosis of FA (51%), but in 2 IC, only a single heterozygous mutation was identified and although reversion may have occurred, revertant mosaicism was not observed. 11 IC were homozygous for their mutations and 7 compound heterozygous. After analysis of all 3 genes, 4 of 5 IC remained mutation negative. Parents of IC were analysed for mutations in 5 families and mutations identified in all; additionally of 11 other relatives analysed, 9 were heterozygous and 2 homozygous affected. TruSight NGS analysis of 7 IC confirmed 100 previously identified sequence variants and mutations (52 unique variants) and identified a homozygous *BRIP1* mutation in one IC and a putative heterozygous splice mutation in *FANCI* in another. Sanger sequencing was used to confirm the identified mutations and to fill 7 small gaps in sequence coverage.
Summary and Conclusion: Previous Sanger sequencing used sequentially on FA IC identified both mutations in only 51% of 35 IC referred. However in 19 IC, only *FANCA* sequencing was requested. The analysis process was slow where a mutation was not originally identified in the *FANCA* gene, with turnaround times (TAT) of 8 weeks/gene. Recent introduction of NGS with an initial 12 week TAT will speed up analysis and should identify mutations in a higher proportion of referred cases where phenotypic diagnosis is sound.

P1022

DIAMOND-BLACKFAN ANEMIA IN RUSSIAN FEDERATION

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Background: Diamond-Blackfan anemia (DBA) is an inherited bone marrow failure syndrome with clinical and genetic heterogenity. It was shown that mutations or deletions of ribosomal proteins genes responsible for desease development.
Aims: To analyze clinical and genetic data from DBA patients in Russian Federation.
Methods: We analyzed retrospective data from 61 cases of DBA (32 male, 29 female) born in Russian Federation in a 20-year period (1993-2013). Direct sequence of 7 ribosomal proteins genes (RPS19, RPS10, RPS24, RPS26, RPL5, RPL11, RPL35a) and transcription factor GATA1 were performed for 30 unrelated probands and their first degree relatives. All coding regions and exon-intron junction sites were studied.
Results: Congenital malformation were observed in 41 patients. The clinical manifestation as severe normochromic hyporegeneratory anemia with normal platlet count on the first month of life was detected in 46 cases, at the age of 4 months - in 11 cases, and at the age of 8 months - in 4 patients. DBA was diagnosed at the age of 1-3 months in 58 cases. Up to 1 year of life 48 patients received regular blood transfusions to keep Hb level 95-120 g/l. Steroid therapy was initiated at the age of 6-8 months in 13 cases. Initially responded to steroid therepy 26 patients. Two non-responded to steroid therapy patients achived spontaneous remission at the age of 15 and 18 years.Transient response was observed in five patients, including one child who was successfully transplanted. All our DBA cases were sporadic. Different mutations in RPS19 were detected in 11 cases (one - compound heterozigote), RPS10 - 1 case, RPS24 - 1 case, and RPS26 - 1 case. Siblings and proband's parents had not have any abnormalities in RPs genes studied.

Summary and Conclusion: The most frequent abnormality was found in RPS19 gene (36,7% of cases) in our cohort of patients.

P1023

TELOMERASE ENZYME ACTIVITY IN EGYPTIAN CHILDREN WITH E ANDBONE MARROW FAILUR RESPONSE TO IMMUNESUPRESSIVE THERAPY

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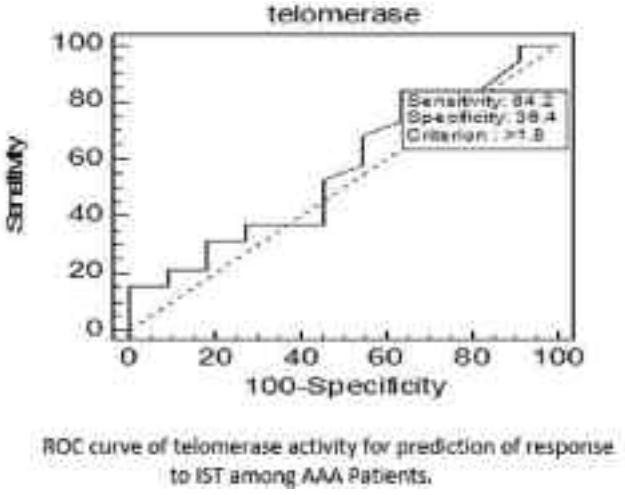
Background: Maintenance of the integrity of telomeres requires the telomerase ribonucleoprotein complex. Abnormal telomere maintenance is a feature of a variety of human diseases including constitutional aplastic anemia. Predisposition to the development of marrow failure has been conferred by genetic alterations results in low telomerase activity, short telomeres in leukocytes and reduced hematopoietic function.
Aims: To evaluate the telomerase functional activity in Egyptian children with inherited and acquired bone marrow failure. The relation of the severity of the acquired disease to telomerase enzyme activity and response to immunosuppressive therapy was studied.
Methods: A case-control study conducted on unrelated children (n=40) with bone marrow failure syndromes (BMFS) and forty healthy age and sex-matched controls. The diagnosis of bone marrow failure was based on the blood-count and bone marrow biopsy criteria of the International Agranulocytosis and Aplastic Anemia Study. Response to immunosuppressive therapy (IST) was evaluated after 6 months of initiation of therapy; complete response (CR) was considered if neutrophil count was >1.5×10^9/L, platelet count >100×10^9/L, and a hemoglobin level >11.0 g/dL. Telomerase activity was measured in mononuclear cells utilizing the Telomeric Repeat Amplification Protocol (TRAP) using the TeloTAGGG Telomerase PCR ELISA.
Results: Patients were acquired aplastic anemia (n=30), Fanconi anemia (n=6). Pure Red Cell Aplasia (n=2), dyskeratosis congenita one case and constitutional aplastic anemia one case. The Mean age was 11.1±4.9 years (range 3.5 to 18 years, median 11 years) and the duration of follow-up mean; 5.14 (±3.84) years (range 1-13 years). Patients with acquired aplastic anemia (n=30) received treatment with cyclosporine A monotherapy (CSA) (n=27) or cyclosporine A and ATG (n=3). The median telomerase activity was significantly lower in inherited BMF syndromes when compared to controls [5.05% (4.60 – 8.70 IQR) *vs* 11.15% (5.90-16.60 IQR), p=0.04]. In AAA the median telomerase activity was insignificantly lower than controls, p=0.228]. Inverse correlation was detected between the telomerase activity and age of the patients ***(r=-0.39, p=0.026),*** but no correlation was found between the telomerase activity and disease duration in hereditary or AAA (r=-0.303, p=0.111 and r=0.305, p=0.156 respectively). In AAA patients 7/30 (23%) had below normal telomerase activity, 6/7 (86%) had severe or very severe disease, 3/7 (43%) responded partially or completely to CSA 7-10mg/kg/day. Twenty seven patients with AAA received cyclosporine therapy, 19/27 (70.4%) were responders *versus* zero % of patients received CSA & ATG (n=3). The median telomerase of responders was 16.5 ± 4.7 % *Vs.* 11.6 ±3.8% of none responders ***(p=0.6).*** sensitivity and specificity of telomerase activity in predicting response of AAA subjects to IST at different cut off values by ROC curve found that area under the curve of telomerase was 0.569 (95% CI 0.377 to 0.748; p=0.540); indicating that the overall predictability of telomerase activity is not significant and on fixing the sensitivity or specificity of telomerase activity, we found that either its sensitivity or specificity became unsatisfactory; making its adoption as a good predictor of response of AAA subjects to IST unlikely.



ROC curve of telomerase activity for prediction of response to IST among AAA Patients.

Figure 1.

Summary and Conclusion: Telomerase activity was low in all hereditary BMFS and in 23% of AAA patients. Evaluation of telomerase activity might not be essential for therapeutic or prognostic aspects of AAA patients. However, It might be useful for selection of stem cell family donors in patients with AAA and telomerase deficiency. Cyclosporin A can be used as a monotherapy in the treatment of AAA even in the presence of decreased telomerase activity

P1024

DECREASED BONE MARROW (BM) LEVELS OF IL-12P40 CORRELATE WITH DISEASE SEVERITY IN ACQUIRED APLASTIC ANEMIA (AA)

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Background: Acquired aplastic anemia (aAA) is an autoimmune disease in which various pro- and anti-inflammatory cytokines play crucial pathogenic roles. These cytokines either can inhibit hematopoiesis directly or can enhance the pro-inflammatory function of autoreactive T cells. Wellknown pro-inflammatory cytokines which were found to be increased in aAA included IFN-γ, TNF-α, IL-2, IL-6, IL-8, IL-12p70, IL-17 and IL-23. Among anti-inflammatory cytokines, TGF-β1 was found to be decreased in AA, while IL-10 and IL-11 serum levels were comparable between aAA patients and healthy controls. Elevated plasma levels of IL-12p70 and IL-23 have already been reported in AA, however, IL-12p40, a natural antagonist of IL-12p70 and IL-23, has not been determined in AA.
Aims: The aim of our study was to evaluate BM plasma IL-12p40 levels in patients with aAA.
Methods: BM plasma samples of 18 newly diagnosed aAA patients (median age 29, range 12-43 years, 7 males), 10 newly diagnosed MDS patients (median age 31, range 19-45 years, 4 males) and 11 healthy subjects (median age 28, range 18-47 years, 5 males) were analysed. All patients provided written informed consent. The BM plasma was stored at -80□. The concentrations of IL-12p40 were detected by commercially available ELISA kit, according to the manufacturer's instructions.
Results: In newly diagnosed AA patients, we found BM plasma IL-12p40 levels were decreased in AA patients and correlated with disease severity. The median plasma IL-12p40 levels in healthy controls, Non-SAA, SAA and VSAA patients were as of 120±17pg/mL, 100±18pg/mL, 85±16pg/mL and 80±6pg/mL, respectively. In addition, we found significant elevated IL-12p40 (168±41pg/mL) levels in MDS patients.
Summary and Conclusion: The influence of IL-12p40 on the immune response may play crucial pathogenic role in the pathogenesis of aAA and MDS. Further studies are investigating why IL-12p40 expression is changed in aAA and MDS, and how the changed IL-12p40 will impact the immune cell of patients.

P1025

INCREASED INCIDENCE OF TRISOMY 8 IN APLASTIC ANEMIA PATIENTS COMBINED WITH BEHCET'S DISEASE COMPARED TO ACQUIRED APLASTIC ANEMIA: A SINGLE INSTITUTE EXPERIENCE

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Background: Behcet's disease (BD) is a disease of unknown cause which is clinically characterized by recurrent oral and genital ulcers, uveitis, polyarthritis, gastrointestinal involvement, and vasculitis. The prevalence of BD is reported to be higher in eastern Asia and Turkey compared to western countries. There is increasing number of case reports of which show BD associated with bone marrow failure syndromes such as myelodysplastic syndromes (MDS) or aplastic anemia (AA) accompanied with trisomy 8 mostly from eastern Asia. It is not clear that trisomy 8 is whether uniquely increased in BD patients or if it is only a chromosomal result which can be observed in a series of aplastic anemia.
Aims: Therefore, we investigated bone marrow results and chromosomal charactertistics of BD patients who underwent bone marrow studies due to various degree of cytopenia.
Methods: The medical records of Behcet's disease patients who visited the Hematology clinic of Severance Hospital from January 2000 to December 2013 were reviewed. The diagnosis of Behcet's disease was made according to the diagnostic criteria of international Behcet's disease study group or Japanese group. After review, total 40 patients, whose reason for bone marrow was peripheral cytopenia of any lineage, were selected. To compare chromosomal aberrancy pattern with acquired aplastic anemia, chromosome results of 159 aplastic anemia patients were retrospectively collected. During the bone marrow study, 10ml of bone marrow blood was aspirated and mononuclear cells from bone marrow blood were separated and cultured for 24 to 48 hours.

Chromosome analysis was done after the preparation for methaphase and Giemsa-banded. Meaningful chromosomal abnormality was defined as at least 2 cells showing same structural aberrancy such as chromosome rearrangement or extra-chromosome or loss of chromosome.
Results: Among 40 patients, 24 patients got specific hematologic diagnosis. Fifteen patients were diagnosed of AA and 2 patients were diagnosed of MDS. Three patients diagnosed of acute myeloid leukemia and 3 patients were diagnosed of immune thrombocytopenia, Chronic myelomonocytic leukemia, hemophagocytic lymphohistiocytosis and NK/T cell lymphoma was diagnosed in each 1 patient. Among 15 BD+AA, 5 showed trisomy 8 (30%) and among 1 BD+nonAA patients 1 showed trisomy 8 (4%, P=0.021). In contrast, among 152 acquired AA patients without symptoms and signs of BD, trisomy 8 was found in 3 patients, and trisomy 9 was found in 2 patients. There was statistically high incidence of trisomy 8 in BD+AA compared to BD+nonAA or acquired idiopathic AA. Intestinal involvement was more frequently observed in BD patients associated with AA of MDS (29.2% *vs*. 50%, P=0.159).
Summary and Conclusion: Trisomy 8 is frequently reported in BD patients showing asplastic feature compared to BD patients without AA or acquired idiopathic AA. The clinical meaning of trisomy 8 should be further determined in BD patients with bone marrow failure.

Myeloproliferative neoplasms - Clinical 2

P1026

PRIOR THROMBOSIS AND CLINICO-BIOLOGICAL CHARACTERISTICS AT DIAGNOSIS IN THE PATIENTS OF THE THROMBOCYTHEMIAS ITALIAN REGISTRY: IS OLDER AGE A PRIMARY OR A SURROGATE PRO-THROMBOTIC FACTOR?

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Background: The identification of the thrombotic risk factors in the Ph-negative MPN is still controversial, due mainly to the heterogeneity of diagnostic criteria and to the potentially confounding effects of the treatments.
Aims: This study in thrombocythemic patients reclassified according to the WHO 2008 criteria aimed to evaluate the pro-thrombotic effects of the main clinico-biological characteristics at diagnosis, by analyzing the prior thrombosis (PrTh) history, *i.e.* the thrombotic events occurring before the start of any cytoreductive treatment.
Methods: The analyzed patients of the Thrombocythemias Italian Registry (RIT) were 1745 (ET 52.5%, ep-PMF 28.3%, PMF 5.0%, PV 2.1%, U-MPN 12.1%), 678 males and 1067 females, with age at diagnosis >60y (42.5%), 40-60y (35.4%) and ≤40y (22.1%). The patients had at least one general cardiovascular risk factor (CVRF) and at least one comorbidity in 66.9% and 50.0% of cases, respectively. The PLT count (median 757 x 10^9/L) was >1000 x 10^9/L in 18.9%, and ≤700 x 10^9/L (low thrombocytosis) in 38.0% of cases. The WBC count was >10000 x 10^6/L (leukocytosis) in 20.8%, and the HCT higher level (>44% in females, and >47% in males) was observed in 22.3% of cases. Hepatomegaly, splenomegaly, and symptoms were present in 24.7%, 25.4%, and 55.6% of cases. The JAK2 V617F mutation was documented in 58.2% of 888 studied cases.
Results: A PrTh, with a median distance from diagnosis of 7 months, occurred in 320 (18.3%) patients, without difference between ET and ep-PMF (19.1 % *vs* 20.5%, p 0.533). As expected, PrTh were mainly arterial (72.8%), and were related to the thrombosis during the follow-up (p<0.001). In univariate analysis, the PrTh rate was higher in patients with male gender (p 0.006), older age (p<0.001), CVRFs (p<0.001), comorbidities (p<0.001), low thrombocytosis (p<0.001), leukocytosis (p<0.001), HCT higher level (p<0.001), and JAK2 V617F mutation (p 0.003). The figure shows, by a *logit function,* that the increasing age at diagnosis was associated to an increasing rate of prior thrombosis, CVRFs, and comorbidities. The significant relationship between PrTh and patient characteristics at diagnosis was confirmed in multivariate analysis, with exception for older age (p 0.061). Nevertheless, when the covariates CVRFs and comorbidities were excluded, the multivariate analysis documented again a significant relationship between PrTh and older age (p<0.001).

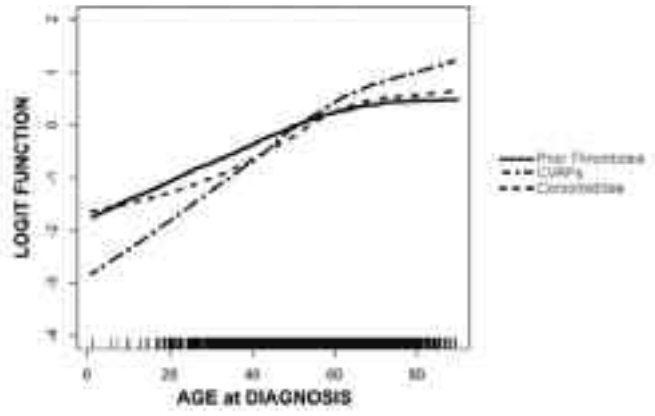

Figure 1.

Summary and Conclusion: PrTh occurrence, in ET as in ep-PMF patients, was related in multivariate analysis to male sex, CVRFs, comorbidities, low thrombocytosis, leukocytosis, higher HCT level, and JAK2 V617F mutation. The older age, whose pro-thrombotic effect appeared *de facto* supported by the CVRFs and comorbidities, seems to be in these patients not a primary but a surrogate thrombotic risk factor. Acknowledgement: this study was partially supported by the Gimema Foundation (promotor of the RIT), and by the AIL Foundation. Umberto Santoro, Rossella Miglio and Paola Monari of the Statistics Dept of the University of Bologna made the statistical analysis. Aurora Rabitti offered the technical assistance.

P1027

PHASE 2 TRIAL OF PRM-151, AN ANTI-FIBROTIC AGENT, IN PATIENTS WITH MYELOFIBROSIS: STAGE 1 RESULTS

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Background: PRM-151 (PRM) is a recombinant form of Pentraxin-2, an endogenous human protein that acts at sites of tissue damage, inducing macrophage differentiation to prevent and reverse fibrosis. PRM has broad anti-fibrotic activity in multiple preclinical models of established fibrotic disease and no dose limiting toxicities in Phase 1 trials.
Aims: This study investigates the potential of PRM in myelofibrosis (MF) to reduce bone marrow (BM) fibrosis and to improve key MF-related disease features including abnormal blood counts, splenomegaly, and symptoms.
Methods: Patients with Intermediate-1, intermediate-2, or high risk MF with grade ≥2 BM fibrosis, either on no current therapy or on a stable dose of ruxolitinib (RUX) for at least 12 weeks were eligible for stage 1 of this open label adaptive trial. Assignment to one of the 4 treatment arms was per investigator and patient choice: PRM IV 1-hour infusion on days 1, 3 and 5, then weekly (QW) or every 4 weeks (Q4W), alone or with continuous oral rux. Primary endpoint is overall response rate by IWG-MRT criteria (spleen by palpation). A decrease in BM fibrosis by ≥ 1 grade with otherwise stable disease is also considered a response. BM biopsies are obtained at baseline, 3 and 6 months, read by local pathologists and a blinded central reviewer. All subjects signed informed consent.
Results: 27 subjects were enrolled in Stage 1: 7 PRM QW, 7 PRM Q4W, 7 PRM QW + RUX, 6 PRM Q4W + RUX. As of January 13, 2014, 15 and 6 subjects have completed 8 and 12 wks of treatment, respectively (none have yet completed 24 wks). There was no apparent treatment-related myelosuppression, and among all AEs, only herpes labialis and anemia occurred in >1 subject. There have been 4 serious adverse events in 3 subjects: 1 with abdominal pain, 1 with hypoxia, and an 85 year old subject who died from gastroenteritis and pneumonia. Improvement in bone marrow fibrosis (Grade 3 to 1; Grade 2 to 0; read by a local pathologist) was observed in 2 of 6 subjects at the 3-month BM mark (1 on PRM Q4W, 1 on PRM Q4W + RUX).
Summary and Conclusion: In stage 1 of this phase 2 trial of MF patients, PRM has shown good tolerability both alone and in combination with RUX. Updated safety and efficacy data on all 27 subjects will be presented at the meeting, including central BM (3 and 6 month) reviews.

P1028

PKC412 (MIDOSTAURIN) IS SAFE AND HIGHLY EFFECTIVE IN SYSTEMIC MASTOCYTOSIS PATIENTS: FOLLOW UP OF A SINGLE-CENTER ITALIAN COMPASSIONATE USE

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Background: Mastocytosis is a myeloid neoplasm characterized by abnormal accumulation and frequent activation of mast cells (MCs) in various organs. The recent WHO classification (2008) includes an indolent form (ISM), an aggressive form (ASM) and a leukemic subvariant (MCL). The *c-kit* mutation D816V is detectable in most adult patients with SM. Treatment of SM usually focuses on symptom relief by histamine receptor antagonists and other supportive therapy. However, in aggressive and leukemic variants, cytoreductive and targeted drugs must be applied.
Aims: To evaluate safety profile (adverse events) and efficacy (remission rates and quality of response) of a cohort of ASM patients, treated with PKC412.
Methods: From 2008, 22 patients (male/female=11/11) affected by SM have been referred to our Institution. Among these, 12 (55%) presented with systemic symptoms associated with signs of organ involvement (skeletal lesions, ascites, liver function impairment or bon marrow disfunction), thus identifying an ASM. Therefore, since a first line therapy (IFNalfa, Imatinib and 2CdA in 56%, 11% and 33% of the patients, respectively) and supportive care with histamine receptor antagonists weren't followed by a significant benefit, a personalized use of PKC412 was asked and obtained for 9 out of 12 ASM patients. Thus, from March 2011 9 (M/F=3/9) patients with ASM have been treated with PKC412, administered orally, at the dosage of 100 mg twice daily, without rest periods. The median age was 60 years (range 39-75); the median time from diagnosis was 6 months (range 2-53). Median serum tryptase level was 100 mcg/L(range 19.3-1160). *C-kit* mutation D816V was present in 8 out of 9 patients. Cytogenetic analysis was normal in all the patients.
Results: Seven out of nine patients were evaluable for response. The median duration of therapy was 517 days (range 327-970+). According to European Criteria, a Major response was observed in one patient, and a partial response in 6 patients. Overall, the drug was well tolerated, and no serious adverse events were observed. All the patients obtained a quick and prolonged improvement of clinical symptoms, in terms of weight gain, bowel function and skeletal pain. At the bone marrow evaluation, the persistence of the D816V *c-kit* mutation was observed, despite a significant decrease of mast cell marrow involvement.
Summary and Conclusion: In a small cohort of ASM patient, the prolonged therapy with PKC412 is safe and effective, mainly on symptoms improvement and haematological profile. Nevertheless, the persistence of the D816V c-kit mutation, despite significant responses, suggests that many other oncogenic factors may be responsible for the pathogenesis of the disease. UDS approaches are needed in order to clarify this issue. Acknowledgments. Work supported by European LeukemiaNet, AIRC, AIL, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

P1029

DIAGNOSTIC ACCURACY OF SERUM EPO LEVEL AND JAK2V617F ALLELE BURDEN IN POLYCYTHEMIA VERA

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Background: The diagnosis of polycythemia vera (PV) is based on criteria defined by the World Health Organization (WHO 2008) with serum erythropoietin (EPO) level below the reference range being a minor criterion. The WHO EPO criterion was established based on studies performed before the description of *JAK2*V617F mutation.
Aims: To evaluate the diagnostic accuracy of serum EPO and *JAK2*V617F allele burden as surrogate markers of PV.
Methods: Serum EPO level at diagnosis was evaluated in 287 patients, 99 of them with untreated PV, 137 with essential thrombocythemia (ET) and 51 with non-clonal erythrocytosis (NCE). Patients were diagnosed at a single center between 1995 and 2013. Before May 2006 serum EPO levels were measured by radioimmunoassay (RIA) whereas after that date it was measured by electrochemiluminescence (ECL). ECL was the technique employed in all patients with NCE. *JAK2*V617F allele burden was measured by quantitative allele-specific PCR. ROC curves were performed to evaluate the diagnostic accuracy of serum EPO level and *JAK2*V617F quantification as markers of PV. The area under de curve (AUC), the sensitivity and the specificity for EPO level and *JAK2*V617F was calculated in the whole cohort of patients and in three different clinical settings: study of erythrocytosis, confirmed myeloproliferative neoplasm (MPN) and *JAK2*V617F-positive MPN. The sensitivity and specificity

values of serum EPO level corresponded to the lower limit of normality whereas for *JAK2*V617F quantification an arbitrary cut-off based on the value that provides the highest specificity ensuring a sensitivity >80% was established.
Results: Median EPO values measured by RIA were 7.7 mUI/mL and 15.1 mUI/mL in PV and ET, respectively (p<0,001) whereas according to ECL method the corresponding values were 2.3 mUI/mL, 7.1 mUI/mL and 8.4 mUI/mL in PV, ET and NCE, respectively (p<0,001). Serum EPO level had a good diagnostic accuracy as a marker of PV as shown in the table. The EPO level measured by ECL showed a better diagnostic accuracy than RIA (AUC for ECL: 0.91, 0.84, 0.80 in erythrocytosis, confirmed MPN and *JAK2*V617F-positive respectively). The *JAK2*V617F showed an excellent diagnostic accuracy as a marker of PV being the AUC 0.98, 0.93, 0.91 in erythrocytosis, myeloproliferative neoplasms and *JAK2*V617F-positive groups respectively.

Table 1.

	No. of patients	AUC	Sensitivity	Specificity
JAK2V617F-positive MPN	194			
EPO RIA	77	0.710	11%	97%
EPO ECL	107	0.833	82%	79%
JAK2V617F	194	0.907	89%	85%
Confirmed MPN, irrespective of JAK2 status	239			
EPO RIA	109	0.765	28%	99%
EPO ECL	130	0.837	82%	79%
JAK2V617F	223	0.851	82%	82%
Study of erythrocytosis	133			
EPO RIA	NA	NA	NA	NA
EPO ECL	87	0.853	85%	89%
JAK2V617F	118	0.983	88%	96%
Whole cohort of patients	287			
EPO RIA	109	0.759	28%	99%
EPO ECL	176	0.869	82%	85%
JAK2V617F	274	0.949	82%	94%

JAK2V617F positive MPN included patients with PV and ET. Confirmed MPN included all patients with PV and ET irrespective of JAK2V617F status. Study of erythrocytosis included patients with hematocrit > 48% and >52% in men and women, respectively. RIA: radioimmunoassay; ECL: electrochemiluminescence. The sensitivity and specificity values corresponded to the lower limit of normality of serum EPO according to each technique. The sensitivity and specificity values of JAK2V617F quantification corresponded to an arbitrary cut-off set at 35% selected in the group including the whole cohort of patients based on the value that provides the highest specificity ensuring a sensitivity > 80%.

Summary and Conclusion: The quantification of the *JAK2V617F* allele burden has a better diagnostic accuracy than EPO serum level as a marker of PV.

P1030

CALR MUTATIONS IN ESSENTIAL THROMBOCYTHEMIA: INCIDENCE, CLINICAL UTILITY AND MOLECULAR DIAGNOSTIC BY HIGH RESOLUTION MELTING

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Background: Around 50-60% of patients with primary myelofibrosis (PMF) and essential thrombocythemia (ET) carry *JAK2* V617F mutations. Mutations in *MPL* have been also described in these neoplasms in 5-10% *JAK2* V617F negative patients. Recently, mutations in calreticulin gene (*CALR*) were described in *JAK2* and *MPL* unmutated cases of ET and PMF, and its clinical, cytogenetic and molecular implications are a challenge.
Aims: We studied the presence of *CALR* mutations in a series of patients with ET and thrombocytosis without *JAK2/MPL* mutations. Among ET patients, we compared clinical, demographic and molecular features of *CALR* mutated *versus JAK2/MPL* mutated subjects.
Methods: In this retrospective study, *CALR* mutation analysis was carried out in patients with wild type *JAK2* and *MPL* in 34 ET, 21 persistent thrombocytosis suggestive of myeloproliferative neoplasia (MPN) and 98 thrombocytosis secondary to other causes and clearly not MPN. We compared clinical characteristics of *CALR* positive ET patients with 45 *JAK/MPL* mutated patients. The diagnosis of ET was according to the 2008 World Health Organization criteria. *CALR* mutation analysis was performed through high resolution melting (HRM) and direct Sanger sequencing. For the statistical analysis, patient's characteristics were compared with the use of chi-square test or Fisher's exact test for categorical variables and t-test or Mann-Whitney U test for continuous variables. All analyses were two sided, and significance was set at a p-value of 0.05. SPSS statistical package (v. 15.0) was used.
Results: All HRM positive samples were confirmed by sequencing analysis. In our series of *JAK2/MPL* negative patients, *CALR* mutations were present in 44% (15/34) of ET, 14.3% of persistent thrombocytosis suggestive of MPN, and in none of the thrombocytosis unrelated to MPN (0/98). The statistical analysis showed that in ET patients: 1) *CARL* mutations were associated with lower hemoglobin levels compared to *JAK2* mutations (p=0.004). 2) *CARL* mutations presented higher platelet count than *JAK2* mutations (p=0.001). 3) Patients carrying *CALR* mutations were younger, although this association was not statistically significant (median 53.33 years for *CALR*+ and 60 years for *JAK2*+, p=0.146).
Summary and Conclusion: a) Results from the present study indicate that HRM method is an accurate, sensitive and rapid method for analyzing *CALR* mutations. b) Mutations were detected in 44% of ET patients with wild type *JAK2/MPL* which is a lower incidence than the one published in other studies. c) As has been described, *CALR* mutations were significantly associated with higher platelet count and normal hemoglobin levels, and marginally associated to younger age of diagnosis. No differences in thrombotic risk were observed in our series.

P1031

POMALIDOMIDE IN MYELOFIBROSIS: LONG TERM FOLLOW UP AND CORRELATION WITH JAK2V617F AND CALR MUTATIONS

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Background: Myelofibrosis (Primary myelofibrosis (PMF), post-polycythemia myelofibrosis and post-thrombocythemia myelofibrosis) are the commonest BCR/ABL negative myeloproliferative neoplasms without good treatments. Risk stratification is useful in identifying patients with shorter survival; and help in deciding who may benefit with medical intervention and/or enrolled to clinical trials. Immunomodulatory therapies have some utility in alleviating transfusion need. Previously we have reported the phase II clinical trial of pomalidomide in myelofibrosis (*Leukemia*. 2011;25(2):301-304)[1].
Aims: To evaluate the long term outcome of pomalidomide in myelofibrosis; and to see if there is any correlation with the newly described mutations.
Methods: After obtaining IRB approval, the data base of patients who received pomalidomide was updated till January 2014. All patients who received pomalidomide for at least one cycle without regard to dose were included.
Results: Ninety four patients took pomalidomide during phase I and II studies at the Mayo clinic from May 2007 to January 2010. The median age was 67 years (range 37-87) and males were 61 (65%). Primary myelofibrosis, post-polycythemic and post-thrombocythemic myelofibrois constitute 73 (77%), 10 (11%), and 11 (12%) of the cases respectively. Thirty six (38%) were in intermediate 2 and 57 (61%) in high risk group according to Dynamic International Prognostic Scoring System Plus risk stratification. Twenty five did have unfavorable karyotype. JAK2*V617F* mutation was identified in 71 (76%), CALR mutation in 5 (5%), and 3 double negative. The anemia response rate was 31 % (29/94), with median duration of response of 21 months (range: 5.5-71 months). Twenty four, two and one of the anemia responders were JAK2*V617F* and CALR positive, and double negative respectively (p=0.2). Five patients maintained their anemia response for more than 4 years; and currently there are 4 patients on treatment. Three of them are JAK2*V617F* positive and one of them CALR positive. Four out of 5 the long term responders did have spleen size less than 10 cm. Sixty three (67%) patients died since the start of the clinical trial. Leukemic transformation was documented in 8 (8 %) patients.
Summary and Conclusion: Pomalidomide may improve anemia in some patients with myelofibrosis, especially those with JAK2*V617F* positive and minimal enlarged spleen. Though the sample size is small, only patients with either JAK2*V617F* or CALR mutation showed long term anemia response to pomalidomide.

P1032

YOUNG AGE IS NOT PROTECTIVE AGAINST THROMBOSIS IN PATIENTS WITH POLYCYTHEMIA VERA

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Background: PV is a chronic myeloproliferative neoplasm affecting people in median/advanced age. Only 4-7% of cases are recognized before the age of 40 and little is known regarding this setting of patients. Thrombosis is the major cause of morbidity and mortality in PV, with an estimated incidence in younger patients of 1.8/100 patients/years.

Aims: The aim of our study is to evaluate in young PV patients the thrombotic risk and its relationship with previous thrombotic event.
Methods: 236 patients with PV diagnosed between 18 and 40 years were studied in 9 Italian Hematological Centers. The PVSG diagnostic criteria were used before 2001 while those of WHO were used later. In most cases, older diagnosis were confirmed with WHO criteria. The study was approved by the institutional review boards at each institution. Nominal variables were compared with the X^2 test. Thrombosis free survival curves were prepared by the Kaplan-Meier method and were compared by the log-rank test. The Cox proportional hazard regression model was used for multivariable analysis. *P* values less than 0.05 were considered significant.
Results: Baseline clinical and laboratory data of PV patients are summarized in the Table.

Table 1.

Tot patients	236
Males/females	154/82
Age (y)	33.5±5.46
Median follow-up (y)	8.95
WBC x 109/L	10.75±4.48
Hb g/L	17.94±2.27
Ht %	53.77±6.62
Platelets x 109/L	529.88±270.24
JAK2V617F/exon 12/WT (available in 171 pats)	143/11/17
thrombotic events at diagnosis	31 (13%)
thrombotic events during follow up	39 (16.5%)

Sixty-three patients out of the 236 evaluated cases (28%) suffered for at least one thrombotic event (incidence 3.8/100 pts/y). In 31 patients (13%) the thrombotic event, 12 in arterial (5 coronary, 5 cerebral, 1 peripheral and 1 splanchnic) and 19 in venous (15 splanchnic and 4 peripheral) vessels occurred at diagnosis. Thirty-nine patients (16.5%) developed a thrombotic event during follow-up: 7 patients had a re-thrombosis (18%), while 32 (9 coronary, 5 cerebral, 2 peripheral artery thrombosis and 5 DVT, 10 splanchnic and 1 cerebral veins thrombosis) did not. The incidence of thrombotic complications during follow-up was similar comparing patients with or without a previous thrombotic event (p=0.64) and no statistical differences were found in parameters at diagnosis between the two groups.
Summary and Conclusion: In the present study, the incidence of thrombotic complications in PV in young age is not different compared to all PV population, estimated 12-39%. The rate of thrombotic complications is similar to that showed in an old French study (25.8%, Najean 1987) but lower than the one found in a small Italian (68%, Frezzato 1993) and in a small Spanish study (58.3%, Perea 2001). Our data confirm that young age per se is not protective against thrombosis in PV patients and that thrombosis as presenting feature of PV seems not to determine a different thrombotic risk during follow-up, in agreement with the observation of Passamonti (Haematologica 2003) in patients younger than 50 years. Therefore, thrombotic risk criteria used for ET seem not to be useful in PV. We think that low dose aspirin may be used in young patient with a suspicion of PV, even during diagnostic evaluation to avoid the risk of thrombosis and that phlebotomies has to be associated as soon as possible. Prospective study may confirm our data.

P1033

AN INTERNATIONAL ASSESSMENT OF STANDARD MEDICAL THERAPY ON SYMPTOM BURDEN AMONG MPN POPULATIONS: PRELIMINARY FINDINGS OF THE MEASURE TRIAL

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Background: Traditional myeloproliferative neoplasms (MPNs) are characterized by myeloid lineage cellular over- or underproduction distinct from acute leukemia. Individuals with these diseases, including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) suffer from excessively burdensome symptom profiles and reduced quality of life. Previous pharmacologic and venosection therapies have targeted specific hematologic responses or survival benefit. To date, the effect traditional pharmacologic therapies on symptom burden has been largely uninvestigated. Furthermore, no studies to date have utilized a seven day, serial assessment of patient-reported outcome (PRO) symptom burden to assess MPN symptom response.
Aims: The MPN Experimental Assessment of Symptoms by Utilizing Repetitive Evaluation (MEASURE) Trial aims to assess symptom change among those undergoing standard MPN therapies. It also aims to validate the daily Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) as an assessment of MPN symptom burden over a seven day period.
Methods: The MEASURES trial is a prospective study evaluating the responsiveness of the MPN-SAF TSS in detecting symptomatic changes in target symptoms for an anticipated 180 ET, PV and MF patients initiating new non-experimental medical therapy. MPN therapies include anagrelide, aspirin, busulfan, cladribine, danazol, hydroxyurea, interferon, lenalidomide, melphalan, thalidomide, phlebotomies, prednisone, and ruxolitinib. Patients complete a packet of metrics at the time of enrollment with a second survey sent out or given in-office between 90 and 180 days after the initiation of treatment. Packet items included the MDASI (*Cancer. 2000;* 89:1634-46), EORTC QLQ-C30 (*J Natl Cancer Inst.* 1993; 85: 365-76), Global Impression of Change (*Psychiatry*. 2007; 4(7): 28–37), as well as the MPN-SAF TSS (*J Clin Oncol*. 2012 Nov 20;30(33):4098-103.). The MPN-SAF TSS assesses 10 of the most pertinent and representative MPN symptoms, and in this study is administered in a daily diary format with a 24-hour recall period for seven consecutive days. Participating institutions acquired demographic, laboratory, physical examination and radiographic data, along with serial response assessments.
Results: The study remains in recruitment phase. To date, 37 patients, including 14 international patients, have been enrolled. Recruitment has yielded 22% ET, 26% PV, and 52% MF patients (including 50% PMF, 8% post-ET MF, and 42% post-PV MF). Median age was 72 (range 39-89) and 69% were male. Most common starting therapies included aspirin (n=8), hydroxyurea (n=7), ruxolinitib (n=6), interferon (n=4), phlebotomies (n=2), anagrelide (n=1), thalidomide (n=1), danazol (n=1), and darbopoietin (n=1). To date, three patients were unsuccessful in starting new therapies, one patient is deceased, and post-treatment surveys have been received for twelve participants (4 ruxolitinib, 3 aspirin, 1 busulfan, 1 anagrelide, and 1 prednisone; Figure 1). Average diary MPN-SAF TSS score was 23.2 for pre-treatment and 19.7 for post-treatment. Further data will be presented during the EHA 2014 meeting.

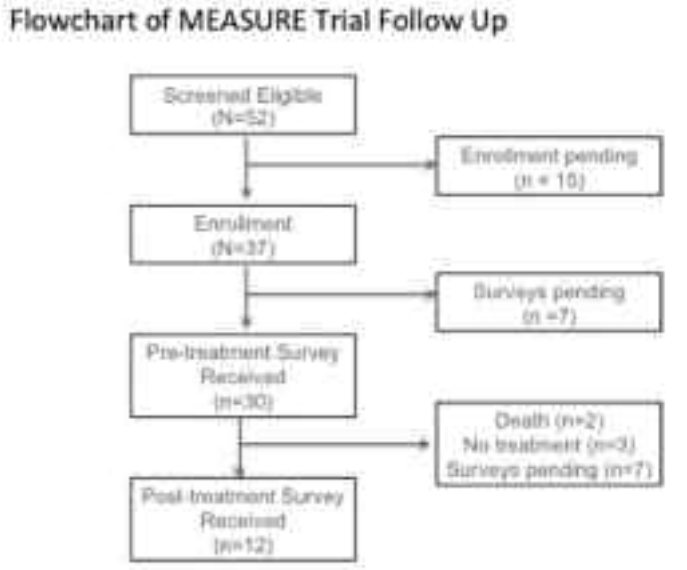

Figure 1.

Summary and Conclusion: Few studies have investigated symptom burden response to standard-of-care pharmacologic and venosection treatments among the MPNs. This study also introduces the daily diary version of the MPN-SAF TSS as a new PRO symptom burden assessment which can quantify symptoms with increased reliability. Although results are still ongoing, few effects of traditional therapies have been observed on symptom response, with the notable exceptions of steroids and ruxolitinib. Future analysis will continue to delineate the effects of traditional MPN therapies on symptom burden.

P1034

CALRETICULIN (CALR) MUTATIONS IN ESSENTIAL THROMBOCYTHAEMIA (ET) AND PRIMARY MYELOFIBROSIS (PMF): SCREENING BY HETERODUPLEX ANALYSIS IS HIGHLY SPECIFIC AND SENSITIVE

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Background: Eight years after the *JAK2*V617F mutation, *CALR* mutations detected in 30% of patients with ET and PMF fill the molecular diagnostic gap in myeloproliferative neoplasms. Assessment of *CALR* mutations advances the current diagnostic approach for ET and PMF and is proposed to be included into the WHO criteria. Mutated calreticulin has an altered c-terminus which is an attractive target for specific treatments, *i.e.* small molecules. More than 50 different deletions and insertions in exon 9 of the *CALR* gene have been described to date, rendering the analysis challenging. Two mutation types are more prevalent: a 52 basepair-deletion (Type 1, 53%) and a 5 basepair-insertion (Type 2, 31%). 20% of mutations are infrequent, most of them being detected only in one patient. In addition to this high diversity of individual mutations, the highly repetitive sequence of exon 9 is another methodological hurdle. Currently, analysis of *CALR* mutations is performed by sequencing or fragment analysis.
Aims: We aimed to develop a fast and specific screening method for *CALR* mutation detection that can be performed with routine laboratory equipment.
Methods: DNA extracted from peripheral blood was amplified by FAST PCR, and PCR products were analyzed by heteroduplex analysis on agarose gels. All samples were sequenced.
Results: To date, 69 samples from *JAK2*V617F-negative patients with ET or PMF were analyzed. 42 patients carried *CALR* mutations of type 1 (n=23), type 2 (n=14) or individual mutations (n=5). Two homozygous mutations were identified. Individual mutations included c.1092-1124del, c.1094_1139del, c.1103_1148del, c.1147_1154delinsTGTC and c.1154_1155insATGTC. Mutant *CALR* was unambiguously represented by 2 or 3 bands while all single bands represented the wild-type sequence. Type 1 and type 2 mutations were identified by a specific pattern. Specificity and sensitivity of our screening assay are 100%, *i.e.* all mutated cases were detected, and none of the negative cases carried mutations. Analysis of more samples is ongoing to identify more rare mutations.
Summary and Conclusion: We present a specific and sensitive screening method for *CALR* mutations that is rapid and inexpensive and can easily be integrated in the routine molecular algorithm for ET and PMF.

P1035

A PROGNOSTIC MODEL TO PREDICT THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA

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Background: Current risk stratification in essential thrombocythemia (ET) is designed to estimate the likelihood of thrombotic complications: high-risk is defined by the presence of age >60 years and/or presence of thrombosis history; low-risk is defined by the absence of these risk factors. Recent data consider some additional risk factors for thrombosis, such as JAK2 mutations and cardiovascular risk factors, but also show a loss of prognostic value of an older age in multivariate analysis.
Aims: The aim of our study was to identify prognostic factors at the time of diagnosis with an impact on prediction of thrombosis in ET patients.
Methods: We retrospectively analysed 244 WHO-defined ET pts who were completely treated and followed in our clinic from January 2000 to January 2012. The median follow-up was 83 months (range 16-156). We analyzed influence of age, gender, laboratory parameters, history of previous thrombosis, spleen size, JAK2 mutation as well as the CV risk factors including arterial hypertension, diabetes, smoking attitude and hyperlipidemia. Patients were treated as follows: 48 pts (19.7%) received low-dose aspirin, 127 (52%) cytoreductive therapy and 69 (28.3%) combination of aspirin and cytoredutive therapy. During the study, 32 patients (13.2%) died.
Results: Mean age was 56 years (range 18-85) (M/F=77/167); median Plt count was $951x10^9$/L (range 497-3672); median WBC count $9.5x10^9$/L (range 4.9-20.6); median Hb 138g/L (range 90-170); splenomegaly had 26 pts (10.7%); JAK2 was analysed in 69 pts and detected in 59.4% of evaluated pts. Distribution of CV risk factors: arterial hypertension had 142 pts (58.2%), diabetes 21 (8.6%), smoking attitude 87 (35.7%) and hyperlidemia 37 pts (15.2%). Previous thrombosis was reported in 42 pts (17.2%) with 58 thrombotic events (45 arterial, 13 venous) distributed as follows: 30 pts had arterial thrombosis, 8 venous and 4 both. During the follow up 35 pts (14.3%) had 41 thrombotic events (22 arterial, 19 venous) including 17 pts with arterial, 16 with venous and 2 with both types of thrombosis with median time to thrombotic events 50 months (range 4-144). Laboratory parameters, age>60 years and JAK2 were not statistically significant for the thrombosis development. Significant predictors of thrombosis were: CV risk factors (p=0.001) and previous thrombosis (p=0.01). Accordingly, we assigned risk scores based on multivariable analysis–derived odds ratios (OR) to 1 CV risk factor (OR=3.9; 1 point), >1 CV risk factors (OR=10.1; 2 points) and previous thrombosis (OR=2.6; 1 point). A new 4-tiered prognostic model (low-risk=0 point; intermediate-1 risk=1 point; intermediate-2 risk=2 points and high-risk=3 points) was developed with the thrombosis risk of 3.8% of pts in low, 14.4% in intermediate-1, 21.2% in intermediate-2 and 60% of pts in high risk group (p<0.001). Regarding the therapy, not using aspirin was a predictor of thrombosis (HR 0.44; p=0.03). Patients with thrombotic complications during the follow-up had a significantly shorter survival (p=0.018).
Summary and Conclusion: The worldwide-recognized prognostic role of previous thrombotic events and older age in ET pts probably deserves some deeper insight. According to our results, the new score was made, based on cardiovascular risk factors and previous thrombosis and allows better discrimination to the prognostic risk groups. The use of aspirin alone or in combination with cytoreductive therapy reduces the risk of thrombosis.

P1036

CLINICAL FEATURES OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA DIAGNOSED BETWEEN THE AGES OF 41-59YEARS IN COMPARISON TO PATIENTS DIAGNOSED AT OR BELOW THE AGE OF 40

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Background: Patients with essential thrombocythemia (ET) below the age of 40 are generally recognized as having a low risk of thrombosis and progression to acute leukemia and myelofibrosis. Patients aged 41-59years have been classified as having an intermediate thrombotic risk in some guidelines but have not been very well defined in terms of their thrombotic risk and natural history.
Aims: In this study, we evaluated the clinical characteristics and outcomes of patients diagnosed with ET between the ages of 41-59years relative to patients diagnosed at age 40 and below.
Methods: 170 patients below the age of 60 at diagnosis were retrieved from our institution's Myeloproliferative Neoplasms (MPN) Registry and their records retrospectively reviewed. 62.4% were between the ages of 41-59 at diagnosis. Diagnosis of ET was made using either the PVSG or WHO 2008 diagnostic criteria depending on date of diagnosis. Statistical analysis was done using standard statistical methods.
Results: Median follow up for all patients was 8.8years. There was no difference in clinical features at diagnosis although more patients in the older group had a prior thrombosis (15.1%) compared to the younger group (4.7%) (p=0.037). There was no difference in the IPSET scores for survival and thrombosis. No difference in use of antiplatelet agents was found: 87.5% of younger patients compared to 91.5% of older patients (p=0.4); but more of the older (92.5%) than younger patients received cytoreduction (79.7%) (p=0.014). Patients in the older group had a worse overall survival (OS) with a median survival of 26.7years compared to 33.8years (p=0.013). 10year cumulative thrombosis rate was 10% for those aged 41-59years, and 0% for patients at age 40 or less (p=0.003). There was no difference in rates of progression to acute leukaemia or myelofibrosis and bleeding events. Incidence rates for the older group compared to the younger group for death, thrombosis, and bleeding (per 100 patient-years) were: 1.11 *versus* (*vs.*) 0.28 (Incidence rate ratio (IRR) 3.93, p=0.03), 1.39 *vs.* 0.14 (IRR 9.82, p<0.005) and 0.74 *vs.* 0.14 (IRR 5.23, p=0.04) respectively. There was no difference in the incidence rates for leukemic or myelofibrotic transformation. On univariate analysis, age 41-59 at diagnosis (p=0.013), presenting platelet count >1000 x 10^9/L (p=0.019), presenting white blood cell count ≥ 11 x 10^9/L (p=0.021) and being in the intermediate IPSET survival risk group predicted a worse OS (p<0.005). Use of antiplatelet or cytoreductive agents had no impact on OS. With multivariate analysis, age 41-59 at diagnosis (Hazard ratio (HR) 9.59, p=0.034) and a platelet >1000 x 10^9/L at diagnosis (HR 6.00, p=0.011) remained as predictors of poorer OS. For thrombotic risk, age 41-59 at diagnosis (p=0.003), prior thrombosis (p=0.015), presence of mutated JAK2 V617F (p=0.008), and presence of one or more cardiovascular risk factors (p=0.005) negatively affected risk of thrombosis (p=0.015). Being in the IPSET thrombosis low risk group also predicted lower thrombotic risk (p=0.004) but use of antiplatelet agents or cytoreduction had no impact on thrombotic risk. On multivariate analysis though, none of these factors were borne out.
Summary and Conclusion: Patients diagnosed with ET between 41-59years at diagnosis constitute a higher risk group with poorer OS and increased incidence of thrombosis. These findings should be confirmed with larger studies. The risk factors for such events and optimal management of this group of patients will also have to be delineated.

P1037

CARDIOVASCULAR EVENTS: RISK FACTORS AND CORRELATIONS WITH THE JAK2V617F ALLELE BURDEN IN PATIENTS WITH PH'-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS. A MONOCENTRIC STUDY

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Background: Myeloproliferative neoplasms (MPN) are a group of clonal diseases associated with JAK2 mutation (V617F) in a percentage varying from 50% (Essential Thrombocytemia and Myelofibrosis) to 95% (Polycitemia Vera). Treatment targets are represented by the maintenance of an adequate blood count, the reduction of the systemic symptoms, and the prevention of the cardiovascular events. Recent papers reported an association between the JAK2V617F mutation and the venous/arterial thromboses, although many studies are still in progress in order to assess whether this parameter can be considered to play an independent role in patients that often present comorbidities or already known cardiovascular risk factors.

Aims: To correlate the presence of the JAK2 mutation, or its mutated allele burden, to the vascular events in MPN patients and to weight the JAK2 significance in respect of the cardio-vascular risk factors.

Methods: We analyzed 384 patients (median age 65 years; range 20-85) affected by Ph'-negative neoplasms for the JAK2V617F mutation by using the *JAK2* Muta*Screen* kit, that is based on the TaqMan allelic discrimination technique. In the mutated cases, the *JAK2* Muta*Quant kit* was used to quantitate the mutated allele (Ipsogen, Luminy Biotech, Marseille, France). The sensitivity of these tests was $1x10^{-4}$. Some of our cases have been also assessed by mean of a new technique, the droplet digital PCR (dPCR, BIO-RAD), that allows the quantitation of the mutated allele without any reference curve.

Results: The JAK2V617F mutation has been reported in 223 (58%) of our patients: in the 57% of the patients with Essential Thrombocythemia (ET), 77% with Polycitemia Vera (PV) and 62% with Myelofibrosis (MF). The mean allele burden was 39.5%: the percentage of the mutated allele was significantly highest in the MF, followed by the PV and then by the ET ($p<0.01$), and it significantly correlated with the WBC count ($p=0.02$). Cardiovascular events have been reported in 124 out of the 223 mutated patients (56%); they were mainly distributed on the arterial side (52%), in particular myocardial infarction (35%), ictus cerebri (24%), and ischemic transitory attack (14%). Among venous events, the deep venous thromboses (44%) were predominant. The events were significantly correlated with the high risk of diseases at diagnosis ($p<0.01$), a PADUA score >4 ($p<0.01$), and dyslipidemia ($p=0.04$). On the contrary, their occurrence was independent from the smoke, diabetes, and hypertension. In addition, vascular events did not correlate with leucocyte count, hematocrit, platelet, and JAK2 allele burden.

Table 1.

Characteristics of the patients

N° patients	384
Age	20-89 (median 65)
Gender	
M	177 (46%)
F	207 (54%)
Diagnosis	
PV	51 (22%)
TE	23 (57%)
MF	14 (13%)
Not Determined	15 (3%)
JAK2V617F	223/384 (58%)
JAK2V617F with vascular events	124/223 (56%)

Summary and Conclusion: Our study showed that more than the half of patients affected by MPN presented cardio-vascular events. Their occurrence was higher in cases with WBC>11000/uL, high risk diseases, and in those showing a high PADUA score. The mutated allele burden, measured by both real-time and droplet PCR, did not significantly condition the occurrence of cardio-vascular events.

P1038

BACK TO LIFE -LIVING, TREATING, MANAGING MYELOFIBROSIS: THE BURDEN OF ILLNESS FOR PATIENTS AND THEIR FAMILIES

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Background: Myelofibrosis(MF) is a chronic myeloproliferative neoplasm that affects approximately 12 individuals per 1 million people a year. The patients' survival depends on the severity and speed of progression of the disease and can vary from 2 years to more than 11 years.

Aims: The objective of the current research was to quantify the burden of illness(BoI) on patients and their families in Italy. The impact of MF on productivity and QoL, the impact of treatments with ruxolitinib or other therapies and the experience of caregivers were evaluated through written interviews consisting of a quantitative part and a narrative medicine part.

Methods: Target of research were patients affected by primary or secondary MF and unpaid caregivers. In 35 Italian haematological centers questionnaires were distributed under the supervision of ISTUD Foundation, between September 2012 and October 2013, with written consent form signed and an Ethic Committee approved protocol. For the quantification of the BoI, 287 questionnaire of patients and 98 of caregivers and, respectively, 210 and 62 their stories were collected.

Results: The patients' mean age was 65 years, 55% were men and 45% women. At the time of diagnosis, the most frequent feelings were fear(70%) and depression(28%); this affected the patients' mindset that is aimed primarily at living in the present tense(50%) or fearing no possible future(27%), as resulted from their stories. 48% of patients were forced to give up the gratifying movement activities mainly because of splenomegaly(70%) and fatigue(64%). In addition, 35% of patients failed to continue their work, with a mean loss of income of 8.065€ per year. Further analyses showed that patients treated with ruxolitinib had a reduction of the spleen in 71% of cases, amounting to 19% if on other therapies. 92% of patients treated with ruxolitinib improved symptoms (59% if on other therapies) and pace of work in 87% of cases(44% if on other therapies). The 98 caregivers interviewed were 41% men and 55% women with a mean age of 55 years. They declared to take care of their relatives for more than 3 hours per day in 45% of cases. As a result of the impact of MF, 87% of caregivers resulted highly stressed, as measured with the "*Caregiver self-assessment questionnaire*" of the AMA. Nevertheless, through narrative it came out that coping was present in 53% of the experience and the success factors were not only love and responsibility, but also the possibility to rely on health professionals, friend or colleagues. In terms of lost revenue due to the care they perform, caregivers declare a quantifiable loss of 4.692€ per year, mainly because only 19% of caregivers manage to maintain their pace of work; to this productivity loss, the mean annual cost of 7.302€ for those who need family support is added.

Figure 1.

Summary and Conclusion: These results suggest that MF causes a heavy loss of income for both patients and caregivers who are still in productive age, as well as high intangible costs determined by the severe limitations on the QoL of people with MF and by the high stress induced by the burden of care. Furthermore, it has been estimated that, to pay an external caregiver the average annual cost would range between 6.606 and 10.590€. Psychological burden affect both patients and caregivers. However from these data the use of ruxolitinib appears to reduce the BoI of patients with MF in terms of restarted activity, work maintenance and social and relational wellbeing. A special reward goes to the Italian hematologies, patients and their families for having partecipated to this project.

P1039

GENDER DIFFERENCES AND MPN SYMPTOM BURDEN: AN ANALYSIS BY THE MPN QUALITY OF LIFE INTERNATIONAL STUDY GROUP (MPN-QOL ISG)

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Background: The role gender plays in the development of myeloproliferative neoplasms (MPNs) and their complications has been a topic of intense investigation. Though notable differences have been observed in rates of disease occurrence, JAK2V617F mutation status and vascular risk profiles, to date no study has investigated the role of gender differences in MPN symptom development.

Aims: We sought to further analyze the relationships between gender differences and MPN disease features, disease risk, individual MPN symptom prevalence and severity, languages and overall quality of life.

Methods: Data was collected among an international cohort of patients with MPNs including essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF). Subjects completed the Brief Fatigue Inventory (BFI) and MPN Symptom Assessment Form (MPN-SAF) instruments. Demographics were compared between males and females using t-tests, Wilcoxon rank-sum tests, and chi-squared tests. Symptoms and TSS were compared between males and females using a general linear model adjusting for MPN type and age.

Results: A total of 2006 subjects with MPNs (ET=830, PV=711, MF=460) were prospectively enrolled and administered the MPN-SAF and BFI. The prevalence of each gender (M=917, F=1089) was similar. Differences between genders were observed in the distribution of MPN type (M: ET=33%, PV=42%, MF=25%; F: ET=49%, PV=30%, MF=21%; p<0.001) and MF subtype (M: PMF=75%, post-ET=14%, post-PV=11%; F: PMF=62%, post-ET=22%, post-PV=17%; p=0.01). Males were slightly older (mean 60.7 yrs [SD 12.6] *vs* mean 59.3 yrs [SD 14.4]; p=0.02), had a higher rate of requirement for red blood cell transfusion (7% *vs* 5%, p=0.02), and had higher mean white blood cell count (mean 9.5x10^9/L [SD 8.2 x10^9/L] *vs* mean 8.5 x10^9/L [SD 6.1x10^9/L]; p=0.004) relative to females. Females had a lower rate of thrombocytopenia (8% *vs* 14%, p<0.001). No differences between the genders were noted in language, MPN risk score (DIPSS, IPSET, PV Risk), anemia, leukopenia, prior thrombosis or prior hemorrhage. Adjusted mean BFI and MPN-SAF items (adjusted for MPN type and age) by gender are listed in Table 1. 15/18 symptom items were higher in females relative to males with 13 reaching statistical significance (p<0.05). Symptom differences between genders were most prominent for fatigue, bone pain, abdominal discomfort, and microvascular dysfunction. TSS was statistically significantly higher in females relative to males (adjusted mean 23.9 *vs* 20.6; p<0.001). Difference in overall quality of life (QOL) did not reach statistical significance (adjusted mean 3.1 *vs* 2.9; p=0.19).

Table 1.

MPN-SAF Item/Scale	Adjusted Mean		P-Value
	Female	Male	
WORST fatigue (BFI)	4.7	4.1	<0.001
Early satiety	2.8	2.4	0.003
Abdominal pain	1.8	1.3	<0.001
Abdominal discomfort	2.2	1.7	<0.001
Inactivity	2.7	2.4	0.03
Headache	2.2	1.8	<0.001
Concentration	2.7	2.4	0.009
Dizziness	2.3	2.0	<0.001
Numbness	2.7	2.2	<0.001
Insomnia	3.5	2.4	<0.001
Sad mood	2.8	2.3	0.009
Sexuality	3.8	3.8	0.32
Cough	1.5	1.4	0.22
Night sweats	2.5	2.0	<0.001
Itching	2.3	2.1	0.16
Bone pain	2.3	1.8	<0.001
Fever	0.5	0.5	0.79
Weight loss	1.4	1.5	0.22
Overall quality of life	3.1	2.9	0.19
MPN-SAF TSS	23.9	20.6	<0.001

Summary and Conclusion: Heterogeneity in disease features and symptom burden exist between the two genders among MPN patients. Overall, females had a statistically significantly higher adjusted symptom score mean in most symptom areas, as well as a statistically significantly higher TSS, though the magnitude of the differences in symptoms and TSS were small in terms of

clinical meaningfulness. This increased symptom burden did not translate into a difference in overall QOL. These data suggest that either females enjoy a higher QOL despite experiencing more symptoms, or, conversely, that both genders enjoy the same QOL because the symptom burden is in fact equal but females are more likely to report their symptoms.As previously reported, females described more abdominal symptoms. However, in distinction to previous reports, these symptoms were not the result of abdominal thrombosis suggesting that abdominal symptoms in females are multifactorial and worthy of additional investigation. Though higher rates of macrothrombosis have been previously documented in females, the high rates of microvascular symptoms and platelet counts suggests females may also suffer from higher rates of microthrombosis.

Hodgkin lymphoma - Clinical

P1040

OUTCOME OF PATIENTS AGED OVER 60 WITH CLASSICAL HODGKIN LYMPHOMA TREATED WITH ABVD

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Background: Approximately twenty percent of classical Hodgkin's Lymphoma (cHL) patients are aged over 60 years. There is no standard of care in this age group. ABVD (Doxorubicin, bleomycin, vinblastine and dacarbazine), proposed for younger patients, is also used in elderly patients but little is known about its toxicity and efficacy. Recently, the German group published his experience for early-stage cHL treated within their trials with ABVD and concluded that four cycles were associated with substantial toxicity leading to dose reduction, delay and mortality.
Aims: In order to evaluate our everyday life practice, we reviewed patients referred to three French hematological departments.
Methods: We retrospectively analyzed efficacy and toxicity of ABVD in 147 patients aged over 60 years referred to Hôpital Saint-Louis in Paris, Institut Paoli Calmette in Marseille and Centre Henri-Becquerel in Rouen between January 1997 and September 2012.
Results: Median age was 68 years (60-88), sex ratio 82M/65F. According to Ann Arbor, stage was I-II-III-IV in 16-47-42-42 patients, respectively. Performance status was 0 in 61 patients, 1 in 50, 2 in 24, and >2 in 5. B symptoms were present in 84 patients. All patients received at least 1 ABVD (1-8), 50 patients received additional radiotherapy. 120 patients achieved a CR (82%), 5 a PR, 15 had refractory disease and 7 couldn't be evaluated. Twenty-five patients relapsed and 11 of them achieved a CR2. Pulmonary toxicity occurred at a median delay of 5 months from the first cycle (range: 1-31). Early pulmonary toxicity (32 patients) led to a treatment regimen modification in 24 patients. There was no significant correlation between pulmonary toxicity and pulmonary history, tobacco use, age, G-CSF, and radiotherapy. Overall survival for the whole group at five years was estimated at 67% (95% CI 58-74). Overall survival was significantly influenced by age ≤ 70 *vs* >70, stage I-II *vs* III-IV and PS 0 *vs* ≥ 1. With a median follow-up of 58 months, 52 patients died, 18 from disease progression, 17 from toxicity, 11 from secondary tumors and 6 from unknown cause.
Summary and Conclusion: Our study confirms the efficacy of ABVD in elderly patients. The high frequency of pulmonary events let us to propose either to remove bleomycin from the regimen or to reduce the dose as no factor appears to predict the occurrence of this toxicity.

P1041

EVALUATION OF THE PROGNOSTIC ROLE OF TISSUE ASSOCIATED MACROPHAGES (TAM) IN HODGKIN LYMPHOMA AND CORRELATION WITH EARLY FDG-PET ASSESSMENT

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Background: Hodgkin Lymphoma (HL) is a highly curable malignancy that mostly affects young adults; despite satisfactory results, about 20% of patients still die of relapsed/refractory disease and late toxic effects rate, often due to over treatment, continue to rise with time. Consequently, the optimal treatment should be designed based on prognostic models, but currently all of them predict outcome with scarce accuracy. Since in the last few years "early FDG-PET" and tissue macrophages infiltration in diagnostic specimens (TAM) emerged as powerful independent prognostic predictors, we previously conducted a pivotal analysis aimed to confirm these issues and looking for a possible link between the 2 tools, concluding that only early FDG-PET was a reliable prognostic factor.
Aims: The primary endpoint of this study was to investigate, in a larger cohort of patients with a longer follow-up, the prognostic role of both early-FDG PET and TAM, while the secondary endpoint was to test if early-FDG PET positivity could correlate with high TAM in diagnostic specimens.

Methods: A cohort of 200 patients (M/F: 105/95; median age 33.5 yrs) diagnosed and treated at 6 Italian hematology institutions between March 2005 and December 2012 was retrospectively analyzed. All patients, diagnosed with classic HL, completed staging with whole body CT scan, FDG-PET and bone marrow biopsy. Eleven patients had stage I disease, 92 stage II, 55 stage III and 42 stage IV. Induction treatment plan consisted, according to staging, of 2-6 courses of ABVD and, if indicated, involved field radiation therapy. Patients repeated CT scan and FDG-PET after 2 cycles and after the completion of therapy. TAM in paraffin embedded diagnostic specimens were determined by immunohistochemistry with a monoclonal antibody (anti-CD68 KP1, Dako®) and classified in 3 groups, based on the percentage of CD68+ cells, as previously reported by Steidl and coworkers (NEJM; 2010).
Results: Overall, 168 out of 200 (84%) patients achieved a complete remission (CR), while 32 (16%) failed first line treatment, with 10 partial responses (PR) and 22 non-responders (NR); among responders 20 pts (10%) relapsed. After 2 cycles of ABVD, FDG-PET was negative in 163 patients (81.5%) and positive in 37 patients (18.5%), showing a high negative predictive value of 93% (95% CI=88-96%) and a significant correlation with the achievement of CR (p<.0001 by Fisher exact probability test). After a median follow-up of 40 months 194/200 patients were alive, progression free survival (PFS) was significantly better for PET negative patients (p<.0001). CD68 expression in diagnostic specimens was low, intermediate or high in 26 (13%), 100 (50%) and 74 (37%) cases respectively; moreover TAM score among responders was low in 23/168 cases (13.7%), intermediate in 85 (50.6%) and high in 60 (35.7%), while in non-responders was low in 3/32 cases (9.4%), intermediate in 15 (46.9%) and high in 14 (43.7%). PFS analysis showed no significant difference in any score group. Analysis aimed to evidence a possible role of TAM as prognostic factor and its possible correlation with early FDG-PET resulted not statistically significant performing the Freeman-Halton extension of the Fisher exact probability test. We observed the same results when we separately analyzed both early (I and IIA) and advanced stage disease.
Summary and Conclusion: The results of this study confirm that early FDG-PET has a high prognostic power, while TAM score doesn't seem to influence the outcome of patients with HL; moreover, in contrast to our original hypothesis, doesn't correlate with FDG-PET assessment.

P1042

ROLE OF BONE MARROW BIOPSY IN HODGKIN LYMPHOMA STAGING IN THE POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY ERA

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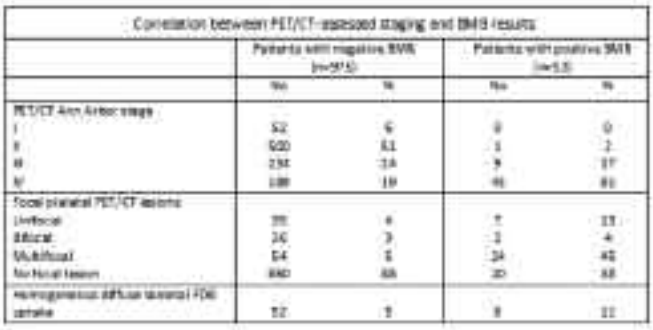
Background: In recent years, several studies investigated the role of routine bone marrow biopsy (BMB) in newly diagnosed classic Hodgkin's lymphoma (cHL) staged with positron emission tomography (PET/CT): recently, a meta-analysis reported data of 955 cases in 9 different studies, to determine whether BMB is still necessary in patients (pts) staged at diagnosis with PET/CT.
Aims: We report data of pts with cHL assessed at diagnosis with both BMB and PET/CT in order to evaluate their concordance in the detection of bone marrow involvement from cHL.
Methods: Data from pts with cHL diagnosed consecutively since 2007 to 2013 at 14 haematology centers of the Fondazione Italiana Linfomi (FIL) were retrospectively collected, excluding those who did not performed both baseline BMB and PET/CT; Ann Arbor stage assessed only with PET/CT was then compared to stage resulting from PET/CT combined to BMB. The predictive significance of PET/CT was determined in terms of positive (PPV) and negative predictive value (NPV), sensitivity and specificity.
Results: In this survey we included 1180 pts; 152 were excluded due to the lack of baseline BMB or PET/CT. 1028 cases were evaluated, median age 33 (range, 14-80 years), 542 male (53%). Nodular sclerosis (70%) and mixed cellularity (20%) were the most common histotypes; bulky disease and B symptoms were present in 27% and 42% of pts, respectively. 148 pts (14%) presented one or more focal skeletal lesions at PET/TC and 53 (5%) had a positive BMB; other patients' characteristics are summarized in table. In 33/53 pts focal skeletal lesions evidenced by PET/CT revealed a positivity of BMB, while in 860/975 pts the absence of skeletal lesions or a diffuse skeletal FDG uptake combined with a negative BMB. Based on these data, PPV and NPV resulted to be 22% and 98%, respectively; sensitivity and specificity were 62% and 88%, respectively. Moreover, a total of 9 patients (1%), one in stage II (0.1%) and 8 in stage III (0,8%) according to PET/CT were upstaged by BMB to stage IV, inducing a change in treatment only in 1 patient (0.1%). Central revision of PET/CTs in BMB-positive cases is ongoing, and will be ready for June 2014.

Table 1.



Summary and Conclusion: Consistently to data previous reported, in stages I-II according to PET/CT, positive BMB were occasional; moreover, the NPV of PET/CT for bone marrow involvement was very high. Furthermore, the influence of BMB on the planning of treatment was minimal. On these grounds, BMB may be omitted in cHL patients staged with PET/CT.

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Abstract withdrawn

P1044

A RANDOMIZED TRIAL OF ROUTINE SURVEILLANCE IMAGING PROCEDURES: ULTRASONOGRAPHY PLUS CHEST RADIOGRAPH *vs* FDG PET/CT FOR DETECTING RELAPSE IN PATIENTS WITH ADVANCED STAGE HODGKIN LYMPHOMA

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Background: Despite the high complete response (CR) rate to induction therapy with ABVD or ABVD-like regimens, about one-third of Hodgkin lymphoma (HL) patients with extensive disease at presentation are expected to relapse over time upon treatment discontinuation. Usually, 30%>50% of relapses are clinically asymptomatic, lacking any physical and/or laboratory sign. For patients at high risk of relapse, a close monitoring plan based on imaging procedures is justified, since early detection of recurrence allows a timely administration of appropriate salvage therapy. Nevertheless, only few clear indications for monitoring such patients are presently available. The existing guidelines are not evidence-based, and post-treatment follow-up is still left to "expert opinions". New imaging approaches are now available, and require validation in a randomized fashion.
Aims: This randomized study compared the benefits and pitfalls of [^{18}F] fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) *vs.* ultrasonography (US) plus chest radiograph (CXR) to systematically follow-up patients with high risk HL. This study was designed as an equivalence trial.
Methods: From January 2001 to December 2009, in this single centre trial, after institutional review board approval and informed consent, patients with advanced stage HL, completely responding to first-line treatment, were randomly assigned (1:1) to either PET/CT-based or US**+**CXR-based follow-up. Follow-up imaging procedures in the US**+**CXR group comprised ultrasonographic scans for the evaluation of superficial-anterosuperior mediastinum-abdominal-pelvic (S-M-A-P) lymph nodes, and frontal and lateral CXR for the evaluation of mediastinum compartments. In the PET/CT group, total-body FDG PET/CT scans were carried out using a combined in-line system. The surveillance schedule for each arm implied 12 checkpoints, including clinical and imaging procedures, at 4, 8, 12, 16, 20, 24, 30, 36, 48, 60, 84 and 108 months after treatment discontinuation. When clinical and/or imaging procedures were positive, recurrence was histologically confirmed. The primary end-point was to compare the sensitivity of the two follow-up imaging approaches. Secondary endpoints were their specificity, positive and negative predictive values, time to recurrence detection, radiation risks and costs.
Results: Overall, 300 patients were randomized in the two arms. The study was closed after a median follow-up of 60 months, with a relapse rate of 27%.

Sensitivity in detecting HL was similar for the two follow-up approaches: 40/40 relapses were identified with FDG PET/CT (100%) *vs.* 39/40 relapses were identified with US plus CXR (97.5%; $p_{equivalence}$=0.0001). US plus CXR showed significantly higher specificity and positive predictive value than PET/CT: 96% (106/110) *vs.* 86% (95/110; p=0.02) and 91% (39/43) *vs.* 73% (40/55; p=0.01), respectively. Exposure to ionizing radiation was estimated to be 14.5 mSv per one PET/CT *vs.* 0.1 mSv per one CXR. Estimated cost per relapse diagnosed with routine PET/CT was 10-fold higher compared with routine US+CXR.
Summary and Conclusion: US and CXR are diagnostic tools that enable effective, safe and low-cost routine surveillance imaging for patients at high risk of HL relapse. This study could have an important impact on practice for the follow-up of patients with advanced stage HL in CR, making surveillance with modern US and CXR routine and decreasing the use of intensive radiological tools.

P1045

PET-ADAPTED TREATMENT MODIFICATION IN EARLY STAGE HODGKIN LYMPHOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Hodgkin lymphoma (HL) is one of the most common malignancies in young adults and, with cure rates of up to 90%, one of the most curable cancers worldwide. Depending on its stage, HL is treated with different regimens of chemotherapy plus radiotherapy. With FDG- PET, HL treatment is in the process of being modified: An interim PET scan, revealing good and poor responders to initial therapy, consequently leads to de- or escalated additional treatment.
Aims: It has been the aim of this systematic review and meta-analysis to assess the potential benefit of PET-adapted treatment in early stage HL patients and the more than interesting resulting question for clinical daily routine whether radiotherapy can be omitted in early stage PET-negative patients.
Methods: All randomized controlled trials (RCTs) comparing FDG-PET-adapted therapy to standard treatment in previously untreated early stage HL patients with a negative PET-scan were included. Two review authors independently screened search results and extracted relevant study data. We used hazard ratios (HR) for time-to-event data and risk rations (RR) for dichotomous data, with 95% confidence intervals (CI).
Results: Three RCTs involving a total of 1480 participants were included in the meta-analysis. Because no HRs for OS were provided, we used RR and did not pool the data. PFS at a median follow-up of 34 months manifested to be inferior in the PET-adapted arm (without radiotherapy) compared to the standard treatment arm. HR: 2.16 (95% CI 1.36 to 3.42; P=0.001) AEs were reported in one study only and showed no significant difference between both arms. There were no data on QoL and TRM available.

Figure 1.

Summary and Conclusion: Our review shows that PFS is significantly decreased if radiotherapy is omitted by performing PET-adapted therapy. Current studies do not provide any evidence for the risk to benefit assessment when balancing the risk of relapse and subsequent salvage therapy against the risk of very late toxicities from radiotherapy. Importantly, there are no data at all on the patients' preferences regarding this difficult question. Therefore, standard therapy based on scientific and academic practice includes both chemo- and radiotherapy in a combined modality approach.

P1046

PROGNOSTICATION BY FDG-PET DETECTED BONE MARROW INVOLVEMENT IN HODGKINS LYMPHOMA - A RETROSPECTIVE ANALYSIS

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Background: Bone marrow involvement is seen in 5–14% of Hodgkin's lymphoma (HL) patients. Currently, BMB is considered gold standard for the evaluation and documenting bone marrow (BM) involvement in HL. However, the chances of missing BM involvement on BMB are a reality especially in the setting of focal disease. With the introduction of FDG-PET into the staging procedures of HL, it became evident that the typical involvement of BM may not be homogenous but, rather, focal. The aim of the current study was to correlate BMB and PET/CT results as part of baseline staging work-up, understand the pattern of BM involvement on FDG-PET and to assess the impact of FDG-PET in the detection of bone marrow disease in patients with HL at baseline diagnosis.
Aims: The identify the concordance between baseline FDG-PET with bone marrow biopsy at initial staging of HL patients. To understand the patterns of bone marrow involvement evidenced on FDG-PET at diagnosis and to correlate outcomes of patient with BMB and FDG-PET evidence of BM involvement in terms of survival.
Methods: Patients diagnosed with HL above 15 years registered in the lymphoma clinic between June 2009 and June 2012 and having undergone both FDG-PET and BMB at baseline staging were included for final analysis. Disappearance of focal lesion in the marrow on interim PET was considered evidence of involvement. BM involvement on FDG-PET was documented as unifocal, bifocal, multifocal, or homogeneous. These were correlated with BMB findings. The log-rank test was performed to compare progression-free survival (PFS) for patients with positive BMB and those with skeletal PET/CT lesions along with negative BMB. Kaplan meier curves were used for survival analysis.
Results: 478 patients with HL were screened with 200 patients meeting the inclusion criteria. These patients were thereafter considered for further analysis. Abnormal skeletal FDG accumulation was noted in 60 (30%) patients. 28 (14%) had focal lesions (unifocal=10; bifocal=4; and multifocal=14). 32 (16%) patients had homogeneous FDG uptake in the axial and appendicular skeleton. All BMB positive patients (n=11) had either focal (n=7) or diffuse (n=3) on FDG-PET. Only one patient was upstaged based on positive BMB with negative PET. 32 patients with diffuse uptake in BM evidenced on PET yielded positivity in only 3 patients on BMB suggesting that diffuse involvement may not necessarily suggest true involvement and low sensitivity for FDG-PET in this situation. The overall sensitivity and specificity for FDG-PET in detecting positive *versus* negative BM were 91% and 73% respectively. Focal FDG-PET positive lesions yielded sensitivity of 64% and specificity of 98.4%. The median follow up period was 23 months and the overall 2 year PFS of the study cohort was 87.5%. The bone marrow biopsy positive patients had a 58.9% two year PFS as compared to 89.3% in bone marrow biopsy negative patients(P=0.08). The two year PFS in FDG-PET positive patients for BM was 73.4% as compared to 94% in FDG-PET negative patients (P=0.0001). There was no significant 2 year PFS (50%) difference between Multifocal PET BM involvement in bone marrow biopsy positive or negative cases, suggesting that these patients were equally burdened by HL.
Summary and Conclusion: Our study reiterates the high sensitivity of FDG-PET in assessing BM disease in HL. Multifocal FDG-PET uptake was a better indicator of BM involvement and correlated with similar outcomes as would for a patient with BMB positive disease. Diffuse involvement on FDG-PET may be non-specific and mostly doesn't correlate with BM involvement. We recommend that FDG-PET could safely replace BMB assessment of BM at baseline staging work up for Hodgkins lymphoma.

P1047

DOSE DENSE ABVD (DD-ABVD) AS FIRST LINE THERAPY IN EARLY-STAGE UNFAVORABLE HODGKIN LYMPHOMA (HL): PRELIMINARY RESULTS OF A PHASE II, PROSPECTIVE, ONGOING MULTI□CENTER STUDY BY FONDAZIONE ITALIANA LIN

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Background: Four cycles of ABVD plus 30 Gy on involved-field radiotherapy is the the standard of care for patients with early unfavorable Hodgkin lymphoma (HL).

Aims: Since dose-density might represent an important factor for achievement of complete remission and long-term survival, we designed a prospective, multicenter, phase II trial to investigate feasibility, safety and efficacy of dose-dense ABVD (dd-ABVD) in patients with early unfavorable HL.
Methods: Fifty-four patients (male 44%, age 18 to 70 years) with newly diagnosed stage I (6%) and II (94%) HL and unfavorable prognostic criteria according to EORTC received dd-ABVD consisting of standard dose ABVD on day 1 and 8 every 21 days instead of days 1 and 15 every 28 days. Patients with stage IIB bulky disease are excluded from the study. Granulocyte colony-stimulating factor (G-CSF) is scheduled as primary prophylaxis. Unless in progression, all patients received 4 cycles of dd-ABVD followed by 30 Gy on IFRT. In standard schedule ABVD, the proportion of patients without a dose intensity reduction is 95%. In order to exclude a dose intensity reduction in more than 10 % of pts treated with dd-ABVD, with a one-side alpha of 0.10 and beta of 0.15, 52 patients are needed. If 5 or more patients will present a dose intensity reduction, the study treatment will not be considered for further evaluation and the enrolment will be ended.
Results: Two out of 54 patients were not evaluable due to screening failure and consent withdrawal, respectively. Thirty sevent (9%) out of 416 evaluable ABVD infusions (208 cycles) were delayed due to hematological toxicity (n=10 cases), non-hematological toxicity (n=10 cases), logistic reasons (n=14), unknown reasons (n=3). One patient suspended bleomycin after first cycle. Four serious adverse events were registered: febrile neutropenia (n=2), erythematous papular skin lesion (n=1), chest pain (n=1). As compared to the planned dose intensity, the median dose intensity for doxorubicin, bleomycin, vinblastine, and dacarbazine were 97.6%, 97%, 95.7% and 97.7%, respectively. Overall 6 patients received a 100% dose-intensity, 24 patient a dose-intensity between 85% and 100% and 4 patients received a dose-intensity below 85%.
Summary and Conclusion: This feasibility analysis confirms the safety of this intensified administration schedule of dd-ABVD with G-CSF support. The main causes of therapy delay are due to logistic reason. The study is ongoing with the aim to evaluate dd-ABVD efficacy.

P1048

THE PROGNOSTIC SIGNIFICANCE OF THE ABSOLUTE LYMPHOCYTE TO ABSOLUTE MONOCYTE COUNT RATIO (ALC/AMC) IN HODGKIN LYMPHOMA (HL) UNDER TREATMENT WITH ABVD OR EQUIVALENT REGIMENS WITH OR WITHOUT RADIOTHERAPY

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Background: Lymphocytopenia and increased CD68+ tumor-associated macrophages (TAMs) are adverse prognostic factors in HL. TAMs are derived from circulating monocytes and are possibly related to AMC. Recently, Porrata et al reported that a low ALC/AMC (<1.1) is an independent prognostic factor in HL. Subsequent studies used different cutoffs for ALC/AMC (1.5 and 2.9).
Aims: The validation of the prognostic significance of ALC/AMC in a group of homogenously treated HL patients (pts) and evaluation of various cut-offs used by different investigators.
Methods: We evaluated 507 pts with HL, treated with ABVD or equivalents (±RT), who had available data for ALC/AMC, determined by an automated blood counter at diagnosis. Median age was 32 yrs (15-87), 54% of patients were male, 36% had B-symptoms, 17% had stage IV disease, 48% had advanced stage (IB,IIB,III,IV), 31% had IPS ≥3, 79% nodular sclerosing histology and 19% mixed cellularity. Failure-free survival (FFS) was defined as time between treatment initiation and failure to achieve remission requiring switch to salvage therapy, relapse after remission or last follow-up; toxic deaths and deaths in remission of unrelated causes were not counted as events. The association between ALC/AMC and FFS was evaluated at all previously published cut-offs of as well as at the median ALC/AMC observed in the present series.
Results: Median follow-up of currently alive patients was 44 months. The median ALC/AMC was 2.24 (range 0.44-20.50, interquartile range; IQR 1.53-3.54). The median AMC was $0.653x10^9$/l (range 0.050-2.070, IQR 0.469-0.836). Lower ALC/AMC was associated with most established markers of increased tumor burden and aggressive biological behavior as well as with monocytosis and lymphocytopenia. In total 449 (89%), 390 (77%) and 183 (36%) patients had ALC/AMC ratio ≥ 1.1, ≥1.5, and ≥2.9 respectively and 20% of patients had monocytosis (≥.$0.9x10^9$/l). Ten-year FFS was 78% *vs* 56% for patients with ALC/AMC ≥1.1 and<1.1 respectively (p=0.0004), 78% *vs* 69% for patients with ALC/AMC ≥1.5 and<1.5 respectively (p=0.06), 79% *vs* 74% for patients with ALC/AMC ≥2.9 and<2.9 respectively (p=0.25) and 81% *vs* 70% for patients with ALC/AMC above and below the median value respectively (p=0.03). Patients with monocytosis (≥.$0.9x10^9$/l) had a 10-year FFS of 70% *vs* 80% for patients without monocytosis (p=0.0008).In early stages (IA/IIA) ALC/AMC had no significant effect on FFS irrespectively of the applied cutoff. In advanced stages ALC/AMC was statistically significant only at the cutoff of 1.1 (10-year FFS 69% *vs* 50% p=0.03). In multivariate analysis, ALC/AMC<1.1 was an independent prognostic factor (p=0.03) along with stage IV (p<0.001), obscuring the significance of all other IPS parameters, B-symptoms, elevated ESR and monocytosis. In a multivariate model including ALC/AMC and IPS (<3 *vs*. ≥3), both factors had independent prognostic significance (p=0.01 and p=0.03 respectively).
Summary and Conclusion: A highly reduced ALC/AMC ratio is an independent prognostic factor in HL treated with ABVD or equivalent regimens ± RT. Its prognostic impact is more profound in advanced-stage disease. In the present series, the cutoff of 1.1 appeared to be more predictive compared to other published cutoffs and the only one that had independent prognostic value in multivariate analysis. However, the prognostically inferior group comprised of only 11% of patients (*vs* ~30% in the initial study of Porrata et al). Further research is needed prior to the widespread use of this easily measurable and reproducible marker.

P1049

ABVD IN TREATMENT OF GENERATIVE AGE FEMALES WITH ADVANCED HODGKIN LYMPHOMA – A RISK FACTOR ANALYSIS

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Background: ABVD and escalated BEACOPP are currently the standard of care in patients with advanced Hodgkin Lymphoma (HL). The use of escalated BEACOPP gives better disease control but it is associated with more acute and late toxic effects, with post treatment infertility as one of the major complications. According to International prognostic score (IPS), patients with score 3-7 are considered as high risk. However, females of generative age who are at risk for infertility can have IPS score of maximum 5.
Aims: The aim of this study was to identify females of generative age with diagnosed advanced HL who should not be considered for more aggressive approach than ABVD.
Methods: A retrospective study was performed on 123 female patients younger than 40 years with advanced classical HL, diagnosed in the period June 1997-December 2008. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of IPS score, ESR≥50 mm/h, and presence of B symptoms, bulky disease and extranodal (EN) disease were examined.
Results: The median age of patients was 27 (range 17-40). The median follow up was 93 months. For the whole group 5-year event free survival (EFS) was 65% and 5-year overall survival (OS) was 82.1%. In univariate analysis, worse OS was found only in patients with IPS≥3 (5-year OS 60.7% *vs*. 88.4%; log rank, p=0.004), while presence of ESR≥50 mm/h, B symptoms, bulky disease or EN disease didn't influence OS (log rank; p=0.194, p=0.982, p=0.064, p=0.089, respectively). Worse EFS was found in patients with IPS≥3 (5-year EFS 46.4% *vs*. 73.7%) and EN disease (5-year EFS 48.5% *vs*.73.4%) (log rank; p=0.005, p=0.012, respectively), while presence of ESR≥50 mm/h, B symptoms or bulky disease didn't influence EFS (log rank; p=0.746, p=0.556, p=0.151, respectively). The multivariate Cox regression analysis identified only IPS≥3 as the independent prognostic factor both for OS (p=0.006) and EFS (p=0.005). Subsequently, we analyzed the subgroup of 95 patients with low IPS. In survival analysis, 29 patients without bulky disease (30.5% of low IPS patients, 23.6% of the entire group) had favorable both OS (5-year OS 100% *vs*. 81.8%, log rank, p=0.026) and EFS (5-year EFS 89.7% *vs*. 65.2%, log rank; p=0.034), with only 2 deaths until the end of follow up (figure). Based on IPS score (0, 1 or 2), presence of ESR≥50 mm/h, B symptoms and EN disease there was no difference both in OS (log rank; p=0.086, p=0.506, p=0.512, p=0.747, respectively) and EFS (log rank; p=0.102, p=0.835, p=0.414, p=0.382, respectively). Bulky disease in the subgroup of low IPS patients retained its prognostic significance in multivariate analysis both for OS (p=0.042; RR=4.674; 95% CI 1.058-20.561) and EFS (p=0.047; RR=2.920; 95% CI 1.012-8.480).

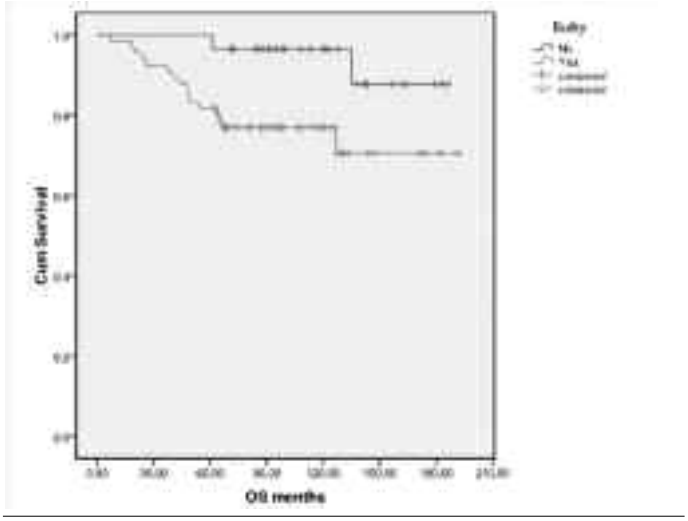

Figure 1.

Summary and Conclusion: ABVD is very effective treatment for female advanced HL patients younger than 40 years with low IPS and without bulky disease.

P1050

COMPARATIVE ASSESSMENT OF BONE MARROW INVOLVEMENT (BMI) BY BM BIOPSY (BMB) OR POSITRON EMISSION TOMOGRAPHY / COMPUTED TOMOGRAPHY (PET/CT) IN HODGKIN LYMPHOMA (HL)

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Background: BMI is observed in ~6% of HL patients (pts) at initial diagnosis and can be predicted by baseline characteristic combinations. PET/CT is a sensitive tool for HL staging. Recent data suggest that few pts may have a positive BMB in the absence of PET/CT evidence, thus questioning the need of BMB.
Aims: (1) To correlate BMB and baseline disease characteristics with BM-PET/CT findings in a series of 172 HL pts; (2) to assess the impact of our previously published clinical prediction rule (Vassilakopoulos et al, Blood. 2005;105:1875-80) on the frequency of BMI detected by either method; and (3) to assess the ability to omit BMB in selected or even all pts.
Methods: After reviewing all baseline PET/CT reports performed in Greek Centers, data regarding BMB, demographics, clinical and laboratory characteristics were retrieved from medical records. Electronic PET/CT data were reviewed by the responsible Nuclear Medicine physicians according to osseous/BM findings and were visually graded as follows: (1) no increased BM FDG uptake; (2) increased BM FDG uptake ≤liver; (3) increased BM FDG uptake >liver; (4) solitary osseous/BM focus without CT correlate; (5) multiple osseous/BM foci. Pts were also classified according to our clinical prediction rule for BMI (based on age, B-symptoms, pre-BMB stage, inguinal/iliac involvement, hemoglobin, leukocyte count) in low-, standard- and high-risk groups.
Results: Combined PET/CT and BMB data were available for 179 pts at the time of the analysis; 166 had full data for risk classification. PET/CT was negative for BMI in 148 pts (83%; 81, 33, and 34 pts with scores 1,2,3) and positive in 31 pts (17%) with multiple foci in the vast majority of them. Only 14 pts had BMI by BMB (7.8%). None of the pts of PET/CT categories 1,2,3 had a positive BMB; 14/31 pts graded as "4" or "5" had positive BMB (45%; all with score "5"). The clinical prediction rule was well validated, since the frequency of BMI by BMB was 0% in low-risk group (44 pts or 27% of the total population), 1.5% in the standard-risk group (1/65 pts or 39% of the total population) and 21.1% in the high-risk group (12/57 pts or 34% of the total population). PET/CT-based BMI was also effectively predicted: it was 0% in low-risk, 6.2% in standard-risk and 38.6% in the high-risk group. The outcome of the 14 pts with BMI by BMB was marginally inferior with 3-yr FFS 66% *vs*. 83% (p=0.16). The difference was more pronounced for the 31 pts with BMI by PET/CT (3-yr FFS 60% *vs*. 86%; p=0.004). Pts with BMI by PET/CT and negative or positive BMB had similar outcomes (3-yr FFS 57% *vs* 66%, p=0.68). Among 148 pts with negative BM by PET/CT, those with diffuse FDG uptake >liver tended to have increased "inflammatory" activity, as reflected by higher leukocyte and platelet counts, higher ESR and CRP levels.
Summary and Conclusion: PET/CT appears more efficient than BMB in detecting BMI, revealing >2 times more cases. Increased diffuse BM FDG uptake is not associated with BMI, but appears to reflect cytokine-related "inflammatory" activity. Our previously published clinical prediction rule was adequately validated regarding the prediction of BMI by either BMB or PET/CT, suggesting that the selected prognostic covariates are valid for BMI irrespective of the method of detection. There was no case of positive BMB in the absence of BMI by PET/CT. These data confirm that BMB can be safely omitted in all HL pts staged by PET/CT, since there was no high-risk group who might obtain a benefit from the combination of PET/CT and BMB.

P1051

EBV VIRAL LOAD DOES NOT IMPACT ON THE CLINICAL OUTCOME OF LOCALIZED HODGKIN LYMPHOMA (HL) – RESULTS FROM A SINGLE REFERENCE CENTER

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Background: Localized forms constitute 40 to 50% of all HL cases. Clinical and laboratory features are variably used by different cooperative groups (GELA, EORTC, GHLSG) to define patients with less favorable outcomes within this generally good prognosis population. EBV is present in about 40% of cases and viral circulating DNA can be detected in these patients, with a good correlation with tumor infection evaluated by EBER-ISH. Recently, plasma EBV viral load before treatment has been shown to have prognostic impact in advanced, homogeneously treated Hodgkin lymphoma.
Aims: With the aim of assessing the impact of pre-treatment plasma/serum EBV viral load in localized Hodgkin lymphoma, we retrospectively evaluated the demographic, histological and clinical characteristics (including staging, B symptoms, Erythrocyte Sedimentation Rate, presence of bulky disease, number of nodal areas involved and extra-nodal involvement) of a series of localized HL patients diagnosed and treated at our institution between 2003 and 2011 and correlated the clinical outcome (failure-free survival, FFS, from diagnosis to relapse, progression or death) with viral load determined by Real Time PCR (qPCR): amplification of 74 bp fragment from BNRF1 gene.
Methods: Wilcoxon rank sum test, Fisher's exact test and Pearson Chi square were used for comparisons between patients with and without detectable viral load (EBV+ and EBV-, respectively) as appropriate. The Kaplan-Meier method was used to estimate FFS and the log-rank test to compare the outcomes between groups. A multivariate analysis was also conducted using Cox proportional hazards model.
Results: 92 patients (51% females, median age 30, 15-76 yo) were identified with stage I (A-10%, B-1%) and II (A-73%, B-16%) HL. Classical HL constituted 97% of cases, with 80% nodular sclerosis, 7% mixed cellularity and 9% NOS. Bulky mediastinal disease was present in 15% and according to the GHLSG criteria 49% patients were early stage-favorable. Treatment was ABVD (median 6 cycles) and IF radiotherapy (30-40Gy) in 79%. Radiotherapy wasn't used in 17 patients due to progression or death during treatment (4), risk of excessive toxicity (6) or physician decision (7) and these patients received a higher number of ABVD cycles (p=0.04). Pre-treatment EBV DNA was detectable (above the laboratory cut off) in 17 patients (18%). Mean viral load was 1008 EBV copies/ml [117; 3386]. EBV+ patients were older than the EBV- (43 *versus* 28yo, p=0.017) but had otherwise similar clinical characteristics and treatment. With a median follow up of 6.2 (0.2-10.6) years, 15 patients relapsed or progressed and 3 died. FFS at 5 years was 83.2% (CI 72%>89%) and was not influenced by EBV status (log rank test stratified by GHLSG risk group p=0.77). This result was confirmed in the multivariate analysis (HR for EBV+ 0.5, CI 0.2%>2.2%) controlling for prognostic stratification (early favorable and unfavorable groups), use of radiotherapy and number of chemotherapy cycles. In this model only early-unfavorable patients and patients not receiving radiotherapy had a significantly worse FFS.
Summary and Conclusion: In this uniformly treated series, EBV DNAemia was less frequently detectable than what has been described in advanced disease and EBV+ patients were older than EBV-. EBV status did not impact in the clinical outcome as measured by FFS. Future prospective studies in larger populations may clarify the prognostic value of EBV viral load before and during therapy for early-stage HL.

P1052

PRELIMINARY RESULTS OF THE TREATMENT OF PATIENTS WITH HODGKIN'S DISEASE FROM INTERMEDIATE RISK GROUP

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Background: Patients with HD from intermediate risk group show unsatisfactory results with ABVD treatment. Intensification of the treatment with BEACOPP-esc/BEACOPP-14 regimens showed better efficacy with high toxicity rate. The attempt to decrease toxicity rate together with preservation of efficacy was made with the inclusion of combined treatment approach of 2 cycles of BEACOPP-esc + 4 cycles of ABVD. This approach showed promising results in previous studies.
Aims: To analyze the efficacy and toxicity of different treatment approaches in patients with HD from intermediate risk group.
Methods: Treatment-naïve patients with HD from intermediate risk group were randomized into three arms: treated with 6-8 cycles of BEACOPP-esc/BEACOPP-14 (55 patients) (group 1), treated with 2 cycles of BEACOPP-esc + 4 cycles of ABVD (48 patients) (group 2) and treated with ABVD regimen (53 patients) (group 3). Recruitment of patients is in progress.
Results: Overall response rate (ORR) was better in the group 1 and group 2 compared to the group 3: 96.36%, 95.83% and 83.02%, respectively (p<0.05). Complete response rate (CRR) was higher in the group 1and group 2 compared to the group 3: 88.68%, 86.95% and 77.27%, respectively (p<0.05). Toxicity was observed in 83.03% of cycles of chemotherapy in the group 1, 50.35% - in the group 2 and 15.09% - in the group 3, p<0.001. Main toxicity type was

hematological in all groups and was detected in 81.21%, 63.19% and 12.89% of cycles, respectively, p<0.001. Anemia grade 3-4 was observed in 24.1% of cases in the group 1, 3.47% of cases in the group 2 and 1.26% of cases in the group 3, p<0.001. Neutropenia grade 3-4 was observed in 58.31% of cases in the group 1, 23.96% of cases in the group 2 and 3.77% of cases in the group 3, p<0.001. Thrombocytopenia grade 3-4 was observed only in the group 1 in 13.36% of cases, p<0.001. Nonhematological toxicity was observed in 67.88% of cycles in the group 1, in 51.39% of cases in the group 2 and in 11.63% in the group 3. 56.67% of cycles in the group 1, 47.92% - in the group 2 and 8.81% - in the group 3 were followed by episodes of gastrointestinal toxicity, p<0.05. 23.64% of cycles in the group 1, 13.54% - in the group 2 and 2.52% in the group 3 were followed by episodes of inflectional complications, p<0.01. Neurotoxicity was observed in 4.54% of cycles in the group 1 and 3.13% - in the group 2 and wasn't detected in the group 3, p<0.05. No deaths due to complications were observed. 46.06% of patients in the group 1, 26.04% of patients in the group 2 and 7.55% of patients in the group 3 had alopecia.

Summary and Conclusion: Patients treated with 6-8 cycles of BEACOPP-esc/BEACOPP-14 and 2 cycles of BEACOPP-esc + 4 cycles of ABVD achieved comparable ORR (96.36% and 95.83% respectively). ORR in the ABVD arm was lower (83.02%). Toxicity rate was 83.03% in the group of -8 cycles of BEACOPP-esc/BEACOPP-14, 50.35% in the group of BEACOPP-esc + 4 cycles of ABVD and 15.09% in the ABVD group.

P1053

SERUM IMMUNOGLOBULINS IN PATIENTS WITH HODGKIN LYMPHOMA (HL): A NEGLECTED POTENTIAL PROGNOSTIC MARKER?

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Background: HL is characterized by cytokine deregulation and elevation of "inflammatory" markers, such as ESR, CRP, serum ferritin as well as a_2-globulin levels. Although clinical experience suggests that hypergammablobulinemia is not infrequent in HL, serum protein electrophoresis (SPE) findings with respect to γ-globulins have not been systematically studied. Similarly, little is known regarding serum immunoglobulin levels (sIgG, sIgA, sIgM). On the other hand, there are recent data implicating serum free light chain concentration (sFLC) in the prognosis of HL, without however the concomitant evaluation of SPE and serum immunoglobulin findings.

Aims: The evaluation of the prognostic significance of SPE-γG, sIgG, sIgA, and sIgM levels and their correlation with sFLC concentration.

Methods: We evaluated 330 pts with classical HL, treated with ABVD or equivalents (±radiotherapy), who had available data for SPE-γG levels. sIgG, sIgA, and sIgM levels were also studied in 267 patients (243 also had SPE-γG levels), while sFLC levels were available in 85 patients. "High" levels were defined as ≥1.6 g/dL for SPE-γG, ≥1400 mg/dL for sIgG, ≥410 mg/dL for sIgA, ≥230 mg/dL for sIgG and ≥57.1 mg/L for the sum of sFLC κ and λ. Failure-free survival (FFS) was defined as time between treatment initiation and failure to achieve remission requiring switch to salvage therapy, relapse after remission or last follow-up; toxic deaths and deaths in remission of unrelated causes were not counted as events.

Results: High SPE-γG were observed in 36% of the patients, high sIgG in 48%, sIgA in 15% and sIgM in 8%. *CORRELATIONS:* Generally, higher SPE-γG, sIgG and sIgA levels correlated with markers of disease burden and aggressiveness, while higher SPE-γG and sIgG also correlated with lower age, female gender and nodular sclerosing histology. Higher sIgM correlated advanced age, male gender, nodular sclerosing disease and lack of B-symptoms. There was a strong correlation between SPE-γG and sIgG (Spearman's rho 0.82, p<0.001). Both SPE-γG and sIgG moderately correlated with sIgA (rho 0.35-0.50, p<0.001) and sIgM (rho 0.25-0.30, p<0.001). SPE-γG, sIgG and sIgA were all moderately correlated with ESR and a_2-globulin levels (rho 0.30-0.45, p<0.001). Finally, SPE-γG, sIgG and sIgA correlated rather strongly with sFLC κ+λ (rho 0.50-0.60, p<0.001). *PROGNOSTIC SIGNIFICANCE:* High SPE-γG levels conferred inferior prognosis in univariate analysis (5-year FFS 82% *vs* 72% p=0.02). The same applied for sIgG as well (5-year FFS 85% *vs* 76% p=0.04). Neither sIgA, sIgM nor sFLC κ+λ concentrations were predictive of the outcome (although only 85 patients had available sFLC). In multivariate analysis of established conventional prognostic factors (including a_2-globulin and ESR), "high" SPE-γG was an independent adverse prognostic factor (hazard ratio 1.8, p=0.046) along with stage IV (hazard ratio 2.6, p=0.003). Similar results were obtained for "high" sIgG as well.

Summary and Conclusion: SPE-γG and serum immunoglobulin levels are frequently elevated in untreated classical HL. "High" SPE-γG or sIgG may be "classic" but neglected independent adverse prognostic factors. Due to their rather strong association with sFLC levels, the prognostic implication of sFLC in HL needs to be examined concomitantly with SPE-γG and/or sIgG, since they may reflect the same biologic process.

P1054

BRENTUXIMAB VEDOTIN FOR HODGKIN LYMPHOMA WHO HAD FAILED ALLOGENEIC STEM CELL TRANSPLANTATION: A MULTICENTER RETROSPECTIVE STUDY

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Background: Brentuximab vedotin (BV) has demonstrated an extraordinary efficacy in heavily pretreated Hodgkin lymphoma (HL) patients due to the selective targeting of CD30-positive cells. The mechanism of action of BV might also involve a cytokine-mediated antitumor immune response, further suggesting that this molecule may play a key role also in HL patients recurring after allogeneic (Allo)-SCT.

Aims: Since limited data have been so far reported on the efficacy of BV in HL patients failing Allo-SCT, we retrospectively evaluated in a multicenter setting efficacy and safety of BV in HL relapsing or progressing after Allo-SCT.

Methods: From June 2011 to January 2014, 16 patients with recurrent HL were included in a compassionate-use program and were treated with BV at four Institutions. BV was given intravenously at the dose of 1.8 mg/kg every 3 weeks. The study protocol was approved by the ethical committee of each participating center.

Results: Main demographic and disease characteristics of the patients are reported in Table 1. Patients received a median of 8 BV cycles (range, 1-17) and all but one are evaluable for response. Best response to BV was achieved after a median of 4 cycles (range, 2-12) and included 5 cases (33.5%) of complete remission (CR) and 5 cases (33.5%) of partial remission (PR), for an objective response rate (ORR) of 67%. Stable disease (SD) was observed in 4 cases and progressive disease (PD) in 1 patient. Patients achieving CR showed a continuous response persisting after a median of 4 months (range, 3-24+) from BV discontinuation. Two of them received either haploidentical allotransplant (n=1) or donor lymphocyte infusion (DLI) (n=1), while the remaining 3 patients did not receive any further treatment after BV discontinuation. All patients achieving PR experienced PD after a median of 8 cycles (range, 5-17). Patients in SD progressed after a median of 3 months (range, 2 - 6). After a median follow-up of 26 months, the median progression-free survival (PFS) and overall survival (OS) were 11 and 25 months, respectively. The 2-yr PFS and OS were 22% and 48%, respectively. At the last follow-up, 10 out of the 16 patients were alive, four of them in continuous CR. Cause of death was PD in 5 patients, pulmonary GVHD while in CR in one patient. The most common adverse events were peripheral sensory neuropathy: grade 1 neuropathy in 3 cases and grade 3 neuropathy in one case. One patient developed a Guillain Barrè syndrome. Two infections (12.5 %) were documented, both sustained by gram positive bacteria. Two patients experienced FUO (12.5%).

Table 1.

Characteristics	N
Median age, y (range)	29 (22-43)
Male sex, n (%)	13(81%)
Bulky disease	3 (18.7%)
Median time from diagnosis to first dose of BV, months	81
Median time from alloSCT to first dose of BV, months	30
Median time from last therapy to first dose of BV, months	2.5
Median n. of prior regimen (range)	8 (4-13)
Median n. of regimen between alloSCT and BV (range)	4 (0-4)
Prior autoSCT	10
Prior alloSCT	10
Prior DLI	8
Prior radiation therapy	16
Refractory to last therapy before BV	14 (87.5%)

Summary and Conclusion: This retrospective study confirms that BV as single agent is safe and effective to achieve a short-term disease control in HL patients progressing after Allo-SCT. Our results do not support the capacity of BV to induce a long-lasting disease control, strongly suggesting that BV should be

used in combination with other agents in order to increase the ORR and enhance disease control.

P1055

POST-TREATMENT RESIDUAL MASSES DETECTED BY COMPUTED TOMOGRAPHY SCAN SIGNIFICANTLY AFFECT THE CLINICAL OUTCOME OF HODGKIN LYMPHOMA PATIENTS WITH NEGATIVE POSITRON EMISSION TOMOGRAPHY SCAN

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Background: Negativity of [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (^{18}-FDG-PET) scan is mandatory for defining complete remission in Hodgkin lymphoma (HL) patients.The persistence of residual masses detected by computed tomography (CT) scans in PET-negative HL patients has been reported to have a controversial impact on the clinical outcome of these patients.
Aims: Thus, we retrospectively analyzed a series of HL patients to investigate whether the persistence of end-of-treatment PET-negative masses detected by CT scans might eventually affect the clinical outcome.
Methods: From May 2001 to May 2012, we identified 133 consecutive patients with primary or recurrent histologically confirmed HL according to the World Health Organization (WHO) classification who were PET negative after completion of first (98 patients) or second line (35 patients) therapy
Results: Median patient age was 33 years (range 17-80), 75 were male, 34 had B-symptoms, and 47 had bulky disease. A residual CT mass ≥2.0 cm in its greatest transverse diameter (CT-positive) was identified in 85 (64%) of 133 PET-negative patients. After a median follow-up of 36 months, 28 of 85 PET-negative/CT-positive patients (32%) relapsed whereas only 3 of 48 PET-negative/CT-negative patients (6%) relapsed (P<0.00000 by Fisher's test). Four-years disease-free survival (DFS) for PET-negative/CT-negative *vs*. PET-negative/CT-positive patients was 94% and 69%, respectively (P=0.001). Statistically significant DFS values for PET-negative/CT-negative *vs* PET-negative/CT-positive patients were detected both for patients receving first- (94% *vs* 72%, P=0.01) and second-line therapy (93% *vs* 61%, P=0.034). The size of the residual CT masses negatively influenced DFS [HR 1.03 (1.02; 1.04), P=0.001] in a continuous fashion. No difference was found in DFS between patients with one *versus* two or more residual masses (67% and 73%, P: 0.5). No difference was found in 4-year overall survival when comparing PET-negative/CT-negative *vs* PET-negative/CT-positive patients (93% *vs*. 91%, P=0.4).
Summary and Conclusion: In conclusion, the presence of a residual mass detectable by CT scan significantly affects the clinical outcome of HL patients, suggesting the that these patients might require a closer follow-up and possibly a consolidation treatment using new agents.

P1056

MYC ONCOGENE IS OVEREXPRESSED IN HODGKIN & REED STERNBERG CELLS (HRS) OF CLASSICAL HODGKIN LYMPHOMA AND CORRELATES WITH P53 EXPRESSION AND PROLIFERATION INDEX

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Background: MYC is a potent transcription factor targeting numerous genes involved in cell cycle progression. MYC oncogene is involved in the t(8;14)(q24;q32) chromosomal translocation, which is detected in Burkitt lymphoma (BL) and in other aggressive non-Hodgkin lymphomas (NHL) including a subset of diffuse large B-cell lymphomas (DLBCL). In addition, MYC oncoprotein can be upregulated in hematologic malignancies in the absence of chromosomal aberrations involving the 8q24 locus. However, detection of MYC protein using routinely processed paraffin-embedded tissues wasn't reliable in the past due to lack of specificity or sensitivity of the available MYC antibodies.
Aims: This study aimed to investigate the expression levels of MYC in HRS cells of classical Hodgkin lymphoma (cHL) by immunohistochemistry using a validated antibody and correlate the findings with p53 status and tumor cell proliferation.
Methods: Seventy two cHLs including 64 nodular sclerosis (NS) and 8 mixed cellularity (MC) cases were included in the study. In addition, a series of 125 NHLs of various histologic types including 8 cases of t(8;14)-positive BL as well as 5 reactive lymph nodes were also analyzed for comparison. The antibody used for MYC detection (MAb Y69) has recently been validated for immunohistochemical studies (G. Cattoretti, J Pathol 2013; 229:430). p53 expression was assessed using the DO-7 antibody that detects both the wild-type and mutated p53 gene products. Any nuclear staining in HRS (identified by CD30 marker) was considered positive irrespective of intensity for both MYC and p53. In selected cases, *p53* mutation analysis was performed for the exons 4-8 of the gene using PCR and direct sequencing techniques. Proliferation index was assessed using the Ki67 (MIB-1) marker.
Results: MYC oncoprotein was detected in all 72 cHL tumors and its expression was restricted in the nucleus of HRS cells. The mean and median percentages of MYC-positive HRS cells were 73% and 80.5%, respectively, ranging from 30% to 88%. MYC expression did not differ statistically among histologic subtypes of cHL (NS *vs*. MC). The median percentage of p53-positive HRS cells was 76%. MYC and p53 expression levels were significantly correlated (Spearman R 0.68, p=0.004). Of note, misense mutations of the p53 gene were found in two cHL cases, both expressing p53 in 100% of HRS, and relatively low levels of MYC (<35% of HRS in both tumors). Moreover, expression of MYC in HRS cells correlated with cell proliferation index. In the group of NHLs, MYC expression was highly variable among various histologic types with the highest level (>90%) observed in Burkitt lymphoma followed by DLBCL with the latter showing high variability in MYC expression patterns. Indolent NHL types expressed MYC at low percentages of tumor cells. In reactive lymph nodes, MYC was detected in a small subset (approximately 2-10%) of germinal center (GC) cells mostly localized at the periphery of the GCs, but also in occasional small lymphocytes in interfollicular areas.
Summary and Conclusion: Our findings show that MYC oncoprotein is overexpressed in HRS cells of all cHL despite the apparent absence of *MYC* translocations. MYC expression is significantly associated with p53 levels and cell proliferation in HRS suggesting that MYC may be involved in cell cycle deregulation in cHL.

P1057

PERIPHERAL BLOOD LYMPHOCYTE/MONOCYTE RATIO AT DIAGNOSIS AS A PROGNOSTIC FACTOR OF SURVIVAL IN CLASSIC HODGKIN LYMPHOMA

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Background: Most patients with Hodgkin Lymphoma (HL) have an excellent prognosis. However, there is a diversity of factors that can affect their outcome. Several studies have investigated the effect of lymphocyte-monocyte ratio at diagnosis on the clinical course of patients with HL, and have demonstrated that a low ratio (with variable cut-off between studies) is associated with a worse outcome.
Aims: The aim of this study was to determine whether peripheral blood absolute lymphocyte count/absolute monocyte count (ALC/AMC) ratio at diagnosis had a prognostic impact on overall survival of patients with classical HL.
Methods: We included 266 patients with classical HL diagnosed between 01/01/1990 and 31/12/2012. Receiver operating characteristic curves and area under the curve were used to determine cut-off values for the ALC/AMC ratio at diagnosis, while Kaplan Meier analysis was used to compare overall survival based on the ALC/AMC ratio.
Results: The median follow-up period was 7.4 years (range, 0.04-22.73 years). An ALC/AMC ratio at diagnosis of 2.37 or more was the best cut-off value for survival (CI 0.509-0.694). ALC/AMC ratio at diagnosis with a cut-off of 2.37 showed a prognostic impact in overall survival (p=0.042). The studied population included 132 patients with a ALC/AMC ratio<2.37 and 134 patients with ALC/AMC ratio >=2.37. There was no significant differences in age (p=0.549), gender (p=0.269), leukocyte count (p=0.078), International Prognostic Score (IPS score) (p=0.402) between the two groups, however differences were found in factors like histologic subtype, stage, albumin and hemoglobin levels. In the population with advanced stage disease and "low risk" IPS score (IPS 0,1 and 2), the addition of ALC/AMC ratio seems to be able to divide this patients into 2 subgroups with different overall survival (although not statistically significant (p=0.351)).
Summary and Conclusion: ALC/AMC ratio at diagnosis inferior to 2.37 was identified as a negative prognostic factor in patients with HL, with impact in overall survival. Therefore, this parameter may contribute to a better stratification of patients with HL. Furthermore, in patients with advanced stage disease and low IPS could potentially add prognostic value in the outcome.

P1058

PNEUMOCYSTIS CARINII PNEUMONIA IN PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: Intensive chemotherapy and T-cell immunodeficiency - important risk factors for Pneumocystis carinii pneumonia (PP) in patients with Hodgkin's

lymphoma (HL). PP may occur in connection with fulminant and it was necessary prevention and early diagnosis.
Aims: The aim of this study was to investigate the incidence of Pneumocystis carinii pneumonia in patients with Hodgkin's lymphoma.
Methods: During the period from 1999 to 2013 PP occurred in 22 (3%) patients of the 741 patients with HL treated with intensive chemotherapy. Ratio of M: F was 1:1.1 and median age was 32 years (range 18-65 years). Advanced stages (IIB-IV) were archived in 82% of patients. PP diagnosis was established according to bacteriological examination (more than 5 Pneumocystis in the preparation with RNIF method) and by computed tomography (CT).
Results: PP developed after 4 or more courses of chemotherapy and has always been associated with other pathogens: Herpes simplex virus in 72% of patients, mixed bacterial and fungal flora in 33% of patients. All patients underwent combined antimicrobial therapy with high-dose intravenous Cotrimoxazole. Mechanical ventilation in the intensive care unit required 10 lung patients (45%). Overall mortality was 32% (7 patients). There was high mortality rate (80%) in patients with relapsed and refractory disease course.
Summary and Conclusion: Patients with Hodgkin's lymphoma during intensive chemotherapy need PCP prophylaxis Cotrimoxazole. When the respiratory failure and CT signs of interstitial pneumonia need for early implementation of flexible bronchoscopy with lavage complex microbiological research for early detection of Pneumocystis Carinii.

P1059

TREATMENT OF ELDERLY PATIENTS WITH HODGKIN LYMPHOMA: EXPERIENCE OF A SINGLE UNIT
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Background: It is estimated that patients over 60 years of age account for about 20% of Hodgkin Lymphoma (HL) patients. Outcomes for these patients are inferior when compared with younger patients. The inferior outcome of elderly HL patients is both related to a biologically more aggressive disease, treatment toxicity and comorbidities. Although ABVD is regarded as the standard of care for most elderly HL patients, a common treatment approach is lacking and innovative approaches incorporating new drugs are awaited.
Aims: The aim of this study was to evaluate treatment approach, outcomes and toxicities in elderly patients with HL.
Methods: We retrospectively analyzed 44 HL patients referred and treated at our institution between 2003 and 2012.
Results: At diagnosis, median age was 71 years (range, 60-86 years), sex ratio 23F/21M; 45% presented in stage I-II and 55% in stage III-IV, 25% had bulky disease and 50% had B symptoms. According to risk stratification, 59% of patients were in the advanced group, and the IPS score was 0-1 for 8%, 2-3 for 46% and 4-7 for 46% of patients. ECOG was 3-4 in 7%, whereas 70% of patients had significant comorbidity. All patients received treatment, 2 with palliative and 42 with curative intent. Combined modality was used in 30% of cases, chemotherapy alone in 61% and radiotherapy alone in 5%. ABVD was the chemotherapy schedule more frequently used (78%), while 18% of patients were treated with ChlVPP. Half patients modified initial treatment and in 29% of patients treated initially with ABVD, treatment was changed to ChlVPP; toxicity was the main reason for treatment modification (81% of cases). Febrile neutropenia occurred in 30% of patients, while bleomycin lung toxicity occurred in 35% of ABVD treated patients, being fatal in 36% of cases; acute cardiac toxicity only occurred in one patient. Overall response rate (ORR) was 77% (complete response - CR 70%) in all treated patients; in 20% of patients response was not available due to early death. In the group of patients treated with ABVD like schedules, the ORR was 84% (CR 78%). With a median follow-up of 6.5 years, overall survival and progression free survival at 4 years were 56.7% and 53% respectively. Eighteen patients died, with 39% of toxic deaths. Late toxicity of treatment occurred in 8 patients, being cardiac toxicity the more frequent.
Summary and Conclusion: Despite its increased toxicity in the elderly HL patients, ABVD is still considered the standard of care, with an ORR of 84% in this study population. The impact of comorbidities and assessment of functional status may help in tailoring treatment, while the use of new drugs may both reduce toxicity and increase outcome in advanced stage disease.

Non-Hodgkin & Hodgkin lymphoma - Biology 2

P1060

IMPAIRED RHOA FUNCTION LEADS TO DEVELOPMENT OF ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA
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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subtype of peripheral T-cell lymphoma with a poor prognosis. Although frequent mutations in *TET2, IDH2*, and *DNMT3A* have been reported in AITL, the precise pathogenesis specific to AITL was unknown. We recently identified recurrent mutations in *RHOA* (p.G17V hotspot mutation, G17del, and A161E) in around 70% of AITL and PTCL-NOS harboring AITL features, but these mutations were undetected in other hematologic malignancies. This finding strongly suggested that dysregulation of RHOA contributes to the pathogenesis of AITL. RHOA is a member of the Rho family small GTPases that regulate a various biological activities. Similar to Ras proteins, Rho GTPases act as molecular switches that shuttle between an active GTP-bound state and an inactive GDP-bound state, and are activated by guanine exchange factors (GEFs).
Aims: To characterize the function of RHOA mutants identified in AITL, we evaluated the activity and the biological influence of RHOA mutants compared to wild-type RHOA.
Methods: To evaluate the level of active GTP-bound RHOA, pull-down assay was performed using GST fusion Rho-binding domain of Rhotekin. For GEF-binding assay, cell lysate was extracted from NIH3T3 cells expressing active form of ECT2, one of the Rho-specific GEFs, and then pulled down with GST fusion RHOA protein. To investigate the biological effects of RHOA mutants, serum response factor-responsive element (SRF-RE) reporter assay was performed and actin stress fibers were visualized using a fluorescence-labeled phalloidin. To analyze the function of RHOA in T cells, we established Jurkat cells inducibly expressing wild-type or G17V RHOA protein, examined cell proliferation, and performed RNA sequencing for gene expression profiling.
Results: Three-dimensional structures of the G17V RHOA predicted compromised binding of GDP/GTP. Actually, when expressed in NIH3T3 cells, a substantial fraction of wild-type RHOA protein bound GTP in the Rhotekin pull-down assay, whereas no GTP-bound form was pulled down for the G17V RHOA mutant. Moreover, the G17V RHOA mutant reduced GTP binding of wild-type RHOA proteins, suggesting dominant-negative nature of G17V RHOA. The G17V RHOA mutant more tightly bound to ECT2 than wild-type RHOA, as was previously described for the G17A RHOA. The G17del and A161E mutants also exert a dominant-negative impact on wild-type RHOA. Consistently, G17V RHOA failed to activate transcription from SRF-RE and attenuated the actin stress fiber formation in NIH3T3 cells. In Jurkat cells, wild-type RHOA suppressed cell proliferation and the G1 to S cell cycle progression, whereas G17V RHOA did not. Gene Set Enrichment Analysis revealed that SRF pathway was enriched in cells expressing wild-type RHOA, but not in those expressing G17V RHOA.
Summary and Conclusion: We demonstrated that all the RHOA mutants identified in AITL abrogate conversion to active form and further inhibit activation of wild-type RHOA in a dominant negative manner, presumably due to sequestration of RhoGEF. Moreover, wild-type RHOA has the potential to serve as a tumor suppressor in T cells, whereas G17V RHOA does not have this effect. These findings suggest that reduced activity of RHOA may play a critical role in the pathogenesis of AITL.

P1061

BRAFV600E MUTATIONS IN HEMATOPOIETIC STEM CELLS OF HAIRY CELL LEUKEMIA PATIENTS SUGGESTS A STEM CELL ORIGIN
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Background: Hairy cell leukemia (HCL) is a chronic lymphoproliferative disorder of mature B cells characterized by somatic *BRAF*V600E mutations, cell-surface expression of the pan-B-cell marker CD19 and monotypic surface immunoglobulins with clonal rearrangements of immunoglobulin heavy and light chains. Despite possessing these stereotypic features, the cell of origin of

HCL has been long debated, and the normal counterpart of HCL cells has not been identified.
Aims: We hypothesized that HCL may originate from immature hematopoietic cells, and therefore we sought to determine at which state of hematopoietic development somatic *BRAF*V600E mutations can be detected in HCL patient samples and how the *BRAF*V600E mutation affects normal hematopoietic development.
Methods: We utilized FACS to evaluate and purify HSCs from HCL patients bone marrow samples to assess for the presence of the *BRAF*V600E mutation, both before and after treatment with BRAF inhibitors. We also evaluated the functional consequences of conditionally expressing of *BRaf*V600E in the mouse hematopoietic system, expressing it from its endogenous locus at different stages of hematopoiesis, including in HSPCs and committed B cells.
Results: We found that the *BRAF*V600E mutation is present in hematopoietic stem cells (HSCs) in HCL patients, and that these patients exhibit marked alterations in hematopoietic stem/progenitor cell (HSPC) frequencies. Quantitative sequencing analysis revealed a mean *BRAF*V600E mutant allele frequency of 4.97% in HSCs from HCL patients. Moreover, tranplantation of *BRAF*V600E mutant HSCs from an HCL patient into immunodeficient mice resulted in stable engraftment of *BRAF*V600E mutant human hematopoietic cells revealing the functional self-renewal capacity of HCL HSCs. Consistent with the human genetic data, expression of *BRaf*V600E in mouse HSPCs resulted in a lethal hematopoietic disorder characterized by splenomegaly, anemia, thrombocytopenia, increased circulating soluble CD25 (sCD25), and increased clonogenic capacity of B-lineage cells - all classic features of human HCL. In contrast, restricting expression of *BRaf*V600E to the B-cell compartment did not result in disease. Treatment of HCL patients with vemurafenib, an inhibitor of mutated BRAF, resulted in normalization of HSPC frequencies and increased myeloid and erythroid output from HSPCs. Likewise, treatment of wildtype mice transplanted with *Mx1*Cre+ *BRaf*V600E mutant bone marrow cells revealed improvement in anemia and hepatosplenomegaly with *in vivo* therapy.
Summary and Conclusion: Overall, these studies demonstrate that specific somatic genetic abnormalities present in hairy cell leukemia are present in immature hematopoietic cells including HSCs, thereby providing support for a model in which mature B-cell malignancies can initiate in the HSC compartment. Moreover, these data suggest that the use of therapies targeting MAP kinase signaling in HCL may lead to durable remissions not only by eliminating the mature leukemic cells but also through targeted inhibition of signaling and survival in HCL initiating cells.

P1062

INTRACELLULAR SIGNALING PATHWAYS IN HAIRY CELL LEUKEMIA (HCL) AND THEIR PROGNOSTIC RELEVANCE

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Background: B-RAF mutations are considered a hallmark of HCL, pointing to constitutive activation of the corresponding signaling pathway.
Aims: To investigate the activation of various signaling pathways interacting with B-RAF mediated cascade in HCL and their potential clinical significance. We therefore evaluated the expression of molecules upstream (Akt, B-RAF, ERK) and downstream (p70, 4EBP1) of m-TOR.
Methods: p-Akt, pm-TOR, B-RAF, pERK, pp70, p4EBP1 were studied by immunohistochemistry in bone marrow trephine biopsies of HCL patients at diagnosis and were correlated to prognosis. The median values of expression of the molecules under study were used as cut-off values for defining low/high expression. The endpoint of the present study was time to 2nd treatment (TT2T).
Results: 77 HCL patients were studied: 60 males, median age: 54 years. The median percentage of infiltration by hairy cells was 80% (10-100%). All cases were annexin-1 positive, while CD11c, CD25 and CD103 were positive in 96%, 98% and 98% respectively. The majority of the patients (82%) received interferon-alpha as initial treatment. Their median follow-up was 10 years (2 months-33 years). 33/77 patients relapsed and required 2nd line treatment. Nuclear expression of pERK, pp70, p4EBP1 was largely absent. Regarding cytoplasmic expression, pAkt median (range) value was 20 (0-90%), with only 3/77 cases being completely negative. B-RAF, cytoplasmic pERK, cytoplasmic pp70 and cytoplasmic p4EBP1 were seen in the vast majority of cases with 100% as the median value for the three former molecules and 80% of positive cells for the latter, respectively. There wasn't any single negative case for B-RAF and pERK and only 5/77 and 2/77 cases were negative for pp70 and p4EBP1. Surprisingly, m-TOR was completely negative in the vast majority of cases (67/77). These findings indicate that pathways other than the classical Akt/m-TOR/4EBP1 are functional in HCL. Pathways emanating either from B-RAF/pERK or pAkt may operate in HCL, accounting for the activation of the effector molecules p70 and 4EBP1, bypassing m-TOR. Thus, among pm-TOR negative/pp70 positive cases, the majority (59/62) expressed pAkt, indicating that pAkt may activate p70 independently from m-TOR. Among pm-TOR negative/ p4EBP1 positive cases, 57/65 were positive for pERK, consistent with activation of 4EBP1 directly by pERK. Still, there were 10 pm-TOR+ cases, all of which expressing pAkt and B-RAF/pERK, indicating that Akt and RAS/B-RAF/MEK/ERK pathways lead to m-TOR activation in a minority of HCL patients. High expression of pAkt (1), pm-TOR (2) and cytoplasmic p4EBP1 (3) was significantly correlated with inferior TT2T (p=0.03, p=0.05, p=0.026, respectively). The combined expression of any two of these 3 molecules could improve the prognostic stratification of the patients [(p=0.01 (1&2), p=0.001(1&3), p<0.0001(2&3)]. Moreover the simultaneous expression of all 3 molecules could identify a subpopulation of patients with a significantly inferior TT2T compared to all other combinations of expression (p<0.0001, median TT2T: 23.4 *vs* 187.9 months respectively).
Summary and Conclusion: The classical pathway Akt/m-TOR/4EBP1 seems to be activated only in a minority of HCL patients. Alternative pathways are operational in HCL, which involve direct activation of the effector molecules p70 and 4EBP1 bypassing m-TOR, by pAkt and pERK respectively. However, the activation of pAkt/m-TOR/4EBP1 pathway confers a significantly inferior prognosis in a disease with a generally benign clinical course.

P1063

EXPRESSION PATTERN AND FUNCTIONAL ROLE OF TOLL-LIKE RECEPTORS IN SPLENIC MARGINAL ZONE LYMPHOMA

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Background: Splenic marginal zone lymphoma (SMZL) is a distinct lymphoid tumor entity that is not associated with a single specific genetic defect, although several genomic abnormalities are described. Recent studies, however, identified recurrent mutations in genes belonging to the B-cell receptor (BCR) and Toll-like receptor (TLR) signaling pathways, thus pointing to their potential central role in the biology of the disease. As for BCR stimulation, antigen selection was previously suggested by the finding of biased immunoglobulin gene usage and distinctive profiles of somatic hypermutation. In contrast, no data are available on the expression pattern and functional role of TLRs in SMZL.
Aims: We aimed at characterizing the expression pattern of TLRs in SMZL cells, and studying their functional role in the activation, proliferation and cell viability of malignant cells *in vitro*.
Methods: Negatively selected primary lymphoma cells circulating in the peripheral blood of 50 patients with SMZL were used for the following analyses: (i) assessment of TLR (TLR1 to TLR10) mRNA expression by RT-PCR and Q-PCR arrays; (ii) determination of the expression of selected TLRs by flow cytometry; (iii) *in vitro* assessment of the activation status of malignant B-cells as defined by the upregulation of CD25 and CD86 by flow cytometry analysis, before and after stimulation of TLR1/TLR2, TLR2/TLR6 and TLR9, with their respective ligands (Pam3CSK4, Malp-2 and CpG-ODN2006, respectively); (iv) analysis of BCR/TLR signaling pathways by Western blot phosphorylated *versus* unphosphorylated ERK, p38, and IKK kinases; and, (v) determination of the proliferation rate and the viability index by Ki67 staining and ATP measurement in cells treated with TLR ligands.
Results: SMZL cells expressed significant levels of TLR1, TLR6, TLR7, TLR9 and TLR10 mRNA. TLR2, TLR4 and TLR8 showed a low variable pattern of expression among patients whereas TLR3 and TLR5 mRNAs were undetectable. SMZL cells also expressed mRNA specific for several TLR signaling molecules and inhibitors (*e.g.* TIR8/SIGIRR). TLR were also studied at protein level by flow cytometry; as expected, TLR1, TLR6, TLR7, TLR8, TLR9 and TLR10 proteins were detected on SMZL cells. Nevertheless, intraclonal variability in the expression of distinct TLR was observed, as well as a certain degree of heterogeneity among different patient samples. Stimulation of TLR1/TLR2, TLR2/TLR6 and TLR9 triggered activation of distinct signaling pathways as demonstrated by the phosphorylation of the IKK and ERK kinases. Moreover, addition of Pam3CSK4, Malp-2 and CpG-ODN2006 to the cell culture induced cell activation as demonstrated by significantly higher expression of CD25 and CD86, although in a heterogeneous manner among different patient samples. Malp-2 and CpG-ODN2006 significantly increased cell viability

compared to untreated cells; Ki67 analysis revealed that cell proliferation was also significantly induced by the same ligands in a proportion of samples.
Summary and Conclusion: SMZL cells show a distinctive pattern of expression of TLR and signaling molecules. *In vitro* stimulation of TLR can induce activation and proliferation of SMZL malignant cells suggesting their possible implication in the disease biology, at least in a proportion of patient samples.

P1064

DROPLET DIGITAL PCR (DDPCR) AND REAL-TIME PCR (RQ-PCR): A HEAD TO HEAD COMPARISON FOR MRD DETECTION IN MYELOMA (MM) MANTLE CELL LYMPHOMA (MCL) AND FOLLICULAR LYMPHOMA (FL)

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Background: Minimal residual disease (MRD) detection is a well described prognostic tool for response evaluation in several hematological malignancies. Real time quantitative PCR (RQ-PCR) has an established role in Mantle cell Lymphoma (MCL), Follicular Lymphoma (FL) and Multiple Myeloma (MM). However, RQ-PCR-based MRD detection is a relative quantification approach, since it requires a reference standard curve. Droplet Digital PCR (ddPCR), based on wide sample partition and Poisson's statistic, allows for a trustworthy absolute quantification.
Aims: This study compares RQ-PCR and ddPCR in the context of IgH and Bcl-2/MBR targets detection for MRD analysis, to assess whether ddPCR could be useful in MRD setting, overcoming some RQ-PCR limits and providing at least similar performances without losing its critical advantages.
Methods: Genomic DNA (gDNA) from 18 MM, 20 MCL and 30 FL patients enrolled in clinical trials and selected for having the IgH (MM and MCL) or Bcl-2/MBR (FL) molecular marker, were employed. Overall 224 samples (179 BM and 45 PB) were analyzed: 95 MM, 69 MCL and 60 FL of whom 70 were taken at diagnosis and 154 after treatment. MRD detection was carried out by RQ-PCR as previously described, [Ladetto M *et al.* BBMT 2000, Ladetto M *et al.* Exp Hem 2001] and results were interpreted according to the Euro-MRD guidelines [van der Velden VHJ *et al.* Leukemia 2007]. ddPCR was performed by the QX100 droplet system (Bio-Rad Inc) on 500ng of gDNA combined with the same Allele Specific Oligonucleotides (ASO) or Bcl-2/MBR primers and TaqMan-probes used in the RQ-PCR. Results were expressed as amount of target copies per 1E+05 cells. ddPCR and RQ-PCR comparability was assessed by means of bivariate correlations between methods analysis (R2.15.1 package irr). Discordances were classified as follows: a positive/negative qualitative discordance was defined as major when the positive result was >1E-04 and minor when ≤1E-04; a quantitative discordance was defined as the presence of two positive results with a quantitative discrepancy >1 log.
Results: MRD analysis was successful in 97% of patients by RQ-PCR (no reliable standard curve was achieved in 2 MCL patients) and 100% by ddPCR. Overall 222/224 samples (99.1%) were evaluated by both methods and a highly significant level of concordance was observed (R^2=0.88) (Fig). MRD detection was fully concordant in 189/222 samples (Cohen's K=0.85) with 137/222 (61.7%) concordantly positive and 52/222 (23.4%) concordantly negative samples. 33/222 (14.9%) were defined as discordant. Only 4/222 (1.8%) samples showed a major qualitative discordance (2MM, 1MCL, 1FL), while 29/222 (13%) were minor qualitative discordances (16MM, 11MCL, 2FL), which most likely reflect Poisson's statistics variation related to the very low number of target copies. 6/222 (2.7%) quantitative discordances were observed (1MM, 4MCL, 1FL). Among qualitative discordances no systematic prevalence of positive cases was observed with either tools (8.6% positive or higher by RQ-PCR, 7.6% positive or higher by ddPCR). Of note, 26/222 samples (11.7%) resulted positive not quantifiable by RQ-PCR. In four of them ddPCR was able to provide a reproducible quantitative result.

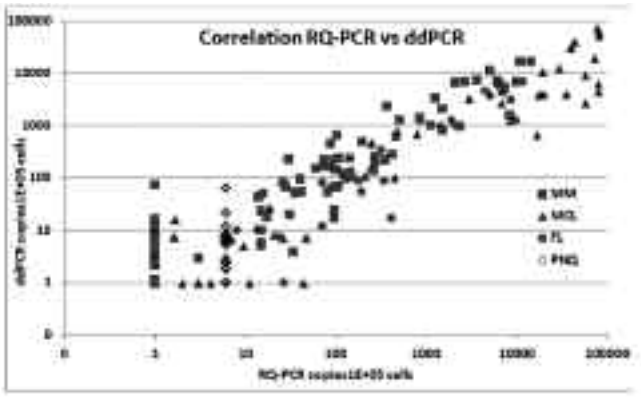

Figure 1.

Summary and Conclusion: This is the first head to head comparison between ddPCR and RQ-PCR in the context of MRD evaluation in lymphoproliferative disorders. We showed that ddPCR is a feasible tool for MRD monitoring in MM, MCL and FL, with similar sensitivity and excellent quantification ability, even at very low MRD levels. Moreover, due to its absolute quantification skill, ddPCR provided considerable technical simplification, bypassing the need of rely on a standard curve, reducing costs and sparing of valuable diagnostic tissue. These features make ddPCR a feasible and attractive alternative method to RQ-PCR for MRD assessment, potentially worthwhile of being considered for multi-lab standardization.

P1065

QUANTIFICATION OF MINIMAL RESIDUAL DISEASE IN CLONAL B-CELL NEOPLASMS USING A HIGHLY SPECIFIC AND SENSITIVE QPCR TARGETING CLONAL IGH REARRANGEMENTS BY USE OF LNA PROBE

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Background: The rearranged immune receptor genes can be used as clonal markers in B-cell neoplasms. Although they are not prognostic for the disease progression, they can nevertheless be employed for identifying clonal populations. In this respect, previous studies have shown that about 85 % of patients with NHL and preB-ALL and the majority of CLL harbor at least one clonal immunoglobulin heavy chain (IgH) gene rearrangement detectable by PCR. A number of quantitative PCR (qPCR) assays have been developed in order to precisely quantify the amount of minimal residual disease (MRD) present in the patients. This tool is now widely used for the tight monitoring of the patients during therapy and numerous studies have shown a close correlation between the amount of MRD after induction therapy and prognosis.
Aims: The aim of this study was to establish a very sensitive and specific qPCR analysis for MRD quantification in clonal B-cell neoplasms taking advantage of the thermodynamic properties of a short locked nucleic acid (LNA) probe. This probe targeted the J genes of the IgH locus enabling short amplicons and high PCR efficiency.
Methods: Peripheral blood was collected from five patients with mantle cell lymphoma (MCL) at time of diagnosis and at follow-up. The clonal IgH rearrangement was PCR amplified and Sanger sequenced in order to design patient specific primers for the predominant IgH rearrangements. The assay encompassed a patient specific forward primer covering the VDJ junction of the IgH receptor while the short LNA probe and the reverse primer targeted the consensus sequence of the J genes.
Results: We here report a highly sensitive and specific qPCR assay for the quantification of clonal IgH rearrangements in B-cell neoplasms with a sensitivity of up to 10^{-5} taking advantage of the thermodynamic properties of LNA. The novelty of this assay is the ultra-short LNA probe that allows for placement of the probe as well as the reverse primer in the consensus part of the J genes. The MRD values obtained using this assay follow the clinical state of the patients.

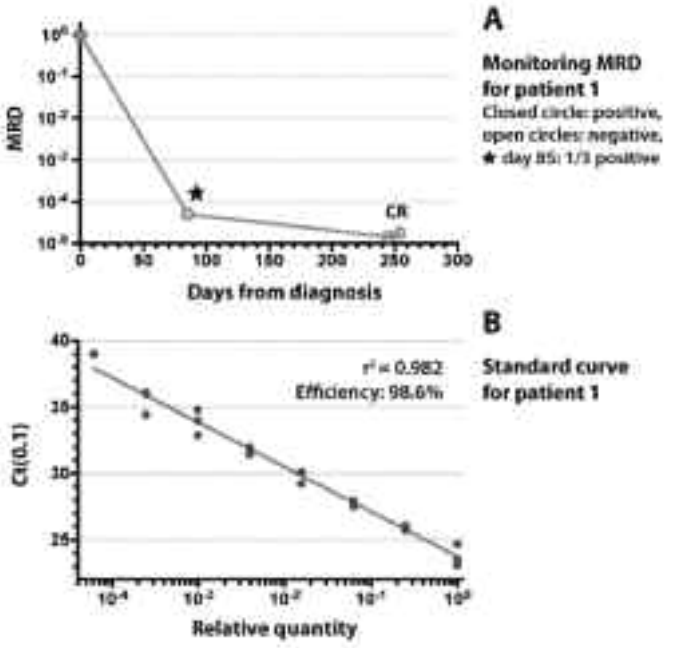

Figure 1.

Summary and Conclusion: We here report a highly sensitive and specific qPCR assay for the quantification of clonal IgH rearrangements in B-cell neoplasms with a sensitivity of up to 10^{-5} taking advantage of the thermodynamic properties of LNA. The novelty of this assay is the ultra-short LNA probe that allows for placement of the probe as well as the reverse primer in the consensus part of the J genes. The MRD values obtained using this assay follow the clinical state of the patients.

P1066

HIGH-THROUGHPUT ANALYSIS OF FULL-LENGTH IMMUNOGLOBULIN TRANSCRIPTS BY TEMPLATE-SWITCHING ANCHORED PCR AND MASSIVE PARALLEL SEQUENCING ON THE PACBIO PLATFORM

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Background: The current BIOMED-2 protocol for the analysis of rearranged immunoglobulin (Ig) genes uses multiplex PCR to amplify the clonally rearranged variable regions. However, somatic hypermutation can impair primer binding, leading from substantial amplification bias to failure to amplify any product. Indeed, BIOMED-2 primers amplify no full-length heavy chain, kappa, and lambda transcripts in 24, 8, and 69% of cases, respectively (van Dongen, 2003). Anchored PCR strategies with sets of nested isotype-specific primers (Osterroth, 1999; Bertinetti, 2006) have excellent success rates in identifying clonally rearranged Ig transcripts.
Aims: To develop a novel method using template-switching anchored PCR (TS-A-PCR) for the amplification and PacBio technology for the analysis of full-length Ig sequences.
Methods: cDNA is synthesized from isolated poly(A)-RNA (Dynabeads; Invitrogen) with gene-specific primers that anneal to the Ig constant region of interest. In the same reaction, the 3' end of the anti-sense cDNA strand is extended by switching to a synthetic oligonucleotide template containing a specific anchor sequence (SMART technology; Clontech). This anchor-tagged cDNA is then PCR-amplified with a forward primer annealing to the added anchor in combination with nested constant region-specific reverse primers. A set of nested barcoding primers further increases target specificity and enables the combination of numerous PCR products in one sequencing run. Amplified cDNA is purified (AMPure® XP beads, Beckman Coulter), dumbbell adapters are added to the termini of 250 ng of DNA and single molecules are sequenced repetitively as rolling circles on the PacBio platform (Pacific Biosciences).
Results: A single TS-A-PCR reaction of 28 cycles reliably yields a distinct band for mu, gamma, kappa and lambda transcripts from $2x10^6$ sorted B cells obtained from cell lines, lymphoma biopsies or peripheral blood. A clonal functional full-length lambda transcript could be readily identified in the Granta-519 mantle cell lymphoma cell line where BIOMED-2 primers failed (Pighi 2013; Koning, 2013). In contrast to the Illumina platform, the insert size (500-700 bp) can be completely covered with the PacBio system with over 20 repetitive reads for a single molecule. Internal alignment therefore yields a highly accurate deduced consensus sequence with very few sequencing errors: the currently observed cumulative error rate for the entire process is $1,26x10^{-4}$ as assessed by sequencing of monoclonal cell lines. Taking advantage of barcoding, just 10% of the capacity of a single PacBio SMRT cell yielded over 2000 B-cell receptor sequences from peripheral blood from a healthy donor, of which >95% were unique. TS-A-PCR performed on serial dilutions of lymphoma or EBV-transformed B-cell lines into a background of other cell lines demonstrated adequate representation of the cellular proportions in the Ig sequences obtained by PacBio sequencing.
Summary and Conclusion: TS-A-PCR is a simple novel method for reliable high-throughput identification of full-length Ig transcripts. Sample preparation can be completed in the course of one day. Avoidance of sequence-specific upstream forward primers appears to prevent potential amplification bias inherent to multiplex strategies, especially for severely hypermutated BCR sequences. Additionally, this method permits in-depth analysis of the BCR repertoire in healthy individuals and patients with lymphoma or autoimmune disease.

P1067

COMPARISON OF CONVENTIONAL CYTOGENETIC ANALYSIS AND WHOLE GENOME SEQUENCING IN GERMINAL-CENTER DERIVED B-CELL LYMPHOMAS IN THE FRAMEWORK OF THE ICGC MMML-SEQ PROJECT

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Background: Germinal–center derived B cell lymphomas (GCB-lymphomas) represent the most common B-cell lymphomas. They include follicular (FL), diffuse large B-cell (DLBCL) and Burkitt (BL) lymphomas. The detection of numerical and structural chromosomal abnormalities in these lymphomas provides essential diagnostic, prognostic and treatment-related information. Moreover, breakpoints of chromosomal translocations can point to genes deregulated in the pathogenesis of lymphomas.
Aims: In the framework of the German BMBF-funded ICGC MMML-Seq-Project, the aim of this study is to compare the detection of chromosomal abnormalities by conventional cytogenetics (CC) and whole genome sequencing. In addition, combined whole genome and transcriptome sequencing is applied to detect hitherto unknown fusion genes.
Methods: Fourteen GCB lymphomas with aberrant karyotype by conventional cytogenetics were analyzed by fluoresence *in situ* hybridization (FISH) (LSI BCL6, LSI MYC (DC, BA), LSI IGH/MYC, CEP8 Tri-color, LSI IGH, LSI BCL2, all from Abbott Molecular), single nucleotide polymorphism (SNP)-arrays (Affymetrix 6.0), whole genome sequencing and transcriptome sequencing according to the standards of the ICGC (www.icgc.org).
Results: A total of 80 chromosomal abnormalities were detected by conventional cytogenetics in the 14 GCB lymphomas. For further analyses, these abnormalities were classified into those detected by sequencing and conventional cytogenetics (group 1; n=28), those detected by conventional cytogenetics but not by sequencing (group 2; n=52) and a third group comprising abnormalities suggested by sequencing but not by cytogenetics (n=960). Comprehensive verification analyses in group 2 were done by SNP arrays and FISH techniques to check breakpoints of abnormalities. This led us to stratify the abnormalities of that group again into two subgroups: aberrations

redefined by sequencing (n=29) and abnormalities not detected by sequencing (n=23). The latter subgroup contained somatic changes involving breakpoints in pericentromeric regions, immunoglobulin V gene breakpoints and subclonal abnormalities. Validation of the aberrations in group 3 is ongoing. From 26 abnormalities yet subjected to validation a total of 19 were verified, from which 8 were associated with fusion transcripts.
Summary and Conclusion: Complete genomic sequencing reveals a complex landscape of somatic mutations and structural aberrations in GCB-lymphomas. The combination of genomic and transcriptomic analysis allows the discovery of potential new fusion transcripts. However, whole genome sequencing might miss some somatic changes detectable by conventional cytogenetics, in particular if these are subclonal or affecting repetitive regions.

P1068

GERMLINE SMALL NON CODING RNAS, PIWIRNAS, ARE EXPRESSED IN HODGKIN LYMPHOMA: THE ROLE OF PIWIRNA-651 AS PROGNOSTIC FACTOR IN HODGKIN LYMPHOMA

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Background: During the last years non-coding RNAs (ncRNAs), have emerged as key regulators of diverse cellular processes including cancer. Piwi-interacting RNAs (piRNAs) are a small ncRNAs (24–35 nt) necessary to protect the genome in germline cells. PiRNAs functions involve transposon repression, DNA methylation, maintaining mRNA stability, protein synthesis and chromatin and genome organization. Recently, piRNA expression has been identified in several tumors, but to date there is no evidence that they are expressed in Hodgkin Lymphoma (HL).
Aims: First, to investigate if HL cell lines and patient lymph nodes express the piRNAs piR-651, piR-20365 and piR-20582, that have been identified disregulated in several tumors. Second, to correlate piRNAs expression levels with clinical characteristics and outcome in HL patients.
Methods: The expression levels of the selected piRNAs were assessed in 5 HL cell lines (L-428, L-1236, L-540, HDLM2, HD-MY-Z), in 12 reactive lymph nodes (RLN) used as controls and 71 HL patients lymph nodes. All patients were diagnosed and treated in a single institution. Median age was 30 yrs [range, 15–89]; 57.7% were male; 32.1% were EBV+. Most frequent histological subtypes were nodular sclerosis (64.2%) and mixed cellularity (16.4%). First-line treatment was ABVD. The piRNA expression was analyzed using custom primers and the PCR product was validated by cloning in topo TA vector (Invitrogen) followed by sequencing. cDNA was synthesized using miScript II RT Kit (Qiagen) and Real time PCR was performed using miScript SYBR Green PCR Kit(Qiagen). PiRNA expression levels were calculated using 2^{-DDCt} method. Statistical analyses were performed using R v2.13 and PASW Statistics 18. Clinical outcomes analyzed were time to relapse (TTR), and overall survival (OS). After a median follow-up of 82 months (range, 2.8–182.2), overall survival was 86%.
Results: The analysis of the piRNAs (piR-651, piR-20365 and piR-20582) showed that all HL cell lines expressed the 3 piRNAs analyzed as well as the patient lymph nodes. piR-651 and piR-20582 were significantly upregulated in LN of HL patients (p=0.0008, and p=0.0001 respectively) in comparison with RLN. Interestingly, the correlation of the piRNAs expression levels with the main clinical characteristics of the patients showed that patients with high B-2-microglobulin levels had significant downregulation of the piR-651(p=0.043), piR-20365 (p=0.017) and piR-20582 (p=0.029). Moreover, a trend for lower levels of piR-20365(p=0.072) and piR-20582 (p=0.054) were correlated to leukocytosis. Interestingly, the expression levels of piR-651 in the patient lymph nodes had an impact on prognosis. First, low expression levels of piR-651 were associated with no achievement of complete response to first line treatment (p=0.007). Secondly, patients with low expression levels of piR-651 had shorter TTR (74 *vs*. 154 months, p=0.004). Finally, patients with low levels of piR-651 had shorter OS (96 *vs*. 169 months; p=0.001). The multivariate analysis identified piR-651 (analyzed as continuous variable) as an independent prognostic marker for TTR (OR, 0.251; 95%CI, 0.083-0.761; p=0.015) and OS (OR, 0.335; 95% CI, 0.11-0.97; p=0.045).
Summary and Conclusion: Low levels of piR-651 were associated with shorter TTR and shorter OS in HL. To our knowledge this is the first evidence that piRNAs are expressed in HL and have a prognostic impact. Nonetheless, further investigation of the piRNAs role in HL is required.
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P1069

ISOLD: A VALUABLE SCORE TO DISCRIMINATE OCULAR LYMPHOMA FROM INFLAMMATORY DISEASES

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Background: Primary vitreoretinal lymphoma (PVRL) is a rare and aggressive subset of primary cerebral tumors associated with poor prognosis. Ocular impairment can also be seen in systemic B-cell lymphomas. Diagnosis of PVRL is challenging and diagnostic delays are frequent. Biological diagnosis is based on cytology of vitreous samples, immunocytochemistry, and molecular biology. The quantification of soluble cytokines represents an attractive alternative and/or complementary technique. Interleukin (IL)-10, produced by lymphoma cells, is a marker of tumoral cell proliferation. In contrast, inflammatory conditions are associated with increased levels of IL-6. Nowadays, IL-10 is assessed by a sensitive flow cytometric multiplex-based technique, the *Cytometric Beads Array*® (CBA) that enables the simultaneous analysis of several cytokines without large sample volumes needed.
Aims: Reevaluating the decisional diagnosis thresholds previously reported in aqueous humor (AH) and vitreous is required for a better and earlier clinical management.
Methods: Our study included all eye fluid samples sent for cytokine analysis for the first time between January 2010 and September 2012. IL10 and IL6 were quantified in 267 AH and 130 vitreous, coming from 23 French hospitals and constituting the screening cohort. Statistical analysis of the results and comparison with final diagnosis, *i.e.* ocular lymphoma (OL) or not (no-OL), allowed us to establish a score based on a linear discriminant function. This score is pondered with a probability of suffering from an OL or not. Because all prediction models must be validated in a new set of patients before clinical practice, we carried out a validation cohort including 59 samples (41 AH and 18 vitreous).
Results: No decisional threshold of IL10 values or IL10/IL6 ratios could be clearly identified because of an overlapping zone, making the discrimination between OL and no-OL difficult. We developed a new diagnostic algorithm to improve the ability to distinguish the two groups allowing to determine a new score named ISOLD (*Interleukin Score for Ocular Lymphoma Diagnosis*). A score value >4.6 is extremely suggestive of an OL diagnosis (probability>99%) and a score value<-4.6 is highly related to a diagnosis of no-OL (probability>99%). In the range between -4.6 and +4.6 ("blurred zone"), each sample is assigned to a probability of presenting an OL which should lead to further investigation. Based on ISOLD, 94% of all samples were correctly classified (probability>99%) in both cohorts and, interestingly, no B-cell OL was underdiagnosed (probability>73%). In the screening cohort, the "blurred zone" included 3 false-positive samples (no-OL with 0<score<4,6). These three patients were considered as having a primary cerebral lymphoma and died before further investigation and treatment. We also report one T-cell OL which did not lead to an increased ISOLD as expected. The validation cohort confirmed the ISOLD relevance, as it classified correctly all OL. The "blurred zone" included 4 cases, out of them two patients were suffering from an ocular toxoplasmosis.
Summary and Conclusion: We validate ISOLD, a new sensitive diagnostic score pondered with a probability, allowing to distinguish OL from inflammatory diseases. This approach would be useful in association with other clinical and biological criteria to reinforce the diagnosis of OL.

P1070

GENOME-WIDE DNA METHYLATION ANALYSIS REVEALS ACTIVATION OF WNT SIGNALING PATHWAY IN PERIPHERAL T-CELL LYMPHOMA-NOT OTHERWISE SPECIFIED

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Background: Peripheral T-cell lymphoma-not otherwise specified (PTCL-NOS) constitutes a heterogeneous group of T-cell malignancy. Resistant to conventional chemotherapy, patients usually have poor disease outcome. Epigenetic silencing of tumor suppressor genes is a major event in the pathogenesis of hematologic malignancies. Searching for biomarkers closely related to tumor progression is critical for better understanding of disease pathogenesis and developing subsequent targeted therapy.
Aims: The purpose of our study is to assess the profile of genetic methylation status in PTCL-NOS.
Methods: Whole genome methylation was analyzed using NimbleGen HG18

Deluxe Promoter HX1 Microarray on six PTCL-NOS patients and six reactive hyperplasia cases as controls. In addition, methylation status of candidate genes was investigated in an extended cohort of 80 patients with T-cell lymphoma using MassARRAY and Methylation-specific PCR (MS-PCR). Expression analysis was conducted in cases with available RNA samples. Biological function of candidate genes was studied in T-lymphoma cell lines Jurkat and H9.
Results: Genome-wide methylation analysis showed distinct DNA methylation profiles in PTCL-NOS comparing with reactive hyperplasia. Principal component analysis clearly discriminated PTCL-NOS patients from control samples. Segregated data showed that aberrant methylated genes were enriched in Wnt signaling pathway, a key signaling cascade in hematopoiesis regulation and carcinogenesis. Among these genes, MassARRAY and MS-PCR confirmed hypermethylation of *SFRP5* and *ASCL1*. *SFRP5* is an important tumor suppressor and antagonist of Wnt signaling cascade. *ASCL1*, a transcription factor, is also implicated in Wnt pathway. In the extended cohort of patients diagnosed with T-cell lymphoma, hypermethylation of *SFRP5* was identified in 32 cases (40.0%), while *ASCL1* was hypermethylated in 37 cases (46.3%). Quantitative PCR identified that gene expression of *SFRP5* and *ASCL1* were accordingly decreased in PTCL-NOS patients compared with controls. In T-lymphoma cell lines Jurkat and H9, treatment of demethylating agent 5-AZA reversed the hypermethylation status of *SFRP5* and *ASCL1*, and retrieved gene expression. Overexpression of *SFRP5* and *ASCL1* by transfection in Jurkat cells resulted in decreased cell proliferation and inhibited Wnt pathway, with decreased expression of targeted genes such as *MYC*, *CCND1* and *LEF1*.
Summary and Conclusion: Taken together, our findings demonstrated a role of aberrant genetic methylation in the pathogenesis of PTCL-NOS. Our data also suggested the involvement of Wnt pathway in tumor progression. Our study provides evidence for targeted therapeutic designs in the future.

P1071

THE RIZ1 GENE EXPRESSION IS SUPPRESSED IN T CELL LYMPHOMA AND THAT EXPRESSION IS CORRELATED WITH THE DECREASED GATA3 GENE EXPRESSION

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Background: We recently reported that aberrant promoter methylation decreases retinoblastoma protein-interacting zinc finger gene (*RIZ1)* expression in adult acute T lymphoblastic leukemia (T-ALL). RIZ1 was isolated as a GATA3-binding protein and showing the characteristics of a negative tumorigenesis regulator. GATA3 is a transcription factor that is expressed very specifically in the T-cell lineage and involved at various stages of thymocyte differentiation. We also found that the *GATA3* expression was decreased in T-ALL, but it was increased by the *RIZ1* re-expression. RIZ1 may be involved in T-ALL development via the *GATA3* repression. T cell lymphoma has a heterogeneous pathogenesis, arising from various stages of T lineage cells. The *RIZ1* and *GATA3* expressions, correlation between these two genes and the *RIZ1* methylation status in T cell lymphoma have not previously been reported. Recently, a highly prevalent somatic mutation of *RHOA* encoding a Gly17Val alteration was reported in AITL and PTCL-NOS. Similar to other epigenetic modulators including *IDH2* and *DNMT3A*, the *RHOA* mutation largely overlapped *TET2* mutations and had similar allelic burdens. We also focused on aberrant *RIZ1* methylation and the *RHOA* mutations in T cell malignancies. Moreover, the *GATA3* mutations cause an autosomal dominant disorder, HDR (*h*ypoparathyroidism, sensorineural *d*eafness, and *r*enal dysplasia) syndrome. The *GATA3* mutation status in T-ALL or T cell lymphoma has not been reported.
Aims: 1) To clarify the relevance of the *RIZ1* and *GATA3* in various T cell lymphomas and compare these findings with those in T-ALL; 2) To assess the *RIZ1* expression and altered promoter methylation status in various T cell lymphomas; 3) To assess the *RHOA* mutation and altered *RIZ1* methylation in T-ALL and various T cell lymphomas; and 4) To examine the hotspot of the *GATA3* mutation in HDR syndrome in these T cell malignancies.
Methods: The *RIZ1* and *GATA3* expressions were examined by quantitative real-time reverse transcription-polymerase chain reaction (PCR) analysis in 39 adult patients newly diagnosed with T-ALL (n=10) and T cell lymphoma (angioimmunoblastic T cell lymphoma; AITL n=12, peripheral T cell lymphoma, not otherwise specified; PTCL-NOS n=9, adult T-cell leukemia/lymphoma; ATLL n=5, lymphoblastic lymphoma; LBL n=2, anaplastic large-cell lymphoma; ALCL n=1). Methylation-specific PCR was performed on the *RIZ1* gene, and mutation of the *RHOA* and *GATA3* genes were screened by direct sequencing. Correlation between the *RIZ1* expression and *GATA3* expression was analyzed by t test.
Results: Mean *RIZ1* expression in all T cell lymphoma cases (n=29) was 2.73 and that of *GATA3* was 3.28. Similar to the *RIZ1* (mean 0.61) and *GATA3* (mean 4.84) expressions in T-ALL (n=10), these gene expressions in T cell lymphoma patients were lower than those in peripheral T lymphocytes from healthy individuals (mean *RIZ1* 6.93, *GATA3* 19.28). Expressions of both *RIZ1* and *GATA3* differed in AITL (n=12, mean *RIZ1* 3.30, *GATA3* 1.75), PTCL-NOS (n=9, mean *RIZ1* 2.35, *GATA3* 2.72) and ATL (n=5, mean *RIZ1* 1.82, *GATA3* 5.35). As in T-ALL, there was a significant positive correlation between the *RIZ1* and *GATA3* expressions in AITL (P=0.004, R2=0.415) and ATL (P=0.038, R2=0.577) but the correlation in PTCL-NOS was not significant. The methylation status of the *RIZ1* in T cell lymphoma differed from that in T-ALL. Methylation of the *RIZ1* promoter was detected in 70% of T-ALL cases (7 of 10), but in only one case of LBL among all T cell lymphomas in this series. Somatic *RHOA* mutations were detected only in AITL (7 of 12, 53.8%). Most of the T-ALL samples showed the *RIZ1* methylation, but none demonstrated the *RHOA* mutations. On the contrary, 53.8% of the AITL samples demonstrated the *RHOA* mutations, but there was no *RIZ1* methylation detected. No *GATA3* mutations were found by sequence analysis in hotspots of the gene in HDR syndrome.
Summary and Conclusion: Similar to that in T-ALL, the *RIZ1* and *GATA3* expressions are frequently decreased in T cell lymphoma. This decreased *RIZ1* expression might be associated with the *GATA3* suppression in AITL and ATL, and the *RIZ1* may be involved in AITL and ATL development via *GATA3* reppression. Although decreased, the *RIZ1* and *GATA3* gene expressions remained higher than those in T-ALL, and may reflect the status of gene expressions in the normal counterpart of T cell malignancies. Even though the *RIZ1* expression was decreased in various T cell lymphomas, the *RIZ1* methylation status differed from that in T-ALL. Moreover, the *RHOA* mutation does not reflect the *RIZ1* methylation status. Silencing of the *RIZ1* may be due to aberrant promoter methylation in precursor T cell malignancies, whereas in mature T cell malignancies, methylation-independent mechanisms of *RIZ1* silencing, such as mutations in promoter sequences, loss of heterozygosity, histone methylation and microRNA, might account for low *RIZ1* expression.

P1072

BACH2 SUPPRESSION IN CD4+ T CELLS MODULATES THEIR RESISTANCE TO APOPTOSIS DEMONSTRATING ITS FUNCTION AS A TUMOR SUPPRESSOR GENE

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Background: Hypereosinophilic Syndromes (HES) cover a wide variety of disorders characterized by sustained hypereosinophilia inducing tissue damage and distinct from secondary causes such as allergic or parasitic conditions. Lymphocytic variant Hypereosinophilic Syndrome (L-HES) has been shown to be caused by the clonal expansion of abnormal T cells producing high levels of IL-5, which drive secondary eosinophil proliferation. Contrasting with the myeloproliferative HES variant (M-HES) associated with PDGFR gene rearrangements, the genetic defect underlying the survival of the L-HES T cell clones is still unknown. Our previous studies have shown a recurrent chromosome 6q deletion leads to repression of specific genes which are located on that deleted segment.
Aims: The goal of this study was to identify potential tumor suppressor gene(s) in the deleted 6q13-22.1 region.
Methods: Cytogenetic analyses of $CD3^{-}CD4^{+}$ T cells from L-HES patients were based on karyotype and FISH. Affymetrix U133 2.0 Plus arrays were used for gene expression profiling of the aberrant $CD3^{-}CD4^{+}$ T cell clones relative to $CD3^{+}CD4^{+}$ T cells from controls. Flow-cytometry, qRT-PCR and shRNA were used to further investigate significant findings.
Results: 6q loss was detected in the CD3-CD4+T-cell nuclei in a third of L-HES patients, thereby indicating that 6q- is the most frequent chromosomal aberration characterizing this disease in our cohort of patients. The established persistence of the 6q13-q22.1 deletion during one patient's disease progression towards lymphoma, also suggested that this abnormality could contribute to the chromosomal transformation. With the final objective of identifying a potential suppressor gene located in the 6q13-22.1 region, a correlation was made between the genes contained in the deletion and their corresponding expression level in CD3-CD4+ T cell clones from 3 L-HES patients, and eleven genes were found to be commonly repressed. As one patient progressed to T lymphoma, only one of the eleven genes, *BACH2,* was continuously repressed, making it the best candidate for tumor suppressor gene in the 6q deletion. Functional analysis using shRNA demonstrated the suppressive properties of BACH2 by showing its apoptosis modulation in $CD4^{+}$ T cells during genotoxic stress. We further demonstrated that one of the suppressive effects of BACH2 on apoptosis in $CD3^{+}CD4^{+}$ T cell lines and $CD3^{-}CD4^{+}$ patient T cells was mediated via transcriptional regulation of the *FAS-L* gene.
Summary and Conclusion: The identification of the 6q-located *BACH2* as a haploinsufficient tumor suppressor gene in $CD4^{+}$ T cells could provide the foundation for a new model of T-cell lymphomagenesis in L-HES. Moreover, by providing evidences that one of the suppressive effects of BACH2 is mediated via FAS-L, in which plays important role in the activated induced cell death (AICD) pathway, thereby suggesting that the deletion of the *BACH2* gene increases the survival of the $CD3^{-}CD4^{+}$ T cells, frequently leading to T-cell lymphoma transformation in L-HES. These data constitute the first experimental evidence that BACH2 exerts a regulatory effect on the FAS-L extrinsic apoptotic pathway in $CD4^{+}$ effector memory T cells. Further investigations on the integrity of the FAS-L pathway modulated by BACH2 in other persisting CD4+ autoimmune, infectious and lymphoproliferative diseases may prove to be rewarding. Finally, our data have demonstrated that BACH2 is a tumour suppressor gene whose repression

modulates apoptosis resistance of CD4+ T cells *in vitro*. To understand further the tumour suppressive role of BACH2 *in vivo*, it would be interesting to follow hemizygous +/- BACH2 knockout mice for autoimmune diseases and neoplastic development in a genotoxic or infectious context.

P1073

SUBMICROSCOPIC GENOMIC REARRANGEMENTS CHANGE GENE EXPRESSION IN T-CELL LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: T-cell large granular lymphocyte leukemia (T-LGL) is a rare lymphoproliferative disorder of cytotoxic lymphocytes. Most patients have a clinically indolent course and a median survival of 10 years, but there is no definitive cure or targeted therapy, and the etiology of T-LGL is still unclear. It is known that malignant cells of T-LGL patients are characterized by dysregulation of important cell signaling pathways that allow them to avoid the activated-induced cell death, and promote cell proliferation and survival. The question remains how those pathways are constantly activated and one of the possibilities may be alterations on the genomic level.
Aims: To better understand the molecular pathogenesis of this disease we decided to search for those genetic alterations in T-LGL patients and MOTN-1 cell line (established from T-LGL patient) that have an impact on gene expression and as a result can influence cell biology.
Methods: mFISH analysis of the MOTN-1 cell line was performed as well as paired-end next generation sequencing (NGS; Illumina HiSeq2000) of this cell line and one T-LGL patient. In addition, chosen 6q region was characterized using high resolution comparative genomic hybridization (FT-CGH) and LM-PCR in three T-LGL patients, as according to previous studies this region is recurrently altered in T-LGL. Gene expression was studied by RNA sequencing (RNAseq; SOLID5500).
Results: mFISH analysis of MOTN-1 revealed several translocations, including t(2;6), t(11;17) and t(12;18), that were confirmed on molecular level using NGS. Moreover, NGS analysis of the whole genome revealed many deletions and amplifications associated with complex rearrangements, most of them within regions 1p, 2q, 6q, 8q, 17q and 18q. RNAseq analysis showed that nine of these rearrangements affected gene expression, for example caused increased expression of *ZEB2* and *GTDC1* (chr2), or decreased expression of *DDI2, CLIC4* (chr1), *IFNGR1*, *MAP3K5, STK39* (chr6), *NFATC1* (chr18). FT-CGH analysis, performed for the 6q23-27 region in three patients, revealed deletion in one of them. It overlapped with the deletion detected in MOTN-1 by NGS and affected *IFNGR1* gene. Deletion was associated with two translocations (with chr14 and 22), and as a result two genes were disrupted: *MAP3K5* and *FAM83F*. In T-LGL patient, analyzed by NGS, few minor alterations were detected, but only two had impact on gene expression: a deletion within Ras oncogene *RAB32* (chr6), and amplification within 14q causing increased expression of 19 genes situated in this region, among them *FON3, RIN3, AKT1, PPP2R5C*. Moreover, whole transcriptome analysis revealed two novel fusion transcripts: *CASP8-ERBB4* in MOTN-1 and *SBF1-PKHD1L1* in one T-LGL patient, and genes differently expressed in studied samples compared to controls, among them many involved in apoptosis (*VAV3, SOCS3, TNFRSF25, BCL2, CASP8, RBM5, TLR2, TRIO, PPP3CC, DAPK2, ARHGEF12, DAPK1*).
Summary and Conclusion: Present study showed that submicroscopic genomic rearrangements change gene expression in T-LGL and thus might have an impact on cell biology. Several genes involved in rearrangements were previously linked to cancer and survival pattern that characterizes T-LGL cells. It would be worth to analyze whole genomes and transcriptomes in larger cohort of patient in order to find the connections between genome, transcriptome and signaling pathways that lead to malignant transformation and T-LGL progression.

P1074

ROLE AND RELATIONSHIP OF INTERLEUKIN-6 AND -15 IN T LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: T large granular lymphocytes leukemia (T-LGLL) is a rare disease characterized by the abnormal expansion of T large granular lymphocytes (LGLs) with cytotoxic activity. Currently, the etiology of this disease is still largely unknown. Several data support the hypothesis that the inciting event is represented by the persistence of antigenic stimulation, maintained by the abnormal release of cytokines, mainly IL-6 and IL-15, establishing an inflammation status not achieving resolution. Recently, we showed that IL-6 and soluble IL-6Ra were highly expressed and released by patients' LGL-depleted peripheral blood mononuclear cells (PBMC), accounting for a trans-signaling process. IL-6 trans-signaling is critically involved in inflammatory disease. Additionally, LGL proliferation is maintained through the activation of many survival signaling pathways, as JAK/STAT and RAS/MEK/ERK pathways. Recently, activating STAT3 mutations have been demonstrated in nearly 30% of LGLL patients.
Aims: We studied the cell source of IL-6 and IL-15, IL-15 contribution to sustain IL-6 trans-signaling, and in turn inflammation, and the role of these cytokines to induce LGL survival.
Methods: Thirty T-LGLL patients were included in this study. Patients' LGLs percentage in PBMCs ranged from 25 to 90%. In order to assess IL-6-producing cells we performed analysis by confocal microscopy using antibodies against IL-6 and CD14. Plasma IL-6 concentration was analyzed by ELISA. We analyzed cytokines expression level by Real Time (RT)-PCR and activation of ERK and STAT3 by Western Blot (WB) analysis. Finally, we assessed LGL viability in PBMCs culture following IL-6 and 15 stimulation by AnnexinV cytometer assay. Sequencing analysis was performed to study STAT3 mutations.
Results: By immunofluorescence assay, we demonstrated that IL-6 is specifically and strongly produced by CD14+ cells, as far as IL-15. According to plasma IL-6 levels, cases characterized by a disease with less than 55% circulating LGLs (low LGL burden) showed significantly higher IL-6 concentrations with respect to patients with LGLs>55% (high LGL burden) ($p<0.05$). Interestingly, we found that patients carrying STAT3 mutations were only included in this last group, characterized by low IL-6 and high LGL percentage. By RT-PCR and ELISA, we found that IL-15 strongly induced IL-6 expression in patients PBMCs, while inhibited IL-6Ra expression in leukemic LGLs, indicating that IL-15 favors IL-6 trans-signaling. By WB analysis we showed that both IL-6 and IL-15 were able to activate STAT3 and ERK, then sustaining LGL survival. To confirm that trans-signaling takes place in T-LGLL we observed that IL-6 increased its pro-survival effect when added to the culture with IL-6Ra, by reducing the percentage of apoptotic cells.
Summary and Conclusion: Our data pointed to the importance of the monocyte/macrophage lineage in LGLL patients, as source of both IL-6 and IL-15 sustaining LGL survival and inflammatory environment. Moreover, our results suggested that the progression of the disease might include two different steps: one characterized by low percentage of LGLs and high secretion of IL-6 and a second one where patients display a high burden disease with low IL-6 concentrations. Accordingly, the first one is mostly sustained by extrinsic factors contributing to the relevant inflammatory background, whereas in the subsequent step, characterized by a high lymphocytosis, LGL disease goes on independently from exogenous stimuli, likely becoming self-maintaining due to the contribution of the emerging STAT3 mutations.

P1075

IDENTIFICATION AND VALIDATION OF POTENTIAL DRUGS INHIBITING CONSTITUTIVELY ACTIVE MUTANT AND WILD TYPE STAT3 IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: Constitutive hyperactivation of the STAT3 transcription factor is often observed in cancer. Recently, we discovered that 40% of patients with T-cell large granular lymphocytic (LGL) leukemia had somatic activating point mutations in the Src Homology 2 (SH2) domain of the *STAT3* gene. Moreover, STAT3 appears to be phosphorylated in all LGL leukemia patient samples indicating that different molecular mechanisms activate the JAK-STAT pathway. We therefore hypothesized that inhibition of STAT3 activating kinases or STAT3 itself could induce apoptosis of LGL cells. However, it is not well known which kinases activate and which drugs inhibit STAT3 in malignant cells and whether hyperactive STAT3 mutants behave differently compared to wild type STAT3.
Aims: Here, we aimed to identify drugs that inhibit STAT3 function and determine if mutant STAT3 and wild type STAT3 react differently to these drugs. In addition, we wished to ascertain if STAT3 inhibition is sufficient to induce apoptosis in cells with constitutively active STAT3, including LGL cells.
Methods: High-throughput drug sensitivity testing was performed with a compound collection containing over 300 approved and investigational oncology drugs including many kinase inhibitors (such as JAK, SRC, VEGFR, mTOR, MEK, and CHK) and small molecule STAT3 inhibitors (Stattic, LLL12, Sta-21). All drugs were tested in eight different concentrations over a 10,000-fold concentration range. In addition to patient LGL cells (n=4), mutant STAT3 (Y640F) transformed Ba/F3 cells and HEK293 cells containing a STAT3 specific luciferase reporter gene element (HEK-SIE) were used in the screens. Patient sample cells and the Ba/F3 cells were incubated in 384-well plates for three

days with the drugs then cell viability measured with CellTiter-Glo. STAT3 induced luciferase activity in the HEK-SIE cells was analyzed after the cells were incubated for 6 or 24 hours with the drugs using the ONE-Glo luciferase assay system.
Results: Preliminary results indicate that PI3K/mTOR inhibitors such as PF-04691502 and INK128 effectively decrease the luciferase signal in both mutant Y640F and wild type STAT3 transfected HEK-SIE cells, demonstrating inhibition of STAT3 functional activity. In addition, PI3K/mTOR inhibitors significantly decreased the viability of mutant STAT3 transformed Ba/F3 cells when compared to wild type cells. Interestingly, JAK inhibitors (*e.g.* Ruxolitinib, Gandotinib) did not effectively inhibit mutant STAT3 activity in HEK-SIE cells, whereas IL6-induced wild type STAT3 activity was completely blocked. A BET family inhibitor (JQ1+), glococorticoids (Dexamethasone, Methylprednisolone) and an Aurora A kinase inhibitor (alisertib) showed specific killing against mutant STAT3 transformed Ba/F3 cells. LGL patient sample cells showed high sensitivity against glucocorticoids, the histone deacetylase inhibitor quisinostat and PF-04691502 when compared to healthy CD8+ T-cells. However, no increased apoptosis was observed with JAK or mTOR inhibitors. Of the tested small molecule STAT3 inhibitors, LLL12 showed the best efficacy against mutant and wild type STAT3 cells.
Summary and Conclusion: Our results suggest that JAK inhibitors will lack efficacy in STAT3 mutated diseases. However, our *in vitro* drug screens highlight some other promising agents including PF-04691502, alisertib and JQ1+. Additional experiments are ongoing to validate whether these drugs clearly function through the STAT pathway when causing cell death. As the STAT3 pathway is activated in many other cancer types as well, the results may be applicable to a variety of different malignancies.

P1076

COMPREHENSIVE CYTOGENETIC AND MOLECULAR GENETIC CHARACTERIZATION OF T-PLL

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Background: T-cell prolymphocytic leukemia (T-PLL) is a rare, mature T-cell lymphoproliferative disorder. Prognosis for patients with T-PLL remains poor, with a median overall survival of less than one year. Only few patients with T-PLL have been analyzed with regard to cytogenetic and molecular genetic aberrations. However, it is known that most T-PLL cases harbor chromosomal abnormalities including mainly chromosome 14 (involving the *TCRA/D* locus and the proto-oncogene *TCL1A*), chromosome 8 and the X-chromosome (activation of the oncogene *MTCP1*).
Aims: 1. Comprehensive cytogenetic and molecular characterization of T-PLL, 2. Identification of potential correlations between the respective markers.
Methods: The cohort comprised 57 T-PLL cases (35 male, 22 female patients). Median age was 70.8 years (range: 32.7-86.6 years). T-PLLs were characterized using immunophenotyping and cytomorphology. 57 patients were investigated using chromosome banding analysis (CBA) and 53 of these in addition by FISH for the detection of deletions of *ATM* and *TP53* and for *TCRA/D* rearrangements. Array CGH (Roche NimbleGen, Madison, WI) was performed in 46 cases, as well as mutation analyses for *BCOR*, *ATM* and *TP53* (n=37, n=42, n=44, respectively; Roche 454, Branford, CT).
Results: With respect to CBA, aberrant karyotypes were observed in 43/57 cases (75.4%), 5 patients showed a normal karyotype, whereas for 9 cases no CBA data was available due to insufficient cell proliferation. In all 5 cases with normal karyotypes and in the 9 cases without metaphases, chromosomal abnormalities or molecular mutations were detected using FISH and sequencing analyses. In more detail, combining CBA and FISH data revealed in 44/57 (77.2%) cases an inv(14)(q11q32)/t(14;14)(q11;q32) (n=40) or t(X;14)(q27;q11) (n=4), respectively. A gain of 8q was observed in 19/57 (33.3%) cases. Based on FISH data, *ATM* deletions were detected in 30/53 (56.6%), *TP53* deletions in 13/53 (24.5%) patients. By array CGH, a deletion within the *BCOR* gene (a BCL6 corepressor located on chromosome Xp11.4) was detected in 3 patients, prompting us to screen for mutations in the *BCOR* gene, which were detected in another 4/37 patients. Further, mutations in *TP53* were observed in 6/44 (13.6%) and *ATM* mutations in 29/42 (69.0%) cases. We correlated mutations in *TP53, ATM* and *BCOR* with chromosomal aberrations. In 4/6 (66.6%) patients with *TP53* mutations an accompanying *TP53* deletion was observed, while only 5/32 (15.6%) *TP53* wild-type cases harbored a *TP53* deletion (p=0.042). 18/29 (62.1%) patients with *ATM* mutations showed an *ATM* deletion, whereas only 2/13 (15.4%) *ATM* wild-type cases possessed an *ATM* deletion (p=0.011). For *BCOR* mutations, no association with any other parameter was revealed. A correlation was observed for chromosomal aberrations involving chromosomes 8 and 14, as the vast majority of cases with gain of 8q harbored a 14q11 rearrangement (17/19, 89.5%).
Summary and Conclusion: CBA, FISH and mutation analysis of *TP53, BCOR* and *ATM* revealed genetic abnormalities in all 57 analyzed cases, most frequently involving the *TCRA/D* locus (14q11) (44/57; 77.2%) activating the proto-oncogenes *TCL1A* on chromosome 14q32 or *MTCP1* on chromosome Xq28. Deletions were detected for *ATM* (56.6%) and *TP53* (24.5%), and a gain of 8q was observed in 33.3% of all cases. *ATM* mutations and *TP53* mutations were detected in 69.0% and 13.6% of patients, respectively. *BCOR* mutations were observed for the first time in a lymphoid malignancy with a frequency of 10.8%. The prognostic relevance of these aberrations has to be determined.

P1077

MIR-150 REGULATES IL-22-CCL20-CCR6 AUTOCRINE PATHWAY IN METASTATIC CUTANEOUS T- CELL LYMPHOMA

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Background: MicroRNA(miRNA)s are involved in regulating diverse cellular pathways. Altered expression of various oncogenic or tumor suppressive miRNAs has been identified in some lymphomas. Among these, a tumor suppressive miRNA, microRNA-150 (miR-150) has proven to be a key miRNA involved in the development of hematological malignancies. We recently showed that miR-150 expression is downregulated in cutaneous T-cell lymphoma. Inoculation of CTCL cell lines into NOD/Shi-scid IL-2γnul (NOG) mice led to CTCL cell migration to multiple organs. However, prior transfection of the cells with miR-150 substantially reduced the invasion/metastasis by directly downregulating CCR6, a specific receptor for the chemokine CCL20.
Aims: The aim of this study is to determine the relationship between a chemokine and a miRNA affecting tumor invasion and metastasis in cutaneous T-cell lymphoma.
Results: We found that IL-22 and its specific receptor subunit, IL22RA1, were aberrantly overexpressed in advanced CTCL, and that productions of IL-22 and CCL20 were increased in cultured CTCL cells. IL22RA1 knockdown specifically reduced CCL20 production in CTCL cells, suggesting IL-22 upregulation may activate production of CCL20 and its binding to CCR6, thereby enhancing the multidirectional migration potential of CTCL cells. CTCL cells also exhibited nutrition- and CCL20-dependent chemotaxis, which were inhibited by miR-150 transfection or CCR6 knockdown. CD4+ T helper cells are divided into T_H1, T_H2, T_H17, and T_H22 subsets. Among these, the TH22 subset produces only IL-22, while T_H17 cells produce both IL-17 and IL-22. Normally, IL-22 activates CCL20 transcription by binding to the IL-22RA1/IL-10RB receptor, which is not expressed in lymphoid organs or lymphocytes. In the present study, we further found that both IL-22 and its receptor subunit, IL-22RA1, are aberrantly overexpressed in advanced CTCL, but IL-17 is not expressed, suggesting CTCL is derived from the T_H22 subset. For molecular targeting therapy, we are currently studying for effects of candidate target molecules by use of anti-CCL20 antibody, siCCR6, siCCL20 or miR-150 injection against CTCL cells transplanted NOG mice.
Summary and Conclusion: We conclude that, in the presence of continuous CCR6 upregulation accompanied by miR-150 downregulation, IL-22 activation leads to continuous CCL20-CCR6 interaction in CTCL cells and, in turn, autocrine metastasis to distal organs. Our data presents the first evidences demonstrating an invasion/metastasis mechanism in advanced cutaneous T cell lymphoma.

P1078

IN VITRO AND IN VIVO EFFECT OF ATO/IFN/AZT FOR ADULT T CELL LEUKEMIA/LYMPHOMA

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Background: Despite of clinical efficacy of combination chemotherapy with arsenic trioxide (ATO), interferon alpha (IFN) and zidovudine (AZT) for adult T cell leukemia lymphoma (ATL), precise mechanism of anti-ATL effect largely remain to be elucidated.
Aims: We herein explore the *in vitro* and *in vivo* effect of ATO/IFN/AZT for ATL using ATL cell line, primary ATL cell and ATL xenotransplantation model.
Methods: ATL leukemic cell line (S1T, non-Tax expressing, HTLV-1 integration site confirmed), HTLV-1-infected cell line (MT2, tax expressing) and primary ATL cells from acute and chronic type ATL patients were employed for this experiment. As an ATL xenotransplantation model, NOD/SCID/Jak3 null mice (NOJ mice) were inoculated with acute or chronic type ATL peripheral blood mononuclear cells and treated with ATO/IFN/AZT.
Results: Cell proliferation analysis against S1T and MT2 at 48 hours was performed with 1 uM of ATO, 1000U/ml of IFN and 5 uM AZT. Despite marginal inhibitory effect of IFN/AZT against MT2, substantial inhibitory effect was observed in S1T cell line In addition, combination of ATO and IFN/AZT showed synergistic effect on S1T. MT-2 cell was only sensitive to ATO treatment. ATO has been known to induce cell apoptosis through mitochondrial stress, we next assessed apoptotic effect of AZT/IFN in S1T. Treatment of S1T with AZT/IFN increased cleaved caspase-3, 7, 8, 9 and cleaved PARP expression and more

pronounced with ATO/AZT/IFN in combination; however treatment of S1T with only ATO did not clearly induced apoptosis. Cytotoxic effect of AZT/IFN was inhibited by pan-caspase inhibitor. NF-kB is known to require ATL cell survival. IFN/AZT treatment decreased phospho-IKK, phopho-IkB,and nuclear transloaction of NFkB. This pathway was mediated by decreased expression of TRAF6. IFN/AZT treatment induced robust phosphor-STAT1 expression, which lead to increased expression of IRF1, IRF7, IRF9 and p53 expression in S1T. We next established a novel experimental model for ATL using NOJ mice. Freshly isolated peripheral blood mononuclear cells from patients with ATL were inoculated intravenously into the tail vein, intraperitoneally, or subcutaneously. For cell doses of 1 x 10^7 PBMSCs/mouse, 100% of the mice succumbed to the ATL within 100 days (n=9). Additionally, increased levels of plasma soluble human IL-2 receptor were detectable as early as 2 weeks after inoculation. Immunohistochemical analyses of ATL-inoculated NOJ mice showed massive infiltration of human CD4/CD25+ leukemia cells in various organs including liver, spleen and lung. Circulating human CD4/CD25 positive cells were also detected in peripheral blood, and were also found in the inguinal region of NOJ mice where ATL cells were inoculated subcutaneously. Using this model, the effect of ATO/IFN/AZT for primary ATL cell was evaluated. 1x10^7 ATL patient PBMSCs/mouse were inoculated into NOJ mice intra-peritoneally and waited for 18 day before ATO/IFN/AZT combination therapy. With only 14 days treatment, treated group showed lower soluble IL2R receptor at day 40 and survived longer than untreated group (n=7 each group, p<0.05).
Summary and Conclusion: Conclusion: While several group reported less *in vitro* effect of AZT/IFN against ATL, we observed induction of apoptosis, inactivation of NFkB pathway and robust up-regulation of intereron related genes in an ATL leukemic cell line (S1T). This can be one of the anti-ATL mechanisms by ATO/INF/AZT. We observed only marginal anti-ATL effect in MT-2 and other ATL cell lines, thus anti-ATL effect of AZT/IFN may limit to subgroup of ATL. In addition, we first demonstrated *in vivo* effect of ATO/IFN/AZT by using NOJ xenograft model transplanted with primary ATL cells. This model can be further utilized for extracting ATO/INF/AZT sensitive ATL patients.

P1079

DOLASTATINS TARGET INTRATUMORAL DENDRITIC CELLS LEADING TO REMODELLING OF THE TUMOR MICROENVIRONMENT AND INDUCTION OF LONG-LASTING TUMOR-SPECIFIC T CELL RESPONSESS
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Background: Antibody drug conjugates (ADCs) are emerging as powerful treatment strategies with outstanding target specificity and high therapeutic activity in cancer patients. Brentuximab vedotin represents a first-in-class ADC directed against CD30-positive malignancies. We hypothesized that its sustained clinical responses could be related to the stimulation of an anti-cancer immune response. Using allogeneic stem cell transplantation of CD30+ lymphomas as clinical model we were able to establish that treatment with brentuximab vedontin +/- donor lymphocyte infusions induces tumor-specific CD4+ and CD8+ T cells leading to longterm remission.
Aims: To dissect the underlying mechanisms we combined the study of murine tumor models with an in-depth analysis of immune responses induced in patients receiving Brentuximab vedotin.
Results: We could demonstrate that the dolastatin family of microtubule inhibitors, from which the cytotoxic component of brentuximab vedotin is derived, comprises potent inducers of phenotypic and functional DC maturation. In addition to the direct cytotoxic effect on tumor cells, dolastatins efficiently promoted antigen uptake and migration of tumor-resident DCs to tumor-draining lymph nodes. Exposure of murine and human DCs to dolastatins significantly increased their capacity to prime T cells. Underlining the requirement of an intact host immune system for the full therapeutic benefit of dolastatins, the anti-tumor effect was far less pronounced in immune-compromised mice. When combining dolastatins with tumor-antigen-specific vaccination or blockade of the PD-1/PD-L1 and CTLA-4 co-inhibitory pathways, we observed substantial therapeutic synergies.
Summary and Conclusion: Our data reveal a novel mechanism of action for dolastatins and provide a strong rationale for clinical treatment regimens combining dolastatin-based therapies, such as brentuximab vedotin, with immune-based therapies.

Aggressive Non-Hodgkin lymphoma - Clinical 2

P1080

ROLE OF INTERIM-PET IN POOR PROGNOSIS YOUNG PATIENTS WITH DLBC LYMPHOMA AT DIAGNOSIS: DATA FROM A PROSPECTIVE ANCILLARY STUDY OF A RANDOMIZED PHASE III STUDY FROM THE FONDAZIONE ITALIANA LINFOMI
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Background: The role of interim-PET (i-PET), after two cycles, in advanced stage Hodgkin's lymphoma is well defined; the same role in diffuse large B cell lymphoma (DLBCL) is still controversial.
Aims: The Fondazione Italiana Linfomi planned an ancillary prospective study during a randomized phase III trial the DLCL04 study addressed to young poor prognosis DLBCL patients at diagnosis.
Methods: The ancillary-PET study was designed to define the prognostic impact of i-PET on progression free survival (PFS) after two cycles of immunochemotherapy. Patients were stratified according to aa-IPI and randomized at diagnosis to receive: R-CHOP14+/- ASCT or R-MegaCHOP14+/-ASCT. A visual dichotomous criteria, according to Deauville, was used to define the i-PET result.
Results: From June 2005 to September 2010 130 patients were enrolled for the ancillary-PET study, 69 in the R-HDC+ASCT and 61 in R dose dense therapy respectively. The clinical characteristics were well balanced among the arms of therapy excluding a selection bias in the group of patients studied with i-PET. The i-PET result was positive in 74 patients and negative in 56 patients. In particular, in R-dose dense arm 27 were negative and 34 positive, in R-HDC+ASCT 29 were negative and 40 positive, according to immunochemotherapy scheme in R-CHOP14 29 were negative and 40 were positive, in MegaCHOP14 27 were negative and 34 were positive. With a median follow-up of 36 months no differences in PFS was reported according to i-PET result. In particular 3-year PFS were 87%(95% CI:75-94) in i-PET negative patients and 76% (95% CI: 65-85) in i-PET positive patients respectively (p: 0.09. The 3-year PFS according to I-PET results in the different treatment arm were: in the R-HDT+ASCT group i-PET negative or i-PET positive patients had a 3-year PFS of 83% (95% CI:63-92) and 79% (95% CI:63-89) respectively; in the R-dose dense i-PET negative or i-PET positive patients had a 3-year PFS of 92% (95% CI:73-98) and 72% (95% CI:54-85) (p: 0.07). I-PET negative and i-PET positive patients had a 3-year OS of 89% and 83% respectively, p=0.219.
Summary and Conclusion: In our multicenter prospective study i-PET, performed after two cycles and analyzed with a visual method, is not able to identify two different risk population. We are now performing a centralized revision of all evaluable PET.

P1081

BONE MARROW INVOLVEMENT AT DIAGNOSIS IS A POOR PROGNOSTIC FACTOR IN YOUNG PATIENTS WITH UNTREATED HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA: SUB-ANALYSIS OF THE PHASE III RANDOMIZED FIL-DLCL04 TRIAL
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Background: The bone marrow (BM) involvement in diffuse large B-cell lymphoma (DLBCL) had an adverse prognostic role (Sehn, J Clin Oncol 2011).
Aims: Aim of this analysis was to evaluate the prognostic role of BM involvement in the prospective randomized phase III trial DLCL04 by the Fondazione Italiana Linfomi (Vitolo, Blood, ASH2012).
Methods: Inclusion criteria were: age 18-65; untreated DLBCL or follicular grade IIIb; age adjusted-International Prognostic Index (aa-IPI) score 2 or 3. Patients were randomized at diagnosis to receive: Rituximab plus dose-dense chemotherapy (R-CHOP14 x 8 cycles or R-MegaCHOP14 x 6 courses), R-dose-dense group, *vs* a brief chemoimmunotherapy followed by high dose

chemotherapy plus autologous stem cell transplantation (R-CHOP14/R-MegaCHOP14 x 4 courses plus Rituximab-MAD and BEAM and ASCT), R-HDC+ASCT group. BM biopsy was mandatory at diagnosis and repeated at the end of treatment if positive. BM involvement was defined as concordant if marrow was involved by large B-cell and discordant by small B-cells.
Results: From 2005 to 2010, 399 patients were randomized to receive: 199 R-HDC+ASCT and 200 R-dose-dense chemotherapy. All patients were evaluable for analysis. BM involvement was reported in 84 patients (21%): 39 (20%) in the R-HDC+ASCT group and 45 (22%) in the R-dose-dense group. Pattern of involvement was concordant in 63 patients, discordant in 14 and not specified in 7 patients. Clinical characteristics in BM positive group compared to BM negative group were well balanced, with the exception of older patients (median age 53 years *vs* 47 years, p<.001) and higher aa-IPI score (aa-IPI 3: 36% *vs* 24%, p.024) in the BM positive patients. With a median follow-up of 49 months, 3-year progression free survival (PFS) for the whole series of 399 patients was 67% (95% CI: 62-72) and 3-year overall survival (OS) was 79% (95% CI: 75-83%). Three-year PFS in BM positive *vs* BM negative were 46% (95% CI:35-56%) *vs* 73% (95% CI:67-77%), p<.001. In a Cox-model for PFS including type of treatment, age, gender, aa-IPI, performance status, histology and BM involvement, the adverse prognostic impact of BM involvement was maintained with an adjusted Hazard Risk (aHR) of 2.22 (95% CI:1.54-3.22, p<.001).Three-year OS rates in BM positive *vs* BM negative were 65% (95% CI:53-74%) *vs* 83% (95% CI:78-87%) with an aHR 1.94 (95% CI:1.23-3.05; p=.004). The adverse prognostic impact of BM involvement on PFS was not affected by the type of treatment: 3-year PFS in BM positive R-dose-dense group was 42% *vs* 69% in BM negative with an aHR of 2.19 (95% CI: 1.35-3.54, p<.001); 3-year PFS in BM positive R-HDC+ASCT group was 51% *vs* 77% in BM negative, with an aHR of 2.27 (95% CI:1.30-3.97, p<.004). Regarding the prognostic impact of the pattern of BM involvement (concordant *vs* discordant), with the limits of small sample size of the discordant group, only concordant BM involvement maintained an adverse prognostic impact on PFS (Figure 1): in a Cox-model assuming BM negative as reference, the risk of progression was significantly increased in the concordant involvement group with an aHR of 2.48 (95% CI: 1.68-3.67, p<.001) and not affected by discordant involvement (aHR 1.05; 95% CI:0.41-2.71, p=.916).

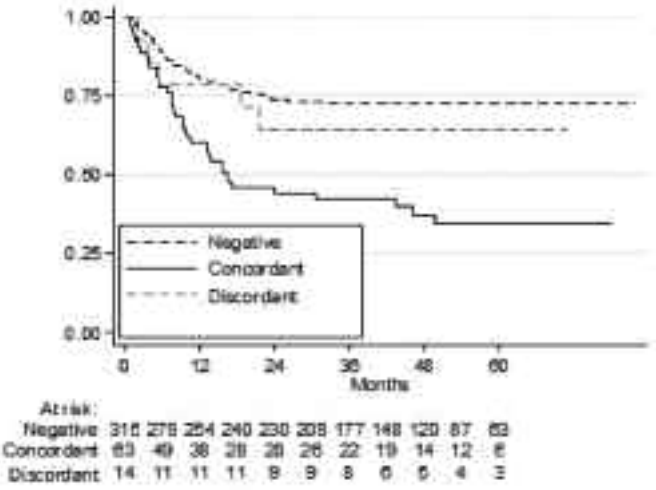

Figure 1.

Summary and Conclusion: BM involvement, namely concordant pattern, is a strong adverse predictor of outcome in young patients with untreated DLBCL at poor prognosis and its negative prognostic role is not overcome by the intensification with R-HDC+ASCT.

P1082

VITAMIN D DEFICIENCY (VDD) IMPAIRS RITUXIMAB-MEDIATED CELLULAR CYTOTOXICITY AND OUTCOME OF DLBCL PATIENTS TREATED WITH, BUT NOT WITHOUT RITUXIMAB (R)

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Background: Vitamin D deficiency has been reported to be associated with a worse outcome in patients with various malignant diseases.
Aims: The aim of this study was to investigate impact and underlying mechanisms of vitamin D-deficiency (VDD) on outcome of elderly patients with diffuse large B-cell lymphoma (DLBCL).
Methods: 359 pretreatment 25-OH-vitamin D serum levels from the RICOVER-60 trial and 63 from the RICOVER-noRTh study were determined by chemoluminescent immunoassay. Rituximab-mediated cellular cytotoxicity (RMCC) was determined by LDH release assay of CD20+ Daudi cells
Results: RICOVER-60 patients with VDD (≤8 ng/ml) treated with R had a 3-year event-free survival of 59% compared to 79% in patients with vitamin D levels >8 ng/ml; 3-year overall survival was 70% and 82%, respectively. These differences were also significant in a multivariable analysis adjusting for IPI risk factors with a hazard ratio (HR) of 2.1 (p=0.008) for event-free and 1.9 (p=0.040) for overall survival. EFS was not significantly different in patients with vitamin D levels ≤8 and >8 ng/ml (HR 1.2; p=0.388) treated without R. This was confirmed in an independent validation set of 63 RICOVER-noRTh patients. While R improved 3-year EFS of VDD patients by only 16%, patients with better vitamin D levels benefitted nearly twice as much from the addition of rituximab (31%). RMCC increased significantly (p<0.001) in 7/7 vitamin D-deficient individuals after substitution and normalization of their vitamin D levels.
Summary and Conclusion: VDD is a risk factor for elderly DLBCL patients treated with R-CHOP. The differential effect of VDD on outcome of patients treated with and without R and the fact that VDD impairs RMCC and substitution improves RMCC strongly suggest that vitamin D substitution might improve the outcome of patients treated with R. Appropriately designed prospective trials must confirm that vitamin D substitution does not only improve outcome in DLBCL, but also in diseases treated with other antibodies (*e.g.* trastuzumab in breast cancer), of which the major mechanism of action is antibody-dependent cellular cytotoxicity. *Supported by Deutsche Krebshilfe.*

P1083

COMBINING SEVERAL MOLECULAR ABNORMALITIES IN IDENTIFICATION OF HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA IMPROVES RISK-STRATIFICATION

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Background: Translocations in both MYC and BCL2 genes, double-hit lymphomas, are associated with poor prognosis in diffuse large B-cell lymphomas (DLBCL). Recent studies have expanded this concept by including double-hit lymphomas diagnosed by increased protein expression detected by immunohistochemistry (IHC). However, none have combined double hit with other abnormalities in trying to identify more patients with poor prognosis.
Aims: We assessed prognostic value of translocations and protein expression of MYC and BCL2, P53 protein expression and deletions of chromosome 17p (del 17p) on available tumor tissue from patients included in a prospective Nordic Lymphoma Group study. We also assessed prognostic value of different combinations of these abnormalities.
Methods: Seventy-four patients with high risk DLBCL or follicular lymphoma grade 3 were included. All patients received R-CHOEP-14 followed by CNS prophylaxis. Immunohistochemistry analyses were performed for anti-BCL2, MYC and P53. Fluorescence *in situ* hybridization (FISH) was performed for translocations in BCL2 and MYC and del17p. Overall survival (OS) and progression-free survival (PFS) were evaluated by Cox regression adjusted for International Prognostic Index (IPI) score.
Results: Seven (13%) patients had simultaneous translocations in BCL2 and MYC. A single translocation of BCL2, present in eighteen patients, was not associated with survival. Simultaneous translocations in BCL2 and MYC was associated with impaired OS (HR 5.5, 95% CI 1.2-24.0, p=0.02) and PFS (HR 3.4, 95% CI 1.0-11.1, p=0.046) in adjusted analyses. Nineteen patients had a high BCL2 and fourteen a high MYC protein-expression. These findings were not associated with survival while the six patients with co-expression of both MYC and BCL2 had impaired OS in adjusted analysis (HR 7.7, 95% CI 2.0-29.9, p=0.003) and PFS (HR 5.5, 95% CI 1.8-16.7, p=0.003). Ten patients (20%) had either a double-hit translocation and/or a double-hit protein expression. This group had impaired OS (HR 6.6, 95%CI 1.7-25.5, p=0.006) and PFS (HR 3.4, 95% CI 1.2-9.4, p=0.021) in adjusted analyses. Five-year OS and PFS was 50% and 37.5%, compared to 90% and 72 % in patients without these abnormalities. Four patients had a deletion in chromosome 17p which was associated with impaired OS (HR 13.9, 95% CI 2.6-74.8, p=0.002) and PFS (HR 6.8, 95% CI 1.7-26.9, p=0.006) in adjusted analyses. A P53 protein expression above 80%, seen in eight patients, was associated with impaired OS (HR 4.7, 95% CI, 1.3-16.4, p=0.017). Combining these two abnormalities yielded a group with higher risk: A group of eleven patients with deletion of 17p and/or high P53 protein expression had impaired OS 9.1 (95% CI 2.4-35.3, p=0.001) and PFS 3.3 (95% CI 1.1-9.8, p=0.028) in adjusted analyses. Seventeen patients had double-hit defined by translocation or protein expression and/or del17p and/or high P53. In adjusted analysis, this group had significantly impaired OS (HR 6.2, 95% CI 1.6-24.3, p=0.009). Analysis of this combined endpoint identified 70% of all deaths.

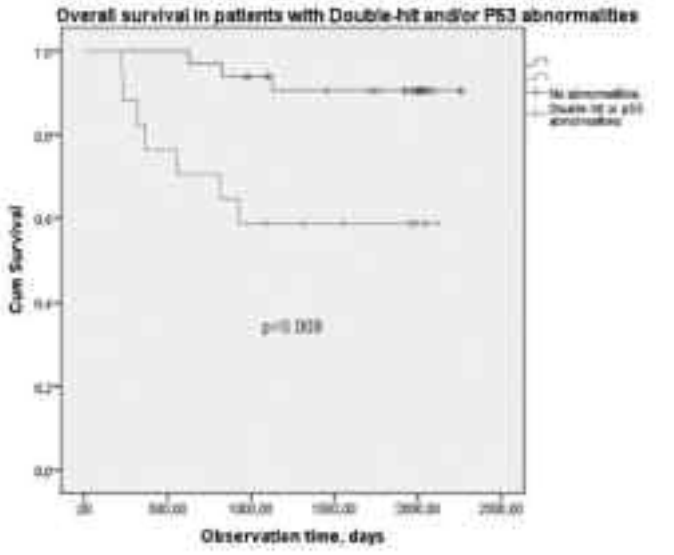

Figure 1.

Summary and Conclusion: Combining detection of MYC, BCL2 and P53 abnormalities identifies a larger proportion of patients with high-risk DLBCL than double-hit abnormalities alone.

P1084

DEFINING ACCURACY AND SENSITIVITY OF T-CELL RECEPTOR REPERTOIRE PROFILING FOR MONITORING RECURRENT/PERSISTENT DISEASE IN PATIENTS WITH MATURE T-CELL NEOPLASMS

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Background: Accurate and sensitive identification of recurrent or persistent disease in T-cell neoplasms is important for proper patient care. While there are several technologies to monitor disease, including flow, allele specific qPCR, and PCR amplification of rearranged receptors, high-throughput sequencing (HTS) of T-cell receptor (TCR) CDR3 chains is an emerging tool that combines the sensitivity of allele-specific qPCR assays with the ease of flow. We developed a method that amplifies rearranged TCR CDR3 sequences and uses high-throughput sequencing (HTS) to sequence tens of thousands of chains simultaneously. Because the technology utilizes gDNA the frequency of sequenced CDR3 chains is representative of the relative frequency of each CDR3 sequence in the sample population of T cells. Thus these assays can describe both the breadth of the T-cell receptor repertoire and quantify individual clones; enabling tracking the presence and frequency of neoplastic clones. However, HTS of TCRs is a relatively new technology with minimal data on sensitivity and accuracy.
Aims: This project aims to demonstrate the potential of this technology to monitor recurrent/persistent disease by first testing the assay's sensitivity and accuracy and then applying the technology to a set of mature T-cell lymphoma samples.
Methods: To test the sensitivity and accuracy of the assay known clones are spiked into a diverse background of lymphocyte and non-lymphocyte cells across a 100,000-fold dilution gradient. The TCR repertoire of 1,000,000 cells from each sample is subsequently amplified and sequenced, and we test if the assay 1) detected the clone across the dilution gradient, and 2) detected the clone at the right frequency. To show utility, we apply the technology to monitor persistent/recurrent disease in a set of mature T-cell lymphoma samples. To identify the neoplasm's CDR3 chain, we sequence the *TRB* and *TRG* repertoire of 35 index samples. Then, to diagnose persistent disease, we sequence the TCR repertoire of follow-up 55 matched follow-up samples and search for the neoplastic sequence. Clone frequencies lower than the sensitivity threshold were excluded.
Results: For the test of accuracy and sensitivity, the expected and observed clone frequencies were highly correlated (r2=0.98) and the clone was detected across the dilution gradient from 10^{-1} to 10^{-5}. Of the 55 samples clinical samples, 4 were MRD negative using either technique, 9 were MRD negative using traditional techniques and MRD positive using HTS, 1 was MRD positive using traditional techniques and MRD negative using HTS, and 41 were MRD positive using either technique.
Summary and Conclusion: These data indicate that HTS is a viable technology relative to currently applied routine clinical testing.

P1085

RETROSPECTIVE ANALYSIS OF TREATMENT OUTCOMES FOR 120 CASES OF NATURAL KILLER/T CELL LYMPHOMA, NASAL TYPE AT THE LARGEST SINGLE HOSPITAL OF NORTH-WESTERN CHINA

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Background: Extranodal natural killer (NK)/T-cell lymphoma (ENKTL), nasal type, is a distinct, heterogeneous histopathologic and aggressive subtype of non-Hodgkin lymphoma (NHL) based on the clinically classification of WHO, with higher frequency in Asia than in Western countries. ENKTL is so aggressive and has high mortality rate and till now there in no standard therapy. In this study, we retrospectively analyzed the 120 cases n recent 5 years.
Aims: To improve the understanding of ENKTL with poor prognosis and provide experiential references for individual therapy via a retrospective analysis of the clinical features at the largest single hospital of north-western China.
Methods: 120 ENKTL cases from Apr.2007 to Oct 2012 in the largest hosptial of north-western China were retrospectively analyzed on their clinical manifestations. Pathological examinations were mainly depended on morphology, immunophenotype and *in situ* hybridization for EBER. PCR for the amount of EBV DNA in whole blood and TCR gene rearrangement were performed. Chemotherapy and or radiotherapy were the main treatments. CR,2 year(2y) OS and PFS according to clinical characteristics were analyzed.
Results: The median age of 120 ENKTL cases was 43.19±13.7 years old. 98 primary nasal ENKTL cases accounted for 81.4%, whose 2y OS/PFS were 88.6%/69.1% respectively. 22 non-nasal ENKTCL accounted for 18.3%, whose 2y OS/PFS were 58.8%/45.5% respectively. It was found that patients with primary intestinal or Ki67 greater than 80% were dead in first year. Patients with primary liver and intestine had higher Ki67 than patients with primary nasal. 2y OS/ PFS of 43 patients with Ki67 from 60% to 80% was 60%/36% as compared to 86.3%/57.5% for OS/PFS of 30 patients with Ki67 from 30% to 50% and 100%/78% for OS/PFS of 7 patients with Ki67 less than 30%. EBER was positive in every case. Among the 13 EBV –DNA samples detected, there were 5 samples with more than $6.1X10^7$ copies/ml with OS 60%. 2y OS and PFS of Patients with normal ferritin or β-microglobulin were longer than that with higher ferritin or β-microglobulin. 64 patients had IPI=0-2, accounting for 53.3%, 56 patients had IPI=3-5, accounting for 46.7%. 56 primary nasal patients with and IPI≤2 were more than 42 patients with IPI-2 and 7 primary non-nasal patients with IPI≤2 less than 15 patients with IPI-2. 2y OS/PFS of 98 patients with primary nasal was 88.6%/69.1%. 2y OS/PFS of 22 patients with primary non-nasal was 58.8%/57.3%. There was different survival outcome between nasal and non-nasal**.** Meanwhile 2y OS/PFS of 27 patients with local IEA was 100%. 2y OS/PFS of 33 patients with local IEB was 100%/90.9%. 2y OS/PFS of 24 patients with invasion I EB was 75%/62.5%. 10 patients with stage I received radiotherapy alone and 2y OS/PFS was 100%. 47 patients with stage II~IV received chemotherapy alone, of which 2y OS/PFS of 23 patients with stage IIEB was 91.3%/ 82.6%, 2y OS/PFS of 17 patients with stage III was 58.8%/47%. Among 34 patients who received more than 3 cycles of CHOPL + radiotherapy, 16 patients received pegaspargase because of allergic reaction of L-asparaginase. 2y OS/PFS of patients with CHOPL was 95%/81% respectively, higher than that of other patients. Among 120 patients, 11 patients received ABSCT after CR still survive and 2 patients after PR relapsed and died.
Summary and Conclusion: It was implied that Ki67, β-microglobulin, EBV-DNA and primary site was related with the prognosis of NKTCL. It needs further and more clinical observation. The chemotherapies containing asparaginase or pegasparaginase are more effective. Autologous stem cell transplantation could make patients long live and pretransplant CR status was a major prognostic factors.

P1086

ABNORMAL IMMUNOPHENOTYPE IS OF GREAT VALUE IN THE DIAGNOSIS OF AGGRESSIVE NATURAL KILLER CELL LEUKEMIA

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Background: Aggressive natural killer cell leukemia (ANKL) is a rare form of large granular lymphocytic leukemia (LGL) with an aggressive clinical course, and a rapid and accurate diagnosis of ANKL is a challenge. Although there have been some reports of small series of cases, reports of a large number of cases are few.
Aims: To describe the clinical features and assess the clonality by flow cytometric (FCM) analysis of Killer-cell immunoglobulin-like receptor (KIR) expression in ANKL, we conducted a prospective study of a series of patients with ANKL in China.
Methods: We described clinical features, checked serum EBV DNA level, analyzed STAT3 mutaion status and immunophenotypes at diagnosis. Nine

samples of chronic lymphoproliferative disorder of NK cells (CLPD-NK) were used as control.
Results: Our study population consisted of 36 consecutive patients recruited between September 2009 and January 2014. The clinical characteristics of 36 patients with ANKL are listed in Table 1. Pleural effusion (PE) was a common complication of ANKL, and lymphocytes observed in the PE were CD3-CD56+ leukemic cells. Thirty-four patients underwent bone marrow (BM) examination, and 32 patients showed varying degrees of BM infiltration of LGLs. Immunophenotypes of the ANKL cells were typically positive for CD2 except one patient, whereas CD1a, CD3, CD4, CD5, CD8, CD10 and CD57 were negative. CD7, CD16, CD56, CD94, CD160 and CD161were positive for 65.6%, 69.4%, 88.9%, 87.5%, 90.5% and 45.8%, respectively. Either restricted KIR expression or complete lack of KIR antigens was observed in all of the patients with ANKL (7 and 17, respectively). In the patients with CLPD-NK, CD3, CD4, CD5 and CD8 were negative; CD2 and CD7 were positive; expression of CD16, CD56, CD57, CD94, CD160 and CD161 were 88.9%, 77.8%, 55.6%, 50%, 85.7% and 0, respectively. NK cells from 5 (5/9) patients with CLPD-NK had homogenous reactivity to a single KIR receptor antibody, and 4 were complete lack of KIR antigens. By comparison, the expression of CD94 and CD161 in the ANKL patients was significantly higher (p=0.047 and p=0.019, respectively) than that in the CLPD-NK patients, but the expression of CD160 was not significant between the two groups. One patient displayed STAT3 mutations (Y640F). 89.7% of patients positive for serum EBV DNA and the quantity of serum EBV DNA ranged from 7.0×103 to 1.6×108 per milliliter at diagnosis. Seven patients who responded to chemotherapy experienced a decrease in EBV-DNA levels; however, the levels increased again when disease progression. Three cases were EBV negative, and the clinical course was less aggressive than that of conventional ANKL. All 36 cases had follow-up data with a mean follow-up of 77.1 days (range 3-720 days), and the median OS was 20 days. Twenty-thee patients received chemotherapy, and a clinical response to treatment was observed in 10 of 23 patients. In univariate analysis, the positivity of EBV-DNA (P=0.015), PS>2 (P<0.001), hemophagocytic syndrome (HPS) (P=0.001), PE/ascites (P=0.017) thrombocytopenia (P=0.021), lymphocytosis (P<0.001), neutropenia (P=0.003) was identified as an unfavorable factor of early death. Therapeutic response (P<0.001), CD56-(P=0.035) and receiving chemotherapy (P=0.038) were associated with better survival.

Table 1. Clinical and biological characteristics of 36 patients with ANKL

Characteristics	No of Patients	%
Median age (years)	43 (14-80)	
Gender		
Male	21	58.3
Female	15	41.7
Performance status (ECOG) 0-2	17	47.2
Performance status (ECOG)3-4	19	52.8
LDH (n=47)		
>245U/L	34	94.4
D-MD (n=42)		
>0.5mg/L	21	50.0
Lymphocytosis	8	22.2
Neutropenia	24	66.7
Anemia	17	47.2
Thrombocytopenia	30	83.3
Fever	36	100
Hepatomegaly	18	50
Splenomegaly	32	88.9
HPS	29	80.6
PE/ascites	18	50
Serum EBV-DNA (n=29)		
>500copies per ml/blood	26	89.7

Lymphocytosis: ALC>4.5×10⁹/L; Anemia: Hb<100 g/L;
Neutropenia: ANC<1.5×10⁹/L; Thrombocytopenia<100×10⁹/L

Summary and Conclusion: Our findings suggest that ANKL is an entity of abnormal phenotypic features and has a clonal KIR expression, and detection of KIR may have important diagnostic value. The detection of serum EBV DNA is a reliable way to monitor treatment response and outcome. To improve the poor prognosis of ANKL, we would like to emphasize three issues from our results: early diagnosis of ANKL, chemotherapy and allogeneic stem cell transplantation.

P1087

CNS PROPHYLAXIS IN PATIENTS WITH DLBCL, ARE WE TREATING OURSELVES? A RESPONSE TO THE RECENT BCSH GUIDELINE

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Background: We were interested to read the recent BSCH guidelines on the role of CNS prophylaxis in the management of lymphoma. The authors concluded patients with Diffuse Large B cell Lymphoma (DLBCL) IPI 3-5 with either, a raised LDH and more than 1 extra nodal site, or involvement of certain anatomical sites (breast, testicular, epidural) should be treated with 3-6 doses of intrathecal methotrexate. The paucity of evidence for all aspects of this guidance was acknowledged. The North Trent Cancer Network covers a population of approximately 1.9 million in the towns/cities of Sheffield, Doncaster, Rotherham, Barnsley and Chesterfield. After a review of available evidence several years ago the clinicians in the network made a unanimous decision not to administer CNS prophylaxis to patients with DLBCL receiving CHOP Rituximab chemotherapy. More recently it was agreed that those with testicular lymphoma should receive high dose systemic methotrexate as part of their treatment regimen with RCHOP. Diagnosis of all new cases of DLBCL are made by the Sheffield Integrated Haemato-Oncology Diagnostic Service (HODS). Since 2006 all cases of diffuse large B cell lymphoma have been discussed at a single weekly teleconferenced region-wide MDT. Therefore centralised diagnostic and clinical data are readily available.
Aims: To determine the CNS relapse rate in North Trent, in an region that does not give CNS prophylaxis to patients with DLBCL, to determine if the policy should be changed in view of current guidelines
Methods: We have reviewed all cases of CNS relapse over a 6 year period and assessed each case with reference to the guidelines identifying those who would have been eligible to receive CNS prophylaxis. Those patients managed palliatively have not been included.
Results: Review of MDT and HODS records reveals that there were 620 de novo cases of DLBCL treated with curative intent over 6 years. 13 patients were diagnosed with CNS relapse, giving a prevalence of 2.1%. Median age was 53 (range 21-79) with 10 male patients 3 of whom had testicular disease. 9 patients had IPI<3, 3 patients did not have an LDH available, resulting in an IPI of 2, 1 other patient had an IPI of 4. According to current BCSH guidelines 4/13 patients would have received CNS directed treatment (3 testicular disease). 8 patients died of CNS disease shortly after presentation, 2 are alive with progressive lymphoma, 3 patients are in remission (2-48 months post relapse), 1 of whom has significant cognitive impairment following brain radiotherapy, reiterating the poor prognosis associated with CNS relapse.
Summary and Conclusion: In the rituximab era the CNS relapse rate in North Trent is 2.1% in de novo DLBCL. This is consistent with published figures despite our conservative approach to CNS prophylaxis. Furthermore less than a third of these cases would have received prophylactic IT methotrexate under the new guidance. This is lower than figures reported by van Besien at el 1998 who used a similar strategy to identify those at risk of CNS relapse before widespread use of rituximab. In this study approximately 50% of patients of the 605 patients meeting the criteria to receive CNS prophylaxis developed CNS relapse. The majority of the published data on CNS relapse are retrospective and of variable quality therefore reaching definitive conclusions is difficult. Evidence collected in the rituximab era suggests a reduction in the frequency of CNS relapse, perhaps because of better initial disease control. An alternative interpretation of the available evidence is that, with the possible exception of testicular disease, there is no reliable strategy for the identification of an individual at risk of CNS relapse. Similarly, the benefit of intrathecal methotrexate prophylaxis alone is unproven. Current data do not support the application of this guidance and we believe that the BCSH should reconsider its adoption. In the meantime we feel our experience may provide reassurance to others wishing to adopt alternative strategies.

P1088

MONOCLONAL AND POLYCLONAL GAMMOPATHY MEASURED BY SERUM FREE LIGHT CHAIN AND IMMUNOFIXATION SUBDIVIDE THE CLINICAL OUTCOMES OF DIFFUSE LARGE B-CELL LYMPHOMA ACCORDING TO MOLECULAR CLASSIFICATION

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Background: Elevated serum free light chain (FLC) is known to be an adverse prognostic factor in diffuse large B-cell lymphoma (DLBCL).
Aims: We hypothesized that monoclonal gammopathy (MG) and polyclonal gammopathy (PG) measured by serum FLC and immunofixation (IF) would have different clinical outcome according to the molecular classification of DLBCL.
Methods: A total of 175 newly diagnosed DLBCL patients with information of serum FLC and IF were retrospectively analyzed. MG was defined as elevated

kappa (κ) or lambda (λ) FLC with abnormal κ to λ ratio or positive IF, PG was defined as elevated κ and/or λ FLC with normal κ to λ ratio and negative IF. Molecular classification of DLBCL such as GCB type and non-GCB type was performed according to the Hans algorithm.
Results: Ninety six (54.9%) patients had elevated FLC. MG was observed in 34 (19.4%) patients and PG was observed in 68 (38.9%) patients. The 2-year overall survival (OS) was 79.0% and the 2-year event-free survival (EFS) was 71.6%. In multivariate analysis, high-intermediate/high international prognostic index (IPI) and elevated FLC showed clinical significance for OS (*P*=0.002, *P*=0.005, respectively) and EFS (*P*<0.002, *P*=0.010, respectively). MG and PG were also associated with inferior OS (*P*=0.002, *P*=0.011, respectively) and EFS (*P*=0.002, *P*=0.013, respectively). Ninety six patients (73.8%) patients were classified to non-germinal center B-cell (GCB) type among 133 evaluable patients. PG showed inferior clinical outcome for OS and EFS in GCB type of DLBCL (*P*=0.006, *P*=0.035, respectively). MG was a significant poor prognostic factor for OS and EFS in non-GCB type (*P*=0.017, *P*=0.004, respectively, figure).

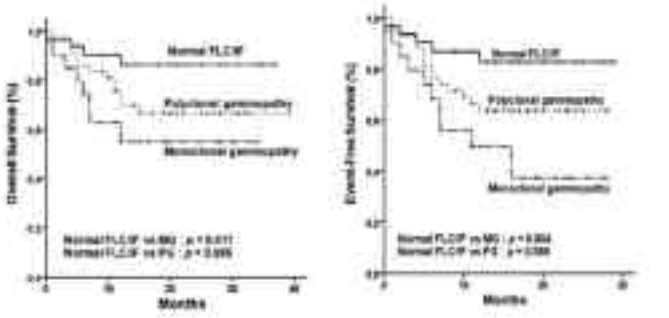

Figure 1.

Summary and Conclusion: MG measured by serum FLC and IF was associated with inferior outcome in DLBCL, especially non-GCB type.

P1089

TORISEL IN MANTLE CELL LYMPHOMA: RESULTS OF RESTOR STUDY (NON INTERVENTIONAL, LONGITUDINAL, RETROSPECTIVE, MULTICENTER AND NATIONAL STUDY ON A COHORT OF 93 PATIENTS WITH MANTLE CELL LYMPHOMA)

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Background: Mantle Cell Lymphoma is an aggressive disease without curative option in most cases. In patients who relapse and not candidate for transplantation, there are few validated options despite a wide range of new compounds under investigation. Treatment with m-Tor inhibitor Temsirolimus (Torisel®) licensed in Europe remains an interesting option mainly in elderly.
Aims: To evaluate the survival of patients with Mantle Cell Lymphoma (MCL) treated with Torisel®.
Methods: Retsrospecive survey in 21 French hematology centers who treated at least 4 patients (pts) with Torisel® between August 1st, 2009 (date of market authorization) and December 1st, 2011. Pts were followed for ≥ 1 year after the first administration of Torisel®. Demographic, clinical and biological data at the diagnosis, treatment regimens, responses and survival data were collected. Data review and analysis were done by a scientific committee on October 31st, 2013.
Results: Ninety three pts responding to inclusion and non-inclusion criteria were identified in the 21 centers. Among these 93 pts, 84 pts (M=65) were evaluable (6 pts did not fulfill inclusion / non-inclusion criteria, 1 pts duplicate, 2 pts with empty CRFs). Median age was 51.4 (73% >65 y), median time from diagnosis to treatment with Torisel® was 4.4y (0-15). Fifty eight pts (69%) had received ≥ 3 prior treatment regimens including 14 pts (24%) who underwent autologous SCT. Sixty four pts (76%) were treated with Torisel® in monotherapy and twenty pts (24%) in combination including seventeen pts (85%) treated with Torisel®+Rituximab. Median treatment duration with Torisel® was 2, 5 months (1 day– 28,5 months). Fifty three pts (63%) received a loading dose of 175 mg. Median duration of follow up was 243, 5 days (16-1143). Among the 84 pts, 32 pts (38%) have had an objective response (IC 95% [27, 7 – 48,5]) with10 CR and 22 PR. There was no significant difference according to age (>or<65 y), number of prior treatment regimens (<ou ≥ 3) or the use of Torisel® either in combination or not. Median survival was 320 days (IC 95% [181 -521]) and median Progression free survival was 100 days (IC 95% [69 -174]). Torisel® tolerability was acceptable, except for toxicities commonly reported in particular gastrointestinal (7, 14%), pulmonary (10, 7%), hematologic (15, 5%) and infection (27, 4%). There was no toxic death attributed directly to Torisel®
Summary and Conclusion: Although this is a retrospective study, it confirms the results previously reported in the pivotal study by Hess et al (*G. Hess, JCO 2009*). These results demonstrate that the administration of Torisel® even in elderly pts for whom chemotherapy is considered potentially toxic (risk / benefit) or contraindicated is feasible. Analysis of long-term data is ongoing and results will be presented at the congress.

P1090

EVALUATION OF THE NOVEL, ORALLY BIOAVAILABLE SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) VERDINEXOR (KPT-335) IN SPONTANEOUS CANINE CANCERS: RESULTS OF PHASE I AND PHASE II CLINICAL TRIALS

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Background: Selective Inhibitors of Nuclear Export (SINE) transiently block CRM1/XPO1, the major nuclear export protein in cells, forcing nuclear retention of key tumor suppressor and growth regulatory proteins ultimately resulting in tumor cell death.
Aims: Here we evaluated the *in vitro* activity of SINE against canine tumor cell lines and investigate the biologic activity of verdinexor (KPT-335) in companion dogs with spontaneous cancers as proof of principle for human clinical studies.
Methods: Cytotoxicity assays were performed in several canine tumor cell lines including those derived from non-Hodgkin lymphomas (NHL). SINE compounds induced growth inhibition and apoptosis. NHL cell lines were particularly sensitive with IC_{50s} of 2 - 42 nM. Phase 1 and Phase 2 clinical trials of oral verdinexor were given to companion dogs with mast cell tumors, osteosarcomas, or NHL at doses of 1 – 1.75mg/kg.
Results: Seventeen dogs with NHL (naive or relapsed) were enrolled in a Phase 1 clinical trial. The maximum tolerated dose was 1.75 mg/kg, given orally twice weekly (Monday/Thursday). Objective responses include Partial Responses (PR n=2) and Stable Disease (SD n=7). Responders had a median Time To Progression (TTP) of 66 days (range 35-256). An additional six dogs with NHL were given verdinexor at a dose of 1.50 mg/kg Monday/Wednesday/Friday; clinical benefit was observed in 4/6 dogs with a median TTP for responders of 83 days (range 35-354). Toxicities were primarily GI-related including anorexia, weight loss, vomiting and diarrhea. Toxicities were manageable with supportive care, dose modulation and "low dose" prednisone. A subsequent Phase 2 study was performed in 58 dogs with either newly diagnosed or relapsed NHL. Verdinexor was administered at 1.25 - 1.50 mg/kg twice weekly (Monday/Thursday). The objective response rate was 34% (1 Complete Response, 19 PR) with an additional 33 dogs experiencing SD for ≥4 weeks. While the median TTP was approximately 5 weeks, 20 dogs (34%) remained on study drug for ≥8 weeks.
Summary and Conclusion: Dogs with T cell lymphoma, a form of disease considered to be biologically aggressive and challenging to treat with cytotoxic chemotherapy, had particularly good objective responses to single agent verdinexor (71% in naïve disease, 57% in relapsed disease). Verdinexor was well tolerated, with anorexia being the most common side effect. Furthermore, the quality of life did not significantly change over the study duration in all dogs enrolled (p=0.13), in dogs that remained on study for at least 28 days (p=0.66) or in dogs that remained on study for at least 56 days (p=0.52), indicating tolerability with both short- and long-term dosing. Together, these data provide robust evidence that the novel orally bioavailable XPO1 inhibitor verdinexor exhibits single agent biologic activity in a relevant spontaneous large animal model of human NHL. It is therefore likely that other SINE compounds, such as the closely related selinexor (KPT-330) will exhibit similar tolerability and biologic activity in humans. Preliminary data on selinexor in patients with advanced NHL support this.

P1091

THE ROLE OF IMAGE GUIDED CORE BIOPSY IN THE DIAGNOSIS OF HAEMATOLOGICAL MALIGNANCY

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Background: Surgical lymph node excision biopsy (EB) has been regarded as the gold-standard for lymphoma diagnosis. Image-guided core biopsy (ICB) is widely used and is included in current UK lymphoma guideline recommendations. However, concern remains regarding the yield and accuracy of this technique.

Aims: To assess the utility of ICB at two large centers, in England and Scotland, and then to survey attitudes of hematologists towards EB and ICB.
Methods: We reviewed all lymph node specimens referred with suspected lymphoma at the Royal Free Hospital (RFH) during 2012. The size of the biopsy, the diagnosis and comments on the report were recorded. We also had access to similar data from the Beatson West of Scotland Cancer Centre (BWoSCC). We composed an e-questionnaire that was e-mailed to 58 hematologists and anonymous responses were scrutinized.
Results: There were 140 samples from RFH;19 (13.6%) were EB, and 120 (86.4%) ICB. Of the ICB 101 (89%) were diagnostic compared to 18 (95%) of the EB. This was similar to the BWoSCC cohort; 83/107 (78%) of ICB were diagnostic. In many of the cases where ICB was non-diagnostic, this was due to disease associated lymph node fibrosis. In the BWoSCC cohort 11/16 (69%) of the non-diagnostic group had fibrosis in association with classical Hodgkin's lymphoma. We received 23 responses (39% response rate) to our questionnaire; of these 9 worked at level 3 centers and 2 were from level 1 centers. All had access to ICB. Sixteen (72%) of respondents estimated that the proportion of ICB providing inconclusive histology was 5-24%. It was reported that ICB could be obtained within one week by 18 (81%) compared with only 5(23%) of respondents reporting availability of EB within 1 week. Factors influencing decision for ICB varied markedly between respondents. Site of biopsy influenced choice of procedure with peripheral lymphadenopathy being more often referred for EB. The strongest factors favoring ICB were a radiology colleague known to consistently obtain adequate biopsy, intra-abdominal lymphadenopathy, speed of access and patient preference. Easily accessible peripheral lymphadenopathy and a trusted surgical colleague were most likely to lead to an EB referral with advanced age of the patient strongly influencing the decision to refer for ICB.
Summary and Conclusion: We conclude that ICB is a widely available procedure often used for lymphoma diagnosis. ICB is diagnostic in the vast majority of cases in the two centers sampled. Responses suggest that ICB can be obtained in a timely fashion, facilitating initiation of appropriate chemotherapy. The attitude of UK hematologists towards ICB is mixed. It is evident that colleagues regard ICB as having the advantage of speed of referral and safety in obtaining deep tissue samples. Expertise of the operator obtaining the specimen is perceived as more influential than the preference of the histopathologist, perhaps reflecting concern regarding adequacy of the sample. Further work is needed to ascertain the risk of diagnostic error of ICB.

P1092

CLINICAL OUTCOME AND PROGNOSIS IN PATIENTS WITH PRIMARY SINONASAL TRACT DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH R-CHOP CHEMOTHERAPY: MULTICENTER RETROSPECTIVE ANALYSIS

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Background: The sinonasal tract is a rare extranodal site of diffuse large B cell lymphoma. Before the era of rituximab, sinonasal tract diffuse large B cell lymphoma (SN-DLBCL) treated with CHOP or CHOP-like chemotherapy represented high relapse rate in central nervous system (CNS) and poor clinical outcomes compared to nodal DLBCL because of its anatomical sites.
Aims: The aim of this study was to evaluate the clinical outcomes and relapse pattern in patients with primary SN-DLBCL in the era of R-CHOP chemotherapy.
Methods: We retrospectively analyzed data from 80 patients, newly diagnosed with primary SN-DLBCL treated with R-CHOP in 22 Korean Institutions between January 2004 and January 2013. Primary SN-DLBCL was defined as DLBCL localized in sinonasal tract with or without nodal disease, which included distant systemic nodal disease as well as regional nodal disease in this study and other multiple extranodal organ involvement was excluded.
Results: Median age at diagnosis was 62.0 years (range, 25-85 years). Forty-five (54.3%) patients were male. Forty-four (55.0%) patients were elderly (≥60 years). Seventy-three (91.3%) patients were Ann-Arbor stage I-II disease. Whereas three (5.8%) patients were high-intermediate to high risk of international prognostic index, nine (11.3%) patients were high-intermediate to high NK/T cell lymphoma prognostic index. Fifty-nine (73.8%) patients received R-CHOP chemotherapy alone without involved field radiotherapy (IFRT) and twenty-one (26.3%) patients were treated with R-CHOP followed by IFRT. No significant difference in response rate and OS between R-CHOP alone and R-CHOP followed by IFRT, regardless of age, sex, ECOG performance status, Ann-Arbor stage, LDH, and NK/T cell prognostic index. Seventy-four (92.5%) patients were treatment responder (CR+PR). Only one (1.9%) CNS relapsed patient and eight (11.8%) local relapsed patients were observed respectively in this study.
Summary and Conclusion: Our results show that primary SN-DLBCL patients treated with R-CHOP seems to be relatively low CNS relapse rate and better OS compared to previously reported literatures treated with CHOP or CHOP-like chemotherapy before the era of rituximab. However, further studies are warranted to validate our retrospective analysis.

P1093

CLINICOPATHOLOGIC CHARACTERIZATION OF IGM-SECRETING DIFFUSE-LARGE-B-CELL LYMPHOMA

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Background: Recently, diffuse-large-B-cell lymphoma (DLBCL) associated with serum IgM paraproteinemia (IgM-Secreting) has been shown to be a very poor prognostic subset although, detailed pathological and molecular data are still lacking.
Aims: In the present study, the clinicopathological features and survival of IgM-secreting DLBCL were analyzed and compared to non-secreting cases in a series of 151 conventional DLBCL treated with R-CHOP.
Methods: This is a retrospective study evaluating the incidence of IgM-secreting DLBCL and comparing this subset with a non-secreting control group for clinicopathological, molecular features and survival. The study was approved by our Institutional Review Board and was conducted in accordance with the regulations of health information protection policies. A hundred and fifty-one patients diagnosed with conventional *de novo* DLBCL were analyzed for serum protein electrophoresis at disease onset, and those who had a likely monoclonal band in the serum were further investigated by serum immunofixation.
Results: IgM paraproteinemia was detected in 19 (12.5%) out of 151 patients at disease onset. In 17 of these cases secretion was likely due to the neoplastic clone, as suggested by the expression of heavy chain IgM protein in the cytoplasm of tumor cells. In IgM-secreting cases immunoblastic features (p<.0001), non-GCB-type (p=.002) stage III-IV(p=.003), ≥2 extra nodal sites (p<.0001), bone-marrow (p=.002), central-nervous-system (CNS) involvement at disease onset or relapse (p<.0001), IPI-score 3-5 (p=.009) and failure to achieve complete remission (p=.005), were significantly more frequent. FISH analyses for BCL2, BCL6 and MYC gene rearrangements detected only two cases harboring BCL2 gene translocation and in one case a concomitant BCL6 gene translocation was also observed. None of the IgM-secreting DLBCL was found to have L265P mutation of MYD88 gene. Thirty-six month event-free (11.8% *vs* 66.4% p<.0001), progression-free (23.5% *vs* 75.7%, p<.0001) and overall (47.1% *vs* 74.8%, p<.0001) survivals were significantly worse in the IgM-secreting group. In multivariate analysis IgM-secreting (p=.005, expB=0.339, CI=0.160-0.716) and IPI-score 3-5 (p=.010, expB=0.274, CI=0.102-0.737) were the only significant factors for progression-free-survival. Notably, four relapsed patients, who were treated with salvage immmunochemotherapy combined with bortezomib or lenalidomide, achieved lasting remission.
Summary and Conclusion: Our data suggests that IgM-secreting cases are a distinct subset of DLBCL, originating from activated-B-cells with terminally differentiated features, prevalent extra nodal dissemination and at high risk of CNS involvement. Authors MC Cox and A Di Napoli equal contribution

P1094

ELDERLY WOMEN WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH RITUXIMAB BASED CHEMOTHERAPY DO NOT HAVE BETTER OUTCOME COMPARED TO MEN

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Background: The DLBCL patient's outcome has been significantly improved by rituximab introduction (Coiffier, 2002). The most powerful and used prognostic system remains IPI designed in pre- rituximab era (Shipp 1993), although some modifications have to be studied - *i.e.* R-IPI (Sehn 2007), NCCN-IPI (Zhou 2014). The recent publications suggest that women could have different outcome compared to men when treated with rituximab either as part of the induction (Pfreundschuh 2013) or maintenance (Jaeger 2013, Gisselbrecht 2012). The different rituximab clearance in older women compared to other gender-age subgroups could be one of the possible explanations (Muller 2012), although the data is not consistently explained by this factor only.
Aims: Evaluate the impact of different prognostic factors including IPI, gender, bulk and BMI in unselected population of DLBCL patients treated with anthracycline based chemotherapy combined with rituximab.
Methods: The unselected cohort of consecutive 611 patients with DLBCL (primary CNS lymphoma were excluded) treated with R-CHOP-like chemotherapy at General University Hospital in Prague between 2001-2012 has been retrospectively analyzed. IPI and its components, gender, bulk (7.5 cm)

and BMI were the factors tested. Statistical analysis was performed by Cox regression model and supported by univariate analysis using Kaplan-Maier estimation model. Patients: The median age was 60 years, 51.6 % men, 48.4 % women. IPI subgroups: good IPI (low or low intermediate risk) 48.8 %, poor IPI (high or high intermediate risk) 46.6 %, unknown IPI 4.6 %. R-CHOP regimen was the most frequent (77.1 %). With median follow up of 4.5 year, the median PFS and OS wasn't reached. The 5-year PFS and OS were 67.0 % ± 2.1 %, 72.6 % ± 2.0 % respectively.
Results: The following factors had significant prognostic impact on PFS and partially on OS: IPI (concurrently significant impact on OS), bulk (significant impact on OS has not been generally confirmed in multivariate analysis), but not the gender or BMI. These factors remain independent in multivariate analysis. When we analyzed younger and older population (60 years cut off) separately, for younger patients the IPI and bulky remain the independent prognostic features, however for elderly only the IPI remains the independent prognostic factor. We did not find linear relationship between BMI and patient's outcome, but for proper evaluation of this factor further analysis ought to be done. We tested separately the gender influence in elderly population of good and poor IPI subgroups. Surprisingly, the data revealed that in good IPI subgroups men (median PFS not reached) have better outcome compared to women (median PFS 6.2 years) - p=0.0347, whereas OS wasn't significantly different, and also that there is no difference in poor IPI risk subgroups.
Summary and Conclusion: We are not able to confirm the findings that elderly women have better outcome compared to men in large series of DLBCL patients. Based on these data we are not strongly convinced that it is worthwhile to change the rituximab treatment strategy according to gender in routine clinical practice. This work was supported by PRVOUK 27.

P1095

IDENTIFYING MALIGNANT LYMPH NODES BY NANOPARTICLE MODELLING AND SIMULATIONS: A PRELIMINARY INVESTIGATION

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Background: The fully and accurate identification and characterization of malignant lymph nodes by imaging has important therapeutic and prognostic relevance, mainly in patients with aggressive non-Hodgkin's lymphoma whose management is based on the pre-treatment staging assessment. Therefore, an accurate evaluation of nodal status and a precise staging of malignant lymph nodes represent an essential adjunct to the clinical investigation for lymphoma staging. This is in fact useful to select the appropriate therapeutic approach, as well as to determine prognosis, monitor response to therapy and provide surveillance after curative treatment. Conventional ultrasound (US) is considered the method of choice to evaluate lymph node disease, due to its high spatial resolution. However, nodal imaging would benefit from more specific and target-sensitive techniques in order to more accurately monitor therapy response and clinical outcome. The development of "smart" contrast agents able to specifically detect the neoplastic lymphoid cells in malignant lymph nodes could provide a significant contribution to the accomplishment of this goal. In this context, the nanoparticles (NPs) interfacing with biological systems have emerged as promising cellular probes for diagnostic imaging, since their surface could be easily conjugated with specific antibodies or ligands for selective recognition and binding to the target cells. Among pathological B cell associated antigens, the CD22 is expressed on more than 90% of B-cell lymphoid malignancies, suggesting that it may be a promising biomarker for the development of novel detection strategies combining US and NPs.
Aims: To test magnetic NPs functionalized with anti-CD22 (anti-CD22-NPs) as possible new contrast agents in a wireless detection system based on the implementation of nanoprobes for ultrasound in a lymph node model, in order to establish wheter this novel imaging strategy could represent a promising support in clinical investigations.
Methods: The NPs will be synthesized by means of a proper choice of silica precursor according with the required functionalization and conjugated with antibody anti-CD22. In order to improve a biocompatibility the silica NPs could be embedded in agarose material. The use of agarose/agarosel gel could be useful for a preliminary "*in vitro*" experimentation stage oriented to the comparison with numerical results. The NPs will be targeted to the "anomalous" lymph node and a high-detected NPs concentration will indicate a possible lymph nodes irregularity. The NPs will interact with the electromagnetic (EM)/ultrasound wave coming from the external ultrasonic source.
Results: The EM/ultrasound interaction is defined by means the wave reflectivity due to the presence of NPs which behave as contrast agent for imaging. The reflected EM/ultrasound wave will be detected by means of an ultrasonic probe able to map the NPs dislocation. The EM/ultrasound characterization can be performed by means of time or frequency domain numerical tools able to provide a mapping of NPs concentration and, consecutively, to indicate a strong signal reflectivity. In Fig.1 is illustrated an example of a 3D model of NPs to simulate.

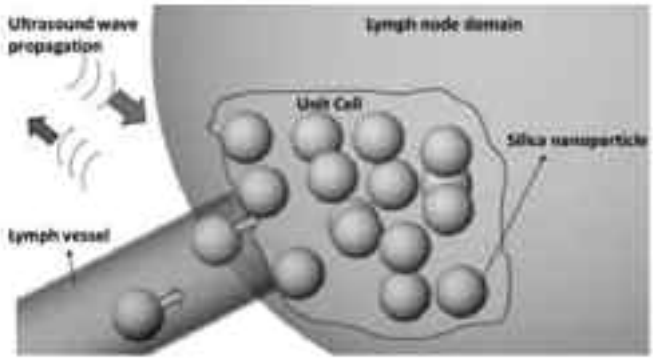

Figure 1.

Summary and Conclusion: The EM/ultrasound characterization represents the first step for the design of the whole nano-system and is useful in order to predict the real lymph node response and to understand how the magnetic NPs interact with all bio-interfaces. The numerical simulations can be used also to analyze the EM/ultrasound response of NPs cluster generated by merged NPs. The biocompatibility and the toxicity concerning the dimension of NPs or NPs clusters will be accurately studied in a future work.

P1096

INCIDENCE OF EBV-POSITIVITY IN THE VERY ELDERLY PATIENTS (AGED ≥ 80 YEARS) WITH DIFFUSE LARGE B-CELL LYMPHOMA: POSSIBLE NEW SUBTYPE OF ELDERLY DLBCL

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Background: Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly has been described in the 2008 WHO classification as a novel provisional entity, and is defined clonal B-cell proliferation associated with EBV that occurs in patients ≥ 50 years of age in whom there is no known immunodeficiency. This unique DLBCL have an activated B-cell immunophenotype and prognosis is significantly inferior compared with EBV-negative DLBCL.
Aims: Recent studies have shown that the population of EBV-associated B-cell lymphoma is increased among patients over 50 years according to aging process. Life expectancy has been increasing consistently over the last century, and is estimated to reach over 80 years by 2030. The probability of DLBCL grows with age, and incidence increases in the elderly. However, little is known about clinical and biological characters in the very elderly patients (aged ≥ 80 years); therefore, the aim of this study was to clarify the incidence of EBV infection and its related clinicopathological features in the very elderly patients with DLBCL.
Methods: We retrospectively analyzed 190 patients with DLBCL, who were diagnosed in our institute from January 2008 to December 2013. Diagnoses were confirmed by immunohistochemistry performed on paraffin-embedded tissue sections, using selected members of a panel of monoclonal antibodies, and all cases were examined for the presence of EBV by the *in situ* hybridization detection technique, and EBV early RNAs (EBER). The comparison between EBV-positive ratio by the detection of EBER in tumor samples and each clinical and immunochemical data according to age were carried out.
Results: Of 190 DLBCL patients, the mean age was 67.2 years (range 22-97 years). The average positivity of EBER by using immunohistological studies for the EBV-latent gene products on paraffin sections was 4.6% in all patients. The mean ratio of EBV positivity of tumor samples was determined for each 10-year aged group. The ratio of EBER was 0% (20-49 years; n=25), 3.6% (50-59 years; n=32), 3.8% (60-69 years; n=38), 8.9% (70-79 years; n=61), and 3.4% (≥ 80 years; n=34), respectively. EBV-positive DLBCL of the very elderly was only one case out of 34 patients. The clinical course of this patient was progressive and died within 7days without any treatment for DLBCL. The mean prevalence of immunostaining for CD5, CD10, CD20, Bcl-2, Bcl-6, and MUM-1 in each aged group was not significant difference, suggesting this cohort was same as general population.
Summary and Conclusion: It has reported that the incidence of EBV-associated lymphoproliferative disorders (LPD) increases with advancing age and EBER-positive DLBCL in aged over 50 years showed more than 20% of patients (Shimoyama Y, *et al.* Pathology Int. 2009; 59: 835-43); however, only 4.6% of patients with DLBCL in aged over 50 years showed EBER-positivity in our study. Interestingly, the peak of EBER-positive ratio was shown in the age

group of 70-79 years (8.9%) as reported, the incidence of EBV-positive DLBCL in the very elderly patients was only 3.4%. The immunophenotypic analysis was similar to each aged group, suggesting the patient population used in this study was not deviated. In conclusion, EBV-positive DLBCL of the elderly has reported mainly identified in patients older than 50 years, the incidence in the very elderly patients is less frequent, and it might be existed in new subtype of B-cell lymphoma. Further clinical and molecular analyses are needed to clarify the pathogenesis of EBV-infection in DLBCL.

P1097

SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR (IL2-R), INTERLEUKIN-6 (IL-6) AND TUMOUR NECROSIS FACTOR (TNF) CORRELATE WITH ADVERSE CLINICAL FEATURES AND POOR OUTCOME IN DIFFUSE LARGE B CELL LYMPHOMA

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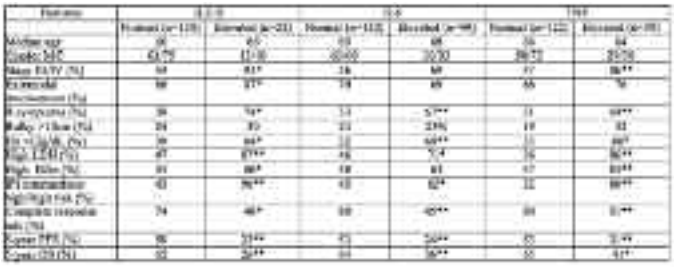
Background: Cytokine and cytokine receptors play an important role in immune regulation and tumour progression in diffuse large B cell lymphoma (DLBCL)
Aims: The aim of this study was to assess the prognostic significance of IL2-R, IL-6 and TNF serum levels in patients with DLBCL
Methods: 181 patients diagnosed with DLBCL in a single institution between 2002 and 2013 were included in the study with the only criterion of availability of cytokine data. HIV+ cases, primary central nervous system and primary mediastinal lymphomas were excluded. All patients received immunochemotherapy, mainly R-CHOP (82%). Complete remission rate, 5-year progression free survival (PFS) and 5-year overall survival (OS) were 71%, 46% and 57%, respectively. Cytokines were determined by standard enzyme-linked immunosorbent assays (ELISA). The best cut-off point for predicting OS was assessed by the Maxstat package (R statistical package)
Results: The best cut-off for OS was 4-fold upper normal limit (UNL), 9-fold UNL and 2-fold UNL for IL2-R, IL-6 and TNF, respectively. The proportion of patients with serum levels above the UNL were 23/161 (14%), 49/182 (27%) and 59/182 (32%) for, the mentioned cytokines, respectively. 50% of the patients had normal cytokines levels, while 30%, 15% and 6% had 1, 2 or 3 elevated cytokines. The main clinico-biological features, response to treatment and outcome according to the cytokines levels are detailed in the table. Regarding histological features, the only significant finding was that patients with high IL-6 levels showed more frequently CD10+ DLBCL (p=.013). In the multivariate analysis, IL2-R (HR 2.2, 95% confidence interval (CI) 1.1-3.8, p=.024) and IL-6 (HR 2.5, 95% CI 2.5-4.2; p=.001) maintained their prognostic significance for OS along with IPI score (HR 2.9, 95% CI 1.04-5.1; p<.001) and serum Beta-2 microglobulin (HR 2.5, 95% CI 1.2-4.5; p=.007).

Table 1. Main clinico-biological features



* p<.05; **p<.001

Summary and Conclusion: IL2-R, IL-6 and TNF serum levels at diagnosis significantly correlated with clinical features and outcome of DLBCL patients. These results warrant further validation in prospective series

P1098

A SERUM NUCLEAR MAGNETIC RESONANCE-BASED METABOLOMIC APPROACH TO DETERMINE POOR PROGNOSTIC DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: The prognosis for diffuse large B-cell lymphoma (DLBCL) patients with early relapse or with refractory disease is very poor. The only prognostic tool used in clinical practice is the International Prognostic Index (IPI) even though it is less useful in the identification of such high-risk patients. Thus, there is an unmet need to determine reliable biological markers that can identify these poor prognostic patients. The functional levels of a biological system include the genome, transcriptome, proteome and metabolome, where the latter is the final down-stream product and therefore is most representative of the system phenotype. The latest years, metabolomics, detecting low-molecular weight molecules in body fluids or tissues, has emerged as a promising tool for diagnostic and differentiating purposes in cancer disease. The prognostic role of using metabolomics in DLBCL is, to our knowledge, not previously addressed.
Aims: To use quantitative ^{1}H nuclear magnetic resonance (NMR) spectrometry, comparing the metabolomic expression in sera from two groups of DLBCL patients treated with modern immunochemotherapy, i) early relapse/refractory patients and ii) cured patients, to determine if differences in the metabolomic profile could be correlated with clinical outcome.
Methods: Serum, prospectively collected at diagnosis and pre-treatment, from 43 DLBCL patients (early relapse, *i.e.* within 12 months or refractory patients, n=19 and cured patients, *i.e.* with a median follow-up of 5 years without relapse, n=24) were analysed using an 800 MHz ^{1}H NMR spectrometer with a 5 mm TCI cryoprobe. After the ^{1}H NMR data acquisition, spectra were aligned and peaks were integrated to a manually defined baseline. A matrix of integrated peaks was constituted and analysed using univariate t-tests and multivariate analysis (Principal Component Analysis; PCA) for a data overview and to clear the data from outliers. In addition, Orthogonal Projection to Latent Structures – Discriminant Analysis (OPLS-DA) was then used to discriminate between relapsed/refractory and cured patients.
Results: We found 69 integrated peaks, corresponding to different serum components, where 15 were significantly different between the two patient groups (t-test, p<0.05) and subsequently identified as the amino acids tyrosine, valine and pyroglutamate (where each metabolite contributes several signals in the NMR spectrum). All the significant components were found to be in higher concentration in cured patients. After correcting for False Discovery Rate (FDR) using Benjamini-Hochberg (B-H) correction, 9 components were still significant. Using OPLS-DA, a model was built with the 15 significantly differentiated components (Q2=0.26). Although not attaining complete separation of the two patient groups, its validation was positively evaluated through a permutation test and a Cross Validation(CV)-ANOVA (p=0.0014). When using only the 9 B-H components, a model was created which could separate the two groups further (CV-ANOVA p=0.0003). A correlation to IPI was found, but when extracting the NMR peaks used in the IPI discriminating model, the cured/relapsed model was still valid. Also, when modelling both factors simultaneously, using OPLS-DA, the most important component was related to the relapsed/cured model (R2X=0.46 and R2Y=0.192; Q2=0.142) while the IPI component was less important (R2X=0.123 and R2Y=0.127; Q2=0.05).
Summary and Conclusion: This study identified a metabolomic discrimination in serum, taken at diagnosis, between DLBCL patients with refractory/early relapsed disease and cured patients. Even though a limited number of included patients, our data suggest that NMR-based metabolomic profiling of serum could play a potential role in the assessment of poor prognostic disease in DLBCL and further exploration of this approach is warranted.

P1099

ASSOCIATION OF EPSTEIN-BARR VIRUS WITH REGRESSION AFTER WITHDRAWAL OF METHOTREXATE AND PROGRESSION OF IATROGENIC IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS IN AUTOIMMUNE DISEASE

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Background: Patients with autoimmune disease (AID) develop lymphoproliferative disorders (LPD) during treatment with immunosuppressive agents, *e.g.* methotrexate (MTX) or biological products. Some of AID-LPD improve by only withdrawal of MTX. However, prognostic factors for AID-LPD remain unclear and standard management and treatment have not been established.
Aims: The aim of this study was to identify characteristics and factors predictive of treatment response and disease progression in patients with AID-LPD.

Methods: Clinical and histopathological data of patients with AID-LPD between 2004 and 2013 were analyzed retrospectively. Factors associated with regression of LPD after withdrawal of MTX and progression free survival (PFS) were analyzed statistically.
Results: A total of 75 cases of AID-LPD were identified. Among this cohort, 69 patients (92%) were with rheumatoid arthritis, and 73 (97%) developed AID-LPD in the course of treatment with MTX. The histology of LPD were as follows; diffuse large B cell lymphoma (n=42, 60%), polymorphic LPD (n=13, 19%), classical Hodgkin lymphoma (cHL, n=6, 9%). Epstein–Barr virus (EBV) was detected by *in situ* hybridization with the EBV-encoded small RNA (EBER) probes in 45% (25/55) of all AID-LPD, 73% (8/11) of polymorphic LPD and 75% (3/4) of cHL. Thirty two patients (43%) had stage IV diseases and 49 (65%) had extranodal diseases. Most frequent extranodal involvement was seen in the lung (12 cases). In 63% (35/55) of patients, withdrawal of MTX induced regression of LPD, and this was significantly found in patients with EBER positive (OR 7.097, 95%CI 1.045-77.86, p<0.05). 2 year PFS and overall survival of all patients were 63% and 83%, respectively. Poor PFS was associated with high-intermediate or high international prognostic index (HR 3.015, 95%CI: 1.155-6.987, p<0.05), soluble IL-2 receptor >1000 U/ml (HR 2.837, 95%CI: 1.098-7.399, p<0.05), abnormal pattern of EBV-related antibodies (HR 3.760, 95%CI: 1.350-28.30, p<0.05) and cHL (HR 4.946, 95%CI: 2.640-79.73, p<0.005).
Summary and Conclusion: EBV positivity with EBER probe is beneficial for predicting the successful regression after withdrawal of MTX, and we speculated that reactivation of EBV may enhance disease progression in patients with AID-LPD.

Stem cell transplantation - Clinical 2

P1100

THE PERIPHERAL BLOOD STEM CELL COLLECTION BY EDAP FOLLOWING INITIAL THERAPY WITH COMBINING BORTEZOMIB AND DEXAMETHASONE IS EFFECTIVE IN YOUNG MULTIPLE MYELOMA PATIENTS

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Background: Bortezomib is first generation proteasome inhibitor which is used as induction therapy for multiple myeloma (MM) patients. Combination of bortezomib and dexamethasone therapy (BD) has significant efficacy in previously untreated myeloma patients. MM is not incurable disease, however, autologous stem cell transplantation is important therapy because of efficacy for the long-term survival. Stem cell mobilization for myeloma patients is primarily performed using granulocyte-colony stimulating factor (G-CSF) alone or after chemotherapy. A minimum of 2 × 10^6 $CD34^+$ cells/kg has been traditionally used for the support of one cycle of autologous stem cell transplantation (ASCT). In addition, the patients who were not obtained at least a very good partial response (VGPR) after the first ASCT have a survival benefit from tandem ASCT. Therefore, it is important to obtain high amounts of $CD34^+$ cells. Most trials suggest that more $CD34^+$ cells can be collected after chemo-mobilization than after G-CSF only mobilization. In addition, the median number of $CD34^+$ cells harvested in the bortezomib–dexamethasone induction therapy was significantly lower without impairing the ability of performing ASCT in comparison with VAD therapy. Therefore, novel mobilization strategies need to improve yields and efficiency. Here, we analyze separately mobilization and collection of HPC using EDAP regimen, which was included to target a more immature myeloma cells.
Aims: To investigate the efficacy of EDAP as stem cell collection protocol is the purpose of this study.
Methods: According to the IMW criteria□patients younger than 65 years with symptomatic multiple myeloma, and signed research consents approved by the Institutional Review Board could be in this study. The exclusion criteria were a serum creatinine level of 2.05 mg/dL or more at time of collection; liver insufficiency, for example, a serum total bilirubin level of 2.0 mg/dL or more, serum aspartate/alanine aminotransferase levels or alkaline phosphatases levels more than 2.5 times the upper limit of normal; poor performance status (grade 3 or worth); and a history of any other malignant disease with the exception of basal cell carcinoma and stage I cervical cancer. All patients received 4 cycles of BD (Bortezomib 1.3 mg/m^2×4d; Dexamethasone 20mg×8d) therapy as induction therapy. EDAP (Etoposide (100 mg/m^2/d×4d); cis-Platin (25mg/m^2/d×4d); Arabinosyl-cytosine (1g/m^2/d×1); Dexamethasone 40 mg d1-5) regimen which was described in total therapy 1 (B. Barlogie *et al.*). All patients received lenoglastim (5μg/kg once a day. Cells were collected on a Fresenius AS-204 apheresis machine.
Results: 62 patients (40 male, 22 female) were enrolled in this trial from November 2008 to December 2012. In all patients, the number of collected cells was sufficient (median 23.4×10^6 $CD34^+$ cells/kg). The overall response rate (ORR) was raised to 88% including 10% CR and 38% very good partial remission according to IMWG criteria. Seven patients had a minimal response. The most common side effects were mucocitis (40%), hematological toxicities (neutropenia and thrombocytopenia), and FN. These were usually mild (almost >grade 3). Peripheral neuropathy was observed in 43 cases after induction therapy, however additive case was not observed during collection phase. 55 patients received high dose melphalan containing pre-conditioning regimen supported by sufficient amount of $CD34^+$ cells.

Table 1. Results of stem cell collection

Median collection day	Day 21 (20-24)
Median $CD34^+$ cells yield (×10^6 /kg)	23.4 (6-36)
Median aphaeresis cycles	2 (1-3)
Median times of G-CSF injection	4 (1-8)

Summary and Conclusion: All patients yielded high numbers of $CD34^+$ cells. Furthermore, the ORR improved from 66% after BD to 88% after EDAP. EDAP following initial therapy with BD produces high response rates, improves stem cell collection, and overcomes the need for intensification before autologous transplantation.

P1101

DEVELOPMENT OF AN ALPHA/BETA T- CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION PROTOCOL FROM MATCHED RELATED AND UNRELATED DONOR GRAFTS IN PATIENTS WITH POOR RISK LEUKEMIA

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Background: αβT- cell depleted allogeneic haematopoietic stem cell transplantations (allo-SCT) have so far been reported in haploidentical stem cell transplantations. First results have been promising with a good engraftment and low incidence of graft-*versus*-host disease (GVHD). Within the context of matched related (MRD) and unrelated (MUD) donors, high rates of life-threatening GVHD are still observed. Therefore we aimed extend αβT- cell depleted allo-SCT to patients with a MRD or MUD.
Aims: To develop an 'innate allo-SCT' protocol defined as αβT- cell depleted stem cell support from MRD and MUD in patients with poor risk leukemia.
Methods: Initial proof runs for the generation of αβT-cell and CD19 depleted grafts have been performed in 4 healthy donors. Grafts for transplantation of the first 5 patients (cohort I) have been depleted with GMP-grade anti-αβTCR and anti-CD19 antibodies. The subsequent grafts for patients in cohort II (n=4) and III (n=5) have been selectively depleted with GMP-grade anti-αβTCRs antibodies. Three conditioning regimens have been investigated (I): fludarabine 120 mg/m^2 + cyclophosfamide 4800 mg/m^2, (II): fludarabine 120 mg/m^2 + busilvex AUC=90 and (III): ATG (Genzyme®) 4 mg/m^2 + fludarabine 120 mg/m^2 + busilvex AUC=90 followed by αβT- cell depleted grafts from matched related or unrelated donors. No additional immune suppression was given after allo-SCT. Main study parameters/endpoints: (1) Feasibility to generate an αβT-cell depleted graft form MRD and MUD. (2) Engraftment and reconstitution of T-cells within the context of different transplantation regimens.
Results: Products for 14 patients have been successfully processed and used for αβT-cell depleted allo-SCT between 2011 and 2013. A ~4 log depletion of αβT-cells has been observed in the product with a recovery of ~75% of CD34+ cells. In cohort I, primary engraftment (chimerism >95%) was 40%. Engrafted patients showed a rapid reconstitution of γδT-cells and αβT-cells with a broad αβT-cell repertoire as determined by spectratyping. Therefore the next cohort was dose-intensified and the CD19-depletion omitted (cohort II). 75% of cohort II showed a swift engraftment. Again a dominance of γδT-cells was observed which associated with a rapidly reconstituting αβT-cell repertoire. Omitting CD19-depletion did not result in severe EBV-reactivations. One patient had an EBV reactivation under a short course of prednisone; however EBV was rapidly cleared after tapering steroids. In order to further increase engraftment cohort III (N=4) was additionally treated with an early application of ATG (day -10/-9). Within this last cohort, all patients showed hematological recovery within 3 weeks and none of these patients experienced serious toxicity within the first 28 days. All patients reached a donor chimerism >95% within 2 months. One patient develop cGVHD of the liver, in the other 3 patients a 'low dose DLI' (1x10^5 T cells) was administered. These patients showed no sign of GVHD ≥ grade II post DLI.
Summary and Conclusion: αβT-cell depletion is feasible in the context of MRD and MUD. αβT-cell depletion associates with a swift and dominant reconstitution of γδT-cells as well as a rapidly restoring αβT-cell repertoire. An intensified conditioning with additional host T-cell depletion seems to be beneficial for a profound engraftment and can be combined with a 'low dose DLI' in 3 months. These results let to the development of a phase I/II study, in which safety of an innate allo-SCT defined as abT-cell depleted stem cell support combined with a 'low dose DLI' is tested in a larger cohort of patients.

P1102

AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS AND PLATELET-RICH PLASMA ENHANCED RECONSTRUCTION OF THE MAXILLOFACIAL BONY DEFECTS

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Background: enriched hematopoietic components and platelet-rich plasma offer potential benefits in oral and maxillofacial surgery including rapid wound healing and bone regeneration.
Aims: The present study was designed to assess autologous bone marrow mononuclear cells and platelet-rich plasma in bone reconstruction of bony defects resulting from enucleation of pathological lesions in maxillofacial region.
Methods: twenty patients diagnosed with acquired maxillofacial bony defects, following conventional surgical excision of the underlying lesion, the selected patients were randomly divided into two equal groups: Group-1 (the study group) ten patients were included, after enucleation of the pathological lesions the bony cavities were grafted by autologous bone marrow mononuclear cells and platelet-rich plasma. Group-2 (control group): ten patients were included, after enucleation of the pathological lesions the bony cavities were allowed to heal spontaneously. Radiographic assessment were carried out using the digital panoramic radiography using diagora software; (postoperatively at one, three, six and twelve months).
Results: Radiographic assessment of the mean bone density in the studied group in comparison to the control group carried out sequentially at: immediate postoperatively and at one, three, six and twelve months showed the following data: immediate postoperative (59.1 ± 13.61, 60.1 ± 15.61 (P-value >0.001) meaning non-significant difference, while significant differences wewr detected in the following assessment sessions: one month (40.2 ± 12.88, 46.3± 13.28 (P-value >0.001), three months: 28.9 ± 8.96, 34.2± 9.91 (P-value >0.001), six months: 19.3 ± 6.98, 24.3 ± 7.78 (P-value >0.001), and after one year :13.5 ± 5.36, 19.1± 5.11 (P-value >0.001).

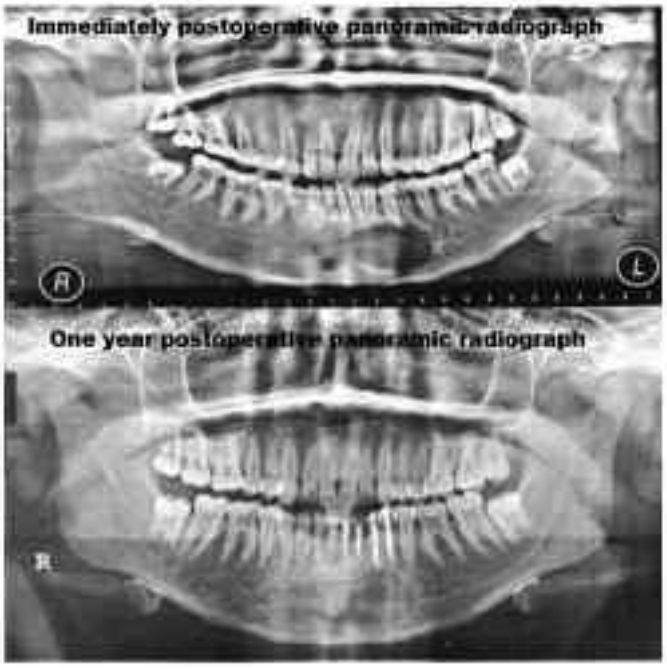

Figure 1.

Summary and Conclusion: Autologous bone marrow mononuclear cells and platelet-rich plasma showed evident neocortex formation that coincided with objective improvements which potentially enhanced reconstruction of the maxillofacial bony defects.

P1103

EPSTEIN-BARR VIRUS INFECTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: THE ROLE OF CYTOMEGALOVIRUS

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Background: Epstein-Barr virus (EBV) infection is a common and severe complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). EBV infection may result in a spectrum of diseases in recipients of allo-HSCT including fatal post-transplant lymphoproliferative disorders (PTLD) and other EBV-associated diseases. Several studies suggested that cytomegalovirus (CMV) might play a role in PTLD.
Aims: In this study, the association between CMV and EBV DNA-emia as well as EBV-associated diseases was evaluated in the recipients of allo-HSCT.
Methods: Four hundred and seven patients undergoing allo-HSCT were enrolled in this study between July 2008 and December 2013. The diagnosis of PTLD was according to the criteria of World Health Organization (WHO). The diagnosis of EBV-associated other diseases, which included EBV-associated fever without tissue involvement and EBV-associated end-organ diseases, was based on the criteria of the European Conference on Infections in Leukemia. The EBV-DNA and CMV-DNA levels in blood and secretion were monitored by quantitative real-time polymerase chain reaction (RQ-PCR) before and after transplantation regularly. EBV and CMV DNA-emia were diagnosed when EBV-DNA or CMV-DNA in the blood was positive twice consecutively.
Results: During the follow-up period, 107 patients (26.3%) developed EBV

DNA-emia and 43 (10.6%) developed EBV-associated diseases including 28 EBV-associated PTLD and 16 other EBV-associated diseases. One hundred and sixty-nine patients (39.3%) developed CMV DNA-emia and 11 (2.7%) developed CMV-associated diseases. Of the 107 patients who developed EBV DNA-emia, 57 had CMV DNA-emia before EBV DNA-emia, and the median time from occurrence of CMV DNA-emia to EBV DNA-emia and EBV-associated diseases were 11 (range, 0-269) days and 23 (range, 0-255) days, respectively. Seven patients developed co-existing CMV DNA-emia at the time of EBV-associated diseases diagnosed. DNA-emia before EBV infection had positive correlation with EBV DNA-emia (r=0.23, p<0.001) and EBV-associated diseases (r=0.19, p<0.001), but both correlation coefficients were weak. There was a strong positive correlation between EBV DNA-emia and EBV-associated diseases (r=0.56, p<0.001). The patients with CMV DNA-emia had a higher risk of developing EBV infection than those without (OR 2.884, 95% confidence interval [CI] 1.8290-4.54627, p<0.001). Besides, CMV DNA-emia was the risk factor for EBV-associated diseases (OR=3.402, 95% confidence interval [CI] 1.737-6.663, p<0.001). After EBV infection occurred, 17 patients developed CMV DNA-emia, including 5 developed CMV-associated diseases, at a median time of 36 days (range, 12-50 days). EBV infection was not related to CMV DNA-emia (p=0.89) or CMV associated diseases (p=0.43) occurring after EBV infection.

Summary and Conclusion: The results suggest that CMV may play a contributory role in the development of EBV DNA-emia and EBV-associated diseases.

P1104

INTENSIFIED CONDITIONING REGIMEN WITH HIGH-DOSE ETOPOSIDE HEMATOPOIETIC STEM CELL TRANSPLANTATION YIELDED PROMISING SURVIVAL FOR HIGH-RISK AND REFRACTORY/RELAPSED ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Allogeneic hematopoietic stem cell transplantation plays an important role in treatment strategy of adult ALL. It's established that GVL effect was weak in ALL and patient shows poor response for DLI.

Aims: Intensified conditioning regimen is aimed to overcome resistance, reduce recurrence rate and improve survival.

Methods: A single-center, open-label, prospective study of intensified conditioning regimen with high-dose-etoposide allo-HSCT for adult ALL in China was performed from 2011 to 2013 in Nanfang hospital according to HDE-ALL-2011 (NCT01457040). The protocol was approved by ethics committee of Nanfang hospital of southern medical university and informed consent was obtained from each subject.

Results: A total of 104 patients were enrolled, 34 cases (32.7%) were refractory and/or relapsed patients. Forty-nine patients (47.1%) received HLA-matched sibling allo-HSCT, 27 patients (26.0%) underwent HLA-matched unrelated donor transplantation, 15 patients (14.4%) received HLA-haploidentical allo-HSCT and 13 patients (12.5%) received HLA-mismatched sibling transplantation. One hundred and one patients (97.1%) achieved complete engraftment and full-donor chimerism with a median of 14 days for neutrophils and 15 days for platelet, 3 patients failed to obtain engraftment. Forty-three patients (41.3%) received one to four pre-emptive and/or MRD-guided DLI from 2 or 3 months after transplantation. Acute III-IV grade graft-*versus*-host disease (GVHD) occurred in 15 patients, including 4 cases of DLI-induced GVHD, as 9 cases occurred in matched-sibling allo-HSCT arm (9/49, 18.4%), 3 cases in MUD allo-HSCT (3/27, 11.1%) and 3 cases in HLA-haploidentical/mismatched allo-HSCT group (3/28, 10.7%). Twelve patients with III-IV GVHD attained durable complete remission with cyclosporine A, tacrolimus, methylprednisolone, cyclophosphamide, methotrexate, anti-CD25 antibody and mesenchymal stem cells (MSC). The overall incidence of EBV viremia was 30.7% (n=32) and 50.0% percent (n=16) of patients received rituximab preemptive therapy. Seven patients (6.7%) developed EBV-related post-transplant lymphoproliferative disorders (PTLD) and durable complete remission was achieved in 6 out of 7 PTLD after integrated treatment with reduction in immunosuppression, rituximab-containing chemotherapies and donor-lymphocyte-infusion. CMV viremia occurred in 41.3% patients (n=43), 6 of them progressed to CMV-related interstitial pneumonia (IP, n=6), which accounted for 17.1% of transplantation-related mortality (TRM). Eleven patients relapsed after transplantation, representing the major cause of TRM (n=11), followed by CMV-IP (n=6), rapidly progressive brochiolitis obliterans (BO) after donor lymphocyte infusion (n=3), severe GVHD (n=3), severe infection (n=3), diffuse alveolar hemorrhage(n=2), thrombotic microangiopathy (TMA, n=2), SOS/VOD (n=1), PTLD (n=1), engraftment dysfunction (n=1), mitochondrial encephalopathy (n=1), and cerebral hemorrhage (n=1). With 2 year of Kaplan-Meier estimate of potential follow-up (KM-PF), 2-year overall survival (OS) of the whole cohort was 60.1%, with no significant difference (p=0.624) between patients in complete remission (59.3%) and refractory/relapsed patients (61.5%) before transplantation.

Summary and Conclusion: Intensified conditioning regimen with high-dose-etoposide allo-HSCT, HDE-ALL-2011, yielded a promising overall survival for high-risk and refractory/relapsed adult ALL.

P1105

Abstract withdrawn

P1106

AUTOLOGOUS OR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH HIGH-RISK AGGRESSIVE LYMPHOMAS: RETROSPECTIVE ANALYSIS IN A SINGLE CENTER IN KOREA

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Background: In relapsed/refractory lymphomas, allogeneic hematopoietic stem cell transplantation (allo-HSCT) was introduced to present graft *versus* lymphoma activity that resulted lower incidence of relapse. However, treatment-related mortality (TRM) is an important factor to overcome. Also, high-dose chemotherapy (HDT) followed by autologous (auto-) HSCT has been considered as a frontline therapy for high-risk lymphomas, while the role of auto-HSCT was already proved for sensitive-relapsed lymphomas.

Aims: We analyzed the clinical outcomes of HSCT in high-risk or relapsed/refractory lymphomas, and tried to introduce our reduced-intensity conditioning (RIC) regimen for allo-HSCT, and a HDT regimen 'BuMelTT' which was dose-reduced by 20% compared to the original protocol [Schiffman *et al.* Biol Blood Marrow Transplant 1997;3:261-266.] to avoid excessive toxicity previously reported.

Methods: From 2007 to 2013, we treated 141 lymphomas (40years, range: 18-65) with HSCT. We treated 57 high-risk lymphomas with frontline auto-HSCT. And we also performed auto-HSCT in 56 sensitive-relapsed lymphomas, and allo-HSCT was performed for 28 relapsed/refractory lymphomas. High-risk patients were selected by aaIPI scoring ≥ 2 mainly based on high LDH level and higher Ann-Arbor stage III~IV at diagnosis. For HDT regimen followed by auto-HSCT, we used 'BuMelTT' which consisted of intravenous busulfan (BU, 2.4mg/kg/day for 3d) and melphalan (MEL, 40mg/BSA/day for 2d) and thiotepa (TT, 200mg/BSA/day for 2d). We used RIC regimen for allo-HSCT which consisted of fludarabine (FLU, 30mg/BSA/day for 6d) and melphalan (MEL, 70mg/BSA/day for 1d) with or without total-body irradiation (TBI, 400cGy/2Fx/day for 2d). Stem cells were collected from fully matched sibling (n=10) or suitably matched (<2 allele-mismatched) unrelated donors (n=18). We used acyclovir and itraconazole for viral and fungal prophylaxes, and ciprofloxacin was also used for prophylactic gut decontamination.

Results: In the frontline auto-HSCT group, 37 patients were B-cell type and 20 were T-cell type, and in the sensitive-relapsed group treated with auto-HSCT, 37 were B-cell type and 19 were T-cell type. In the relapsed/refractory group treated with allo-HSCT, there were 8 DLBCL, 7 PTCL, 5 various T-cell lymphomas, 4 T-lymphoblastic lymphoma (TLL), 2 MCL, 1 BLL and 1 plasmablastic lymphoma. Among the relapsed/refractory group, 13 were primary refractory, 5 were relapsed after chemotherapy with refractoriness and 10 were relapsed after auto-HSCT. Only 4 patients (14.2%) were in complete response (CR) before allo-HSCT, and CR was achieved in 22 patients (78.5%) after allo-HSCT. We calculated clinical outcomes for the 3 groups (frontline auto-HSCT, sensitive-relapsed group treated with auto-HSCT and relapsed/refractory group treated with allo-HSCT) after a median follow-up of 25.6 months (3.5-84.0). Five-year OS was 85%, 66%, and 63% and 5-year DFS was 76%, 47%, and 55% respectively. Cumulative incidence of relapse at 5-year was 23%, 53%, and 30% respectively. TRM at 5-year was 21% after RIC-allo-HSCT, whereas there was only 1 case of TRM (0.8%) after using 'BuMelTT' regimen. The benefit of frontline auto-HSCT was significant for B-cell lymphomas, but the survival outcomes of sensitive-relapsed group treated with auto-HSCT or relapsed/refractory group treated with allo-HSCT were comparable with frontline auto-HSCT in the T-cell lymphoma subgroup.

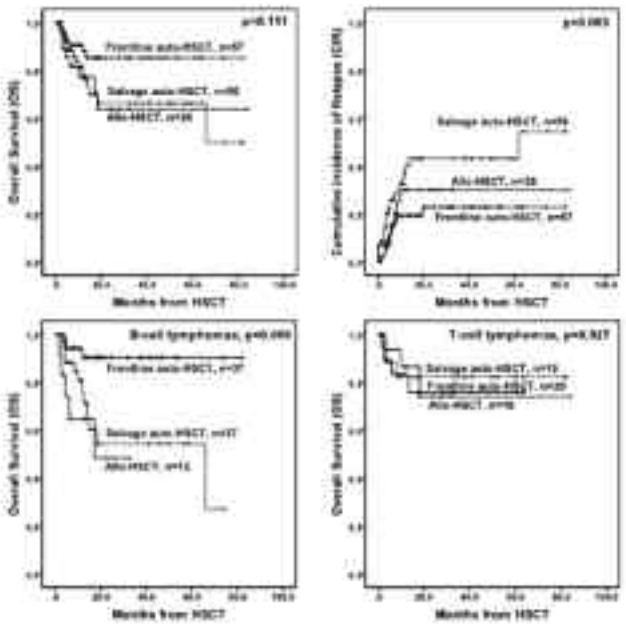

Figure 1.

Summary and Conclusion: Our data showed acceptable clinical outcomes of FLU+MEL+TBI for RIC-allo-HSCT and 'BuMelTT' for HDT for high-risk and sensitive-relapsed aggressive lymphomas. Further risk-adapted treatment approach using HSCT according to the type of lymphomas should be validated.

P1107

PROPHYLAXIS OF CMV INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH NILOTINIB : A PHASE II TRIAL

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Background: Cytomegalovirus (CMV) infection is an important complication which can arise after allogeneic stem cell transplantation (allo-SCT), especially in Taiwan, where the CMV sero-positive rate is more than 90% in adults. (Lu SC, Kaohsiung J Med Sci. 1999) Platelet-derived growth factor-alpha (PDGFRa) activation has been reported to be critical for CMV infection, (Soroceanu L, Nature 2008) a finding that provides a possible prophylactic approach to address CMV infection.
Aims: This trial is aimed to test whether PDGFRa inhibition by nilotinib could effectively protect patients from CMV infection after allo-SCT.
Methods: Patients with signs of engraftment, undetectable CMV copies, and no other anti-CMV medicine exposure were eligible. Nilotinib (200 mg/day) was given continuously. Plasma CMV DNA-copies were monitored at least once a week by real-time, quantitative-PCR in which the lowest detection limit was 150 copies/mL. Failure of prophylaxis by nilotinib was defined as plasma CMV copies higher than 10,000 copies/mL or, regardless of levels, initiation of anti-CMV treatments for definite or clinically suspected CMV disease during nilotinib administration. Primary endpoint was defined as rate of successful prophylaxis, *i.e.* free from failure of prophylaxis, by day+100 after transplant. A Simon two-stage design, in stage 2, considered the treatment unfeasible if CMV infection was successfully prevented in less than 22 patients among a total of 31 evaluable patients. The ClinicalTrial.gov identifier for this study is NCT01252017.
Results: Between Dec. 2010 and Jan. 2014, 37 patients were enrolled. All patients and donors were CMV sero-positive. Six patients were not evaluable because of their early withdrawal from the trial within 10 days of nilotinib administration: five were for adverse events (AEs) not related to nilotinib, and one, nilotinib-associated idiosyncratic reaction. The 31 evaluable patients, median age 46 years, contained 17 males and 14 females. Twenty-six patients received grafts from matched-siblings; fourteen patients received non-myeloablative transplant; and antithymocyte globulin (ATG) was used in 12 patients. The median time of starting nilotnib administration was day+23; the median duration of nilotinib administration was 79 days. Among the evaluable patients, none had nilotinib-associated grade 3/4 AEs or had early trial discontinuation for nilotinib-associated AEs. Twenty-five patients fulfilled the pre-defined successful prophylaxis criteria: in 12, plasma CMV was continuously undetectable; and in the other 13, there were detectable, but less than 10,000 copies/mL of CMV which later resolved spontaneously without development of CMV diseases. For the six patients whose plasma CMV copies were higher than 10,000 copies/mL, one elected to continue nilotinib treatment, four switched to ganciclovir treatment, and one received add-on ganciclovir therapy. Among them, one patient developed CMV colitis, whereas the other five patients' CMV levels declined smoothly later without development of CMV disease during follow-ups.

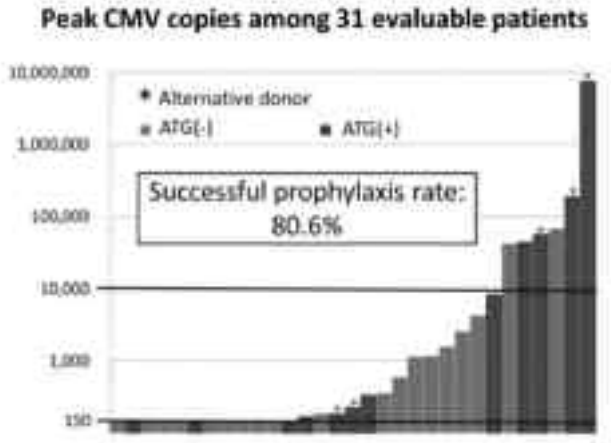

Figure 1.

Summary and Conclusion: Nilotinib is well tolerated and effective for prophylaxis of post allo-SCT CMV infection. Accordingly, the current result warrants further large, confirmatory studies.

P1108

BEEAM (BENDAMUSTINE, ETOPOSIDE, ARA-C, MELPHALAN) PRIOR TO AUTOLOGOUS STEM CELL TRANSPLANT IS SAFE AND EFFECTIVE IN AGGRESSIVE B-CELL NON HODGKIN LYMPHOMA: A PHASE II MULTICENTER STUDY

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Background: We previously demonstrated (Visani et al, Blood 2011) the safety of a new conditioning regimen with bendamustine, etoposide, cytarabine, and melphalan (BeEAM) prior to autologous stem cell transplant (ASCT) in resistant/relapsed lymphoma patients. The regimen showed long-lasting significant anti-lymphoma activity, with a 3-year PFS of 75%.
Aims: We designed a phase II study to confirm the effectiveness of BeEAM as a preparative regimen for autologous stem cell transplantation in resistant/relapsed aggressive B-cell non-Hodgkin lymphoma patients. The study was registered at EMEA with the EUDRACT no 2011-001246-14.The primary end-point of the study is to evaluate the 1-year complete remission rate. Fixing the lowest acceptable rate as 55% and the successful rate as 70%, with a significance level a=0.05 and a power 1-b=0.90, the sample size was estimated in 88 patients.
Methods: until now, 37 patients (median age 56 years, range 19-69) with resistant/relapsed diffuse large B cell (32) or grade III B follicular (5) non-Hodgkin lymphoma were consecutively enrolled in the study. Briefly, 27 patients had advanced stage disease (III-IV), 12 were primary refractory and 25 had relapsed after a median number of 2 lines of therapy (range: 2-3). Thirty-three patients had good performance status (WHO 0-1), and 11 patients presented with 1 or more relevant comorbidities (range: 1-5). Nineteen patients were in II or subsequent CR after salvage therapy, whereas 16 were in PR and 2 had progressive disease.
Results: A median number of $5.84x10^6CD34^+$/kg cells (range 2.8-8.8) collected from peripheral blood was reinfused to patients. All patients engrafted, with a

median time to ANC>0.5x10^9/l of 10 days. Median times to achieve a platelet count >20x10^9/l and >50x10^9/l were 12 and 16 days respectively. Eight out of 37 patients presented a fever of unknown origin (21.6%), whereas 19 patients (51%) presented a clinically documented infection. All patients received G-CSF after transplant for a median time of 8 days (range: 8-13). One patient died due to an incomplete hematological recovery after transplant, producing an overall transplant related mortality of 2.7%. Twenty-seven out of 37 patients are evaluable up to now for response to treatment. 22/27 (81.5%) obtained a CR, 2/27 a PR, whereas 3/27 did not respond to therapy. After a median follow-up of 9 months from transplant (range 2-24), 5/24 patients relapsed, whereas 19/24 (79.1%) are still alive, in continuous CR.

Summary and Conclusion: The BeEAM regimen preliminary confirmed its safety (TRM 2.7%) and its promising efficacy in resistant-relapsed aggressive B-cell lymphomas.

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P1109

USE OF AZATHIOPRINE FOR GRAFT-VERSUS-HOST DISEASE IS THE MAJOR RISK FOR DEVELOPMENT OF SECONDARY PRIMARY MALIGNANCIES AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Since 1983, hematopoietic stem cell transplantation (HSCT) has been widely used to treat and cure many malignant and benign hematological diseases in Taiwan. As more patients are treated by hematopoietic stem cell transplantation (HSCT), the development of secondary primary malignancy (SPM) is an increasingly common issue in long-term survivors.

Aims: To explore the incidence and identify the risk of SPM for patients treated with HSCT.

Methods: A nationwide population-based study of the Taiwanese population was performed to analyze patients who received HSCT between January 1997 and December 2010. Standardized incidence ratios (SIRs) were used to compare the risk of SPM in HSCT patients and the general population. Multivariate analysis was performed to identify independent predictors of SPM. A time-dependent covariate analysis was used to evaluate the association between cumulative doses of immunosuppressant and SPM.

Results: Patients receiving HSCT had a significantly greater risk of developing SPM (SIR 2.00; 95% confidence interval [CI] 1.45–2.69; p<0.001). Specifically, SPM incidence increased for cancers of the oral cavity (SIR 14.18) and esophagus (SIR 14.75) after allogeneic HSCT. Multivariate analysis revealed an increased SIR for cancer in patients who received the immunosuppressant azathioprine. The risk of SPM also increased with greater cumulative doses of azathioprine, especially those with exceeding 15,100 mg. Figure 1 demonstrated the cumulative incidence of SPM after transplantation.

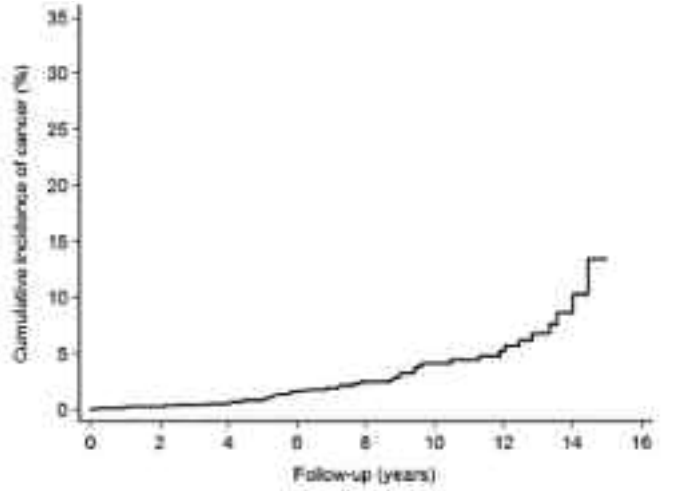

Figure 1.

Summary and Conclusion: This study demonstrates an increased incidence of SPM in Taiwanese patients who received allogeneic HSCT, especially for cancers of the oral cavity and esophagus. This finding is different from results in populations in Western countries. Physicians should be cautious about azathioprine use for graft-*versus*-host disease after HSCT. Long-term survivors who received HSCT should be carefully monitored for cancer development and targeted with preventive care strategies.

P1110

NEW FACTORS PREDICTING GRAFT FAILURE AND THE LOSING UNIT AFTER DOUBLE UMBILICAL CORD BLOOD ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULTS

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Background: Only few factors that predict engraftment or single-unit predominance after double umbilical cord blood (dUCB) allogeneic stem-cell transplant (allo-SCT) have been reported so far.

Aims: Our main objective here was to consider new factors, such as HHV-6 reactivation, age of the CB unit or the unit/unit matching, together with other well known factors, to examine if they can influence engraftment and single-unit predominance after dUCB allo-SCT.

Methods: We made a retrospective, single-center study included all patients receiving dUCB allo-SCT in Nantes hospital (France).

Results: This retrospective study included 77 patients (male: n=40; median age: 52 years, myeloid diseases n=43; complete remission at transplant n=46, previous autograft n=20) who had received a dUCB allo-SCT between June 2006 and December 2012 at the CHU of Nantes. The majority of patients received a reduced-intensity conditioning regimen (n=69) with a median interval between diagnosis and transplant of 11 months. Our main objective was to exhaustively determine factors associated with engraftment and single-unit predominance after this type of graft. With a median follow up of 40 months for survivors, 3-year OS, DFS, RI and TRM were 55+-6%, 44+-6%, 33+-5% and 23+-4%, respectively. Cumulative incidence (CI) of engraftment was 78±4% at day 60. CI of grade II-IV and III-IV acute GVHD were 27+-5% and 9+-3%, respectively. 3-year CI of chronic GVHD was 26+-5% (limited n=14; extensive n=5). In multivariate analysis, HHV6 reactivation during aplasia (HR=2.63; 95% CI: 1.64-4.17; p<0.001), younger recipient age (<53 years; HR=1.97; 95% CI: 1.16-3.35; p=0.012) and lower HLA matching between the 2 units (3/6 or 4/6, HR=2.09; 95% CI: 1.22-3.59; p=0.013) were factors independently associated with graft failure. Regarding single-unit predominance, multivariate analyses identified younger CB unit age (continuous, HR=1.01; 95% CI: 1-1.02; p=0.03), lower infused CD34+ cell doses (<=0.8 10^5/Kg; HR=2.55; 95% CI: 1.05-6.16; p=0.04) and ABO incompatibility between a CB unit and the recipient (OR: 2.53; 95% CI: 1-15-5.53; p=0.02) as unfavorable predictive factors.

Summary and Conclusion: Thus, HHV-6 reactivation during aplasia and younger cord blood age may represent new parameters to take into account after dUCB allo-SCT. These results have to be confirmed prospectively as they may influence units selection and outcomes of patients.

P1111

A SINGLE-CENTER RETROSPECTIVE ANALYSIS OF RECOMBINANT HUMAN SOLUBLE THROMBOMODULIN FOR THE TREATMENT OF HEPATIC SINUSOIDAL OBSTRUCTIVE SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Hepatic sinusoidal obstructive syndrome (SOS) is recognized as a lethal complication of stem cell transplantation (SCT). There is no specific therapy directed at this sinusoidal pathology, which affects sinusoidal endothelial cells and causes sinusoidal vasoconstriction and ischemic hepatocyte necrosis. Recently, recombinant human soluble thrombomodulin (rTM) has been approved for the treatment of disseminated intravascular coagulation in Japan.

Aims: The purpose of this study was to examine the effect of rTM on SOS prognosis and to identify other prognostic factors for SOS.

Methods: We retrospectively analyzed 16 patients with SOS after SCT (allo-SCT, n=13; auto-SCT, n=3) who were treated at Akita University Hospital between February 2001 and December 2013. SOS was defined according to Modified Seattle criteria and/or Baltimore criteria. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional review board of Akita University Hospital.

Results: We analyzed 16 patients who received SCT for the treatment of hematological malignancies. The median age was 48.5 years. The ratio of men to women was 12:4. All patients were treated with ursodeoxycholic acid and heparin as prophylaxis for SOS. Of these, 7 patients were administered rTM via intravenous drip injection at a dose of 380 units per kg for 7 days. The recovery rate from SOS was 75% at 100 days after hematopoietic SCT, and the median recovery time was approximately 40 days after the diagnosis of SOS. The administration of rTM was significantly correlated with recovery from

SOS (*P*=0.044). Moreover, several risk factors for SOS among the 12 factors previously reported, including hepatic or renal dysfunction before SOS; prior infection; administration of busulfan, cytarabine, cyclophosphamide, or methotrexate for SCT; allo-SCT; human leucocyte antigen-mismatch transplantation; lung disease complication; second transplantation; and radiation therapy to the abdomen before SCT, were significantly associated with recovery from SOS (*P*=0.020). Hyperbilirubinemia (maximum value) during SOS was also significantly associated with recovery from SOS (*P*=0.020).

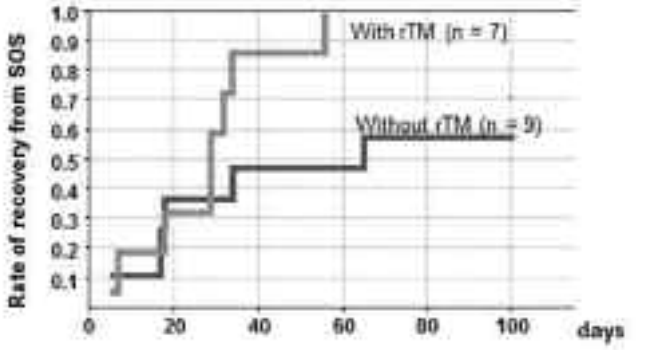

Figure 1.

Summary and Conclusion: According to previous reports, rTM directly repairs vascular endothelial injury or improves inflammation via the anti-high-mobility group protein B1 effect. Although a prospective study will be necessary to confirm our results, this retrospective analysis indicated that rTM might be a promising therapy for patients with SOS.

P1112

OUTCOME OF PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKAEMIA RELAPSED AFTER RELATED AND UNRELATED STEM CELL TRANSPLATATION. SINGLE CENTER EXPERIENCE

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Background: Disease relapse is the most common cause of treatment failure after stem cell transplantation (SCT) for advanced myeloid malignancies, and generally carries a poor prognosis with short median survival without active treatment.
Aims: We describe treatment and outcomes for patients (pts) who relapse following SCT and compare the outcome of the group of pts transplanted with related and unrelated donor.
Methods: In a retrospective single center study we analyzed 204 pts (73 pts after related and 131 pts after unrelated SCT) with high risk AML transplanted between the years 2000 and 2013. 24 pts (33%) relapsed after related SCT and 37 pts (28%) relapsed after unrelated SCT (p=0.5288). Median of relaps was 3 months in the group of related SCT(1-28 months) and 8 months (1-52) in the group of unrelated SCT (p=0.0031). Both groups were comparable according to age, disease status, prognostic factors and the type of conditioning (myeloblative *vs*. reduced intensity conditioning, p=0,3401). 33 pts (54%) received aggressive treatment for relapse (12/24 and 21/37, p=0,7929) including donor lymphocyte infusion alone (DLI) (9/24 and 6/37, p=0.0740), chemotherapy alone (2/24 and 6/37, p=0.4621), chemotherapy with DLI (0/24 and 7/37, p=0.0358) and second transplantation (1/24 and 2/37, p=1,0).
Results: 11 pts (18%) archieved complete response (CR) after treatment for relapse (10 pts after DLI with or without chemotherapy and 1 pt after second transplantation), 1 pts transplanted with related donor and 10 pts transplanted with unrelated donor, p=0.0380. 5 pts still alive (1 from group of related SCT and 4 from group of unrelated SCT) 4-99 months after SCT with median of overall suvival(OS) 5 months after related SCT and 11 month after unrelated SCT, the probability of 1 year OS 8% and 22% (p=0,0293). 50 pts died, most of them due to disease progression (47/50, 94%), 1 pts died due to GVHD after DLI, 1 pts due to respiratory failure and 1 pts due to suicide. We did not observe difference in the outcome among pts treated with DLI alone, chemotherapy alone and second transplantation, better outcome had group of pts relapsed after unrelated transplant treated with chemotherapy and subsequent DLI.
Summary and Conclusion: Salvage chemotherapy, cellular therapy or second transplantation for AML relapse after SCT is feasible with low treatment-related mortality, but optimal management of the therapy for AML relapsing after SCT still remains to be defined. Comparing the group of pts transplanted with related donor and pts transplanted with unrelated donor better outcome was found in the group of unrelated transplantation treated with chemotherapy and DLI for AML relapse.

P1113

UNRELATED BONE MARROW TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING REGIMEN FOR ELDERLY PATIENTS WITH ADULT T-CELL LEUKEMIA/LYMPHOMA, FEASIBILITY STUDY WITH TWO-YEAR FOLLOW UP DATA

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Background: Adult T-cell Leukemia Lymphoma (ATL) is a peripheral T-cell malignancy that is caused by human T-lymphotropic virus type 1 (HTLV-1) infection and commonly affects individuals at an average age of 67 years. Since the prognosis of elderly patients with the disease has been unsatisfactory by conventional chemotherapy, we had so far conducted two clinical trials of allogeneic hematopoietic stem cell transplantation (allo-SCT) using G-CSF-mobilized peripheral blood stem cells from HLA-identical sibling donors combined with reduced-intensity conditioning regimen (RIC) and reported promising results (Okamura *et al*, *Blood*, 2005; Tanosaki *et al*, *Biol Blood Marrow Transplant*, 2008; Choi *et al*, *Bone Marrow Transplant*, 2011). The availability of suitable sibling donors, however, becomes difficult probably due to aging as well as complications of donors. Therefore, we conducted a clinical trial to evaluate the feasibility of an alternative strategy of allo-SCT using bone marrow cells from unrelated donors, and we reported the results in ASH meeting 2011 (San Diego, CA). Here we report the results of two-year follow up.
Aims: We conducted a clinical trial to evaluate the feasibility of an alternative strategy of allo-SCT using bone marrow cells from unrelated donors, and we reported the results in ASH meeting 2011 (San Diego, CA). Here we report the results of two-year follow up.
Methods: Fifteen patients, median age of 58 (range, 51-62), were transplanted between February 2009 and April 2011. Seven were male, 12 were acute type and 3 were lymphoma type. At the time of registration, seven were in complete and 8 were in partial remission after chemotherapy. The conditioning regimen consisted of fludarabine (180 mg/m^2), intravenous busulfan (6.4 mg/kg) and low dose total body irradiation (2Gy). Bone marrow grafts from the Japan Marrow Donor Program, whose HLA-A, B and DR loci were genotypically matched or DR one locus-mismatched donors, were transplanted on day 0. To prevent graft *versus* host disease (GVHD), tacrolimus (0.03 mg/kg/day) and short-term methotrexate were administered. The degrees of donor-recipient chimerism and HTLV-1 proviral DNA in peripheral blood mononuclear cells were quantified by published methods.
Results: Thirteen of 15 patients achieved the primary objective, achievement of complete donor chimerism before day 100 and survival at day 100 after SCT and we concluded that SCT using bone marrow cells with RIC from unrelated donors is a feasible therapeutic procedure for elderly patients with ATL. In the results of two-year follow up after transplantation, relapse was observed in 4 patients days 39, 59, 161, and 336 after transplant, respectively and all died of disease. Acute GVHD (aGVHD) was observed in 10 of 15 patients where 2/6/2 patients experienced grade III/II/I, and chronic GVHD was observed in 10 of 14 patients who survived more than 100 days after transplantation where 6 patients experienced extensive type. The two-year progression free survival (PFS) and overall survival (OS) were 65.5±25.0% and 66.7±23.9% respectively. The HTLV-1 proviral load after SCT decreased to an undetectable level (<0.5 copies) at least at once in 13 of 15 patients, and 9 of 10 were remaining undetectable in two years after transplantation. The presence of aGVHD grade I-II was to be favorable prognostic factor for PFS or OS in the patients with ATL undergoing allo-SCT from HLA-identical sibling donors, but in the present study, we could not confirm them in the setting of unrelated bone marrow donors, probably because the event was too small.
Summary and Conclusion: This study indicated that allo-SCT using bone marrow cells with RIC from unrelated donors is a feasible and potent highly effective therapeutic procedure for elderly patients with ATL. (UMIN000001355)

P1114

BIOSIMILAR VERSUS ORIGINATOR G-CSF FOR AUTOLOGOUS STEM CELL MOBILIZATION IN HEMATOLOGIC PATIENTS. AN EXPERIENCE FROM THE PHARMACOVIGILANCE GROUP OF "RETE EMATOLOGICA LOMBARDA" (FARMAREL)

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Background: The use of G-CSF biosimilars in the context of stem cell harvest was approved by the European Medicines Agency (EMA), even if its therapeutic equivalence has been extrapolated in the absence of "ad hoc" studies.
Aims: To assess the efficacy and safety profile of biosimilar compared with originator G-CSF on PBSC autologous mobilization in association with chemotherapy (CT) in hematologic pts treated in the Hematology Units of REL.
Methods: Data were retrospectively collected from consecutive pts with Hodgkin disease (HD), non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) undergoing PBSC harvest after CT with biosimilar filgrastim between July 2012 and December 2013; furthermore lymphoma cases were compared with a historical control group treated with originator filgrastim between August 2007 and December 2008. The Shapiro-Wilk test was used to assess normality of data: p-values were derived from Mann–Whitney U-test.
Results: One hundred-five leukapheresis from 96 consecutive pts including 3 HD, 50 NHL (45 HIV-negative and 5 HIV-positive) and 43 MM cases were analyzed. Median age was 57 yrs (19-73); 56 cases were male (58 %). Mobilization was planned as part of first line CT in 65% pts; only one case was mobilized during 3rd line CT. Fourty-five cases (47 %) had been previously treated with alkylating drugs (47 %), three (3 %) with fludarabine-containing regimens. Biosimilar filgrastim was used at doses ranging from 5 (MM and lymphoma HIV-negative) to 10 mcg/kg/d (lymphoma HIV-positive) subcutaneously, starting from the day after the end of CT until the end of leukapheresis. Mobilization started a median of 13 days after CT (range 10-21) and was completed in a median of one procedure (range 1-4); median number of CD34+ cells x 10^6/Kg/pt collected was 9, ranging from 3,05 to 47. Planned end-point was reached in all but 2 cases (3 %). During mobilization, median peak of circulating white blood cells (WBC) and of CD34+ cells count were 12,740 x 10^9/l (range 2,160-66,580) and 90,5 x 10^9/l (range 15-1435), respectively. Biosimilar filgrastim use was generally well tolerated: mild bone pain (WHO grade 1-2) was frequently reported (16 pts, 23 %); two pts needed treatment with acetaminophen for headache (1) and bone pain (1), both WHO grade 3. Data from 53 lymphoma pts were compared with a control group of 58 pts mobilized with originator G-CSF (filgrastim or lenograstim) between August 2007 to December 2008 (Table, part A), differing for a higher frequency of mobilization with high-dose cyclophosphamide (24 % *vs*. 4 %, p<0.001; Table, part B) only. Median number of procedures, CD34+ count peak and CD34+ collected were similar in both groups, however a statistically significant difference in median WBC count peak during leukapheresis was documented (Table, part C).

Table 1.

Summary and Conclusion: We confirm efficacy and safety of biosimilar filgrastim for autologous PBSC mobilization in lymphoma and myeloma pts. The lower WBC count peak after biosimilars compared to reference products in spite of similar efficiency in CD34+ cells mobilization is interesting and needs to be confirmed in larger studies.

P1115

COMPARISON OF UNRELATED CORD BLOOD TRANSPLANTATION AND HLA-MATCHED SIBLING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN ADVANCED STAGE

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Background: Patients with chronic myeloid leukemia (CML) in accelerated phase (AP) and blast crisis (BC) are generally considered as having a poor prognosis, and allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative option. However, the majority of the published studies are based on the results obtained by human leukocyte antigen (HLA)-matched related allogeneic peripheral blood stem cell (PBSC) or bone marrow (BM) transplantation (allo-PBSCT/BMT), and few studies have evaluated the treatment effect of unrelated cord blood transplantation (CBT).
Aims: In the present retrospective single-center study, we report a clinical comparison of unrelated CBT and HLA-matched sibling allo-PBSCT/BMT for patients with CML in the advanced stage.
Methods: A total of 35 consecutive patients with advanced CML received unrelated CBT (n=18) or HLA-matched sibling allogeneic peripheral blood stem cell or bone marrow transplantation (allo-PBSCT/BMT) (n=17) between 22 March 2002 and 11 November 2012.
Results: The median day to neutrophil engraftment and platelet engraftment were longer in the unrelated CBT group (21.3d of CBT *vs* 12.8d of allo-PBSCT/BMT, p=0.01; 42.8d of CBT *vs* 15.9 d of allo-PBSCT/BMT, p=0.006). The cumulative incidence of grades I-II acute GVHD (aGVHD), III- IV aGVHD and chronic GVHD (cGVHD) did not differ significantly between the 2 cohorts: the day +100 cumulative incidence of grades I-II acute GVHD (aGVHD) was 30.4% [95% confidence interval (CI), 21.6- 39.5%] for CBT patients and 36.9% (95% CI, 27.6- 45.8%) for allo-PBSCT/BMT patients (p=0.65), and III-IV aGVHD was 23.8% (95% CI, 13.9-32.6%) for CBT patients and 16.9% (95% CI, 8.4-24.2%) for allo-PBSCT/BMT (p=0.19). The cumulative incidence of chronic GVHD (cGVHD) was 18.2% (95% CI, 11.4-28.9%) in the CBT group and 32.6% (95% CI, 21.8-41.5%) in the allo-PBSCT/BMT group (p=0.14). The cumulative incidence of transplant-related mortality (TRM) at d+180 (early TRM) was higher in CBT group (36.2% *vs* 12.5%, p=0.015). The risk of relapse was lower in CBT patients compared with that of allo-PBSCT/BMT patients (12.8% *vs* 39.6%, p=0.02). The long-term survival in CBT group patients was slightly better than allo-PBSCT/BMT group although the difference did not reach statistical significance: the 5-year overall survival (OS) for CBT patients and allo-PBSCT/BMT patients was 63.4% and 50.1%, respectively (p=0.11), while the 5-year leukemia-free-survival (LFS) rate was 54.8% and 44.3%, respectively (p=0.15).
Summary and Conclusion: Our comparisons suggested that patients with advanced CML receiving unrelated CBT had a lower relapse rate, a slightly better long-term survival, but a higher early TRM than HLA-matched related allo-PBSCT/BMT.

P1116

SEQUENTIAL CHEMOTHERAPY FOLLOWED BY REDUCED INTENSITY CONDITIONING AND ALLOGENEIC HSCT FOR HIGH RISK AML PATIENTS IN FIRST COMPLETE REMISSION: A PROSPECTIVE PILOT STUDY

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Background: High-risk acute myeloid leukemia (AML) patients still have a poor outcome, the only therapeutic strategy with curative potential remains allogeneic HSCT.
Aims: With the aim to improve the effect of allo-HSCT by sequential use of chemotherapy followed by reduced intensity conditioning (RIC), we conducted a prospective pilot study in high-risk AML patients in first complete remission (CR1).
Methods: The chemotherapy sequential regimen consisted in fludarabine 30 mg/m², high-dose cytarabine 2 g/m², and amsacrine 100 mg/m² from days -12 to -9 (FLAMSA). After 3 days of rest, RIC consisted of 4 Gy total-body irradiation (TBI) on day -5, cyclophosphamide (40 mg/kg with HLA-identical sibling, 60 mg/kg for unrelated or mismatched donors) on days -4 and -3, and rabbit antithymocyte globulin (ATG, Genzyme) (5 mg/kg total dose) from day -3 to day -1. As a new experimental approach, we replaced TBI by iv. busulfan (BU) (Busilvex, Pierre Fabre) 3.2 mg/kg/d during either 4 or 2 days according to patient age (>55 years) (from day -7 to -4 or from day -5 to -4). GvHD prophylaxis consisted in ciclosporine from day −1, and mycophenolate mofetil (15 mg/kg bid), starting from day 0. Except for cord blood transplantation, patients received 3 prophylactic increased doses of donor lymphocyte infusions (DLI) if they were in CR and GvHD-free at day +120 or 30 days after discontinuation of immunosuppressive agents starting at 1x10^6 CD3+ cells/kg.

Results: Between August 2010 and March 2013, 26 consecutive patients were included; 11 males and 15 females with a median age at allo-HSCT of 55 years (24-67), 19 (73%) were *de novo* AML and 7 (27%) secondary AML. According to cytogenetics and molecular markers, 22 (85%) were unfavourable and 4 (15%) were in intermediate II category. Stem cell source was PBSC for 23 (88%) patients, CB for 2 and BM for 1 patient. Donors were 10/10 HLA matched siblings in 9 (35%) patients, 10/10 HLA matched unrelated in 8 (31%) patients and HLA mismatched for the rest of patients [unrelated 9/10 (n=7), CB 4/6 (n=2)]. For ABO compatibility, 13 (50%) were compatible, 5 (19%) had minor incompatibility and 8 (31%) had major incompatibility. For conditioning, 6 (23%) patients received TBI, 13 (50%) received 4 days BU and 7 (27%) received 2 days BU. After transplantation, 23 (88%) patients engrafted, 3 patients died early (1 at day 1 and 1 at day 2 both from septic shock; 1 at day 8 from pneumonia and pericardial effusion). At day 90 post-allo-HSCT, 18 (78%) showed total donor chimerism and 5 (22%) had mixed chimerism and all patients were in CR. There were 6/23 (26%) patients with acute GvHD [2 gr I, 2 gr II and 2 gr III] and 5/23 (22%) chronic GvHD [4 limited and 1 extensive], all before DLI. After a median follow-up of 9 months (0.03-35), the 2-years probability of overall survival (OS) for the whole population was 58% (47-69) and the 2 years cumulative incidence of relapse was 18% (confidence interval: 17-19). At the latest follow-up, 16/23 (70%) engrafted patients were alive, 4/23 (17%) patients relapsed and died later and 3/23 (13%) patients died from transplant related infectious complications. No statistical difference in terms of OS and relapse incidence was found between the 3 types of conditioning.
Summary and Conclusion: FLAMSA-RIC regimen followed by allo-HSCT showed promising results in high-risk CR1 AML patients. Because of some early severe infections, an efficient prophylactic anti-infectious strategy is recommended. The use of BU instead of TBI does not impact on transplant outcomes.

P1117

"REAL LIFE" USE OF PLERIXAFOR: A MULTICENTER NORTH EAST ITALY EXPERIENCE

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Background: Plerixafor is a reversible inhibitor of chemokine stromal cell derived factor 1 alpha (SDF1a) binding to chemokine (C-X-C motif) receptor 4 (CXCR4). Interruption of the CXCR4/SDF 1-a interaction enhances CD34+ haematopoietic stem cells (HSCs) mobilization with G-CSF alone or G-CSF plus chemotherapy to the peripheral blood where stem cells can be collected. The efficacy of Plerixafor has been demonstrated in patients affected with Hodgkin Lymphoma (HL), non Hodgkin Lymphoma (nHL) and Multiple Myeloma (MM). Besides, there is a lack of standardized criteria on how and when to use Plerixafor and each center develops its own treatment plan.
Aims: The aim of our survey was to study the "real life" use of plerixafor.
Methods: A questionnaire for each dose of Plerixafor has been filled by hematologists and apheresis physicians of each center. The retrospective survey involved all the patients treated between January 2010 and June 2013 in 7 centers of HSCs collection and transplantation in north-east Italy.
Results: Plerixafor was administered at the dose of 0.24 mg/kg. 65% of patients were affected with nHL, 24% with MM, 5% with HL, 3% with AL Amyloidosis, 2% with POEMS syndrome, 1% with Acute Lymphoblastic Leukemia. 76% were "proven poor mobilizers" and 24% were "predicted poor mobilizer" according to the GITMO criteria. 31% of patients were mobilized by G-CSF alone, 69% received G-CSF plus chemotherapy. According registration studies, 70% of patients collected at least 2.0 x10^6 cells/kg, and 66% reached a CD34+ peak of more than 20 cells /µl. We did not find any statistically significant difference in CD34+ peak between predicted and proven poor mobilizers. 48% of patients reached the target with one dose of the drug and 48% with two doses. When baseline CD34+ cell count was more than 6 cells/ml we observed a four-fold increase of baseline CD34+ cell count after Plerixafor administration. When baseline CD34+ count was below 6 cells/ml we observed a seven-fold increase in CD34+ cell count. Interestingly, a baseline CD34+ count below 2 cells/ml was associated, in our experience, with failure to achieve the endpoint of collection of at least 2.0x10^6 CD34+/kg. A ROC curve has allowed to estimate a discrimination threshold of 6 CD34+ cells/µL for a successful mobilization with Plerixafor (collection of more than 2.0x10^6 CD34+/kg). Only one serious adverse event was reported: a spontaneous splenic rupture in a patient affected with AL amyloidosis. 34% of our patients underwent stem cell transplantation, and neutrophils and platelets engraftment were on expected time (10,8 days and 18,9 days, respectively).
Summary and Conclusion: Our study showed that, in our experience, plerixafor has the same efficacy as the registration studies With the limitations of a retrospective study, our work documented that a CD34+ cell count below 2.0x106 cells is associated with collection failure in 100% of patients. Considering the cost, the use of Plerixafor in such patients may require careful evaluation. The development of standardized criteria for patients selection and timing of Plerixafor administration is needed.

P1118

TREATMENT OF CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) WITH RITUXIMAB AND NILOTINIB, FIRST RESULTS OF ONGOING CLINICAL TRIAL

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Background: About 60% of all patients receiving an allo-SCT and surviving beyond day 100 develop cGvHD, assuring high morbidity within this patient group. For this reason, chronic GvHD is associated with a substantial impairment in quality of life and loss of employment in long-term allo-SCT survivors. For physicians, cGvHD poses a big challenge to treat adequately and satisfactory. The current treatment relies heavily on corticosteroids, however refractoriness is a big problem as well as the unwanted effects of longterm corticosteroid use. Therefore, new therapies are urgently needed. Earlier studies by others and us[1] have demonstrated amelioration of chronic GvHD symptoms by B-cell depletion. In addition inhibition of the PDGF pathway by tyrosine kinase inhibitors also provided provocative data. However, complete resolution of cGVHD is usually not reported with single drug therapies and cGvHD with ulcera has so far been refractory to most experimental therapies.
Aims: We aim to test whether the sequential therapy of the anti-CD20 antibody Rituximab followed by a 6 month period of treatment with the tyrosine kinase inhibitor Nilotinib can further improve response rates.
Methods: 30 patients are treated with a combination of 4 weekly infusions of the anti-CD20 antibody Rituximab followed by a 6 month period of treatment with the tyrosine kinase inhibitor Nilotinib. Patients have been evaluated monthly.
Results: 4 patients have completed the study period whilst 11 patients are still being treated. Three out of four patients who completed the study showed a partial response. Two patients showed complete resolution of ulcerative skin lesions. Response rates for the remaining patients will be available shortly.
Summary and Conclusion: The sequential therapy of B-cell depletion and tyrosine kinase inhibition might show for the first time a complete resolution of ulcerative cGvHD and provides a new and interesting alternative treatment option for this difficult patient category.

P1119

INCREASED RISK OF SEVERE ACUTE GRAFT-VERSUS-HOST DISEASE IN LOW BODY MASS INDEX PATIENTS UNDERGOING HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Previous investigations reported miscellaneous impacts of body mass index(BMI) on outcomes in allogeneic stem cell transplantation(allo-HSCT).
Aims: To investigate the impact of pretransplantation BMI on clinical outcomes in haploidentical allo-HSCT.
Methods: We performed a retrospective cohort study of 253 adult patients with acute or chronic leukemia from Aug 2008 to Dec 2011. All patients received bulsufan based myeloablatvie conditiong regimen. Patients were stratified according to BMI values (low BMI: BMI<18kg/m2; normal BMI: 18≤BMI<25kg/m^2, overweight: BMI≥ 25kg/m^2). Other possible factors correlated with the occurence of major events also included recipient and donor age,donor and recipient gender, HLA disparity, relationship between donor and recipient, diagnosis, the status of the disease, ATG dose in conditioning regime (10mg/kg, 6mg/kg), mononuclear cells (MNC), $CD34^+$ and $CD3^+$ cell dose from granulocyte colony-stimulating factor (G-CSF) primed bone marrow grafts (G-BM) and G-CSF mobilized peripheral blood grafts (G-PB).
Results: Median age of 253 patients was 31 years (18-56) years. Among these patients, there were 128 (50.6%) with AML, 95 (37.5%) with ALL, and 30 (11.9%) with CML. According to primary disease, 185 (73.1%) cases were classified in the normal-risk group and 68 (26.9%) were in the high-risk group. Median follow-up was 929 days (range: 48-1762 days) post-transplantation. 252 (99.6%) recipients attained engraftment and the median time for granulocyte recovery and platelet recovery was 12 days (ranging from 9 to 45 days) and 16

days (ranging from 5 to 86 days), respectively. Cumulative incidences of acute GVHD was 33.2% with median time of 25 days (range: 13-88 days). Cumulative incidences of acute GVHD accoding to BMI group were similar for grades II–IV (low BMI: 52.9%; normal: 31.8%; overweight: 31.6%; *P*=0.252), but significantly different for grades III-IV (low BMI: 35.3%; normal: 8.9%; overweight: 10.16%; *P*=0.040). Multivariate analysis identified low BMI was associated with an increased risk of grade III-IV acute GVHD (*P*=0.003, HR=5.736, 95% CI: 1.779-18.491). There were no difference in the occurrence of other graft-*versus*-host reactions, treatment related mortality (TRM), relapse or overall survival (OS) among BMI groups.

Summary and Conclusion: Our findings demonstrate a correlation between pre transplant BMI and clinical outcome post-transplant. An unhealthy BMI was associated with increased risk of severe acute GVHD in haploidenctical allo-HSCT. Meticulous supportive care with nutritional support pre-transplantation should be required for these patients.

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P1120

DEFIBROTIDE AS PHOPHYLAXIS OF LIVER TOXICITY DURING ALLOGENEIC HSC TRANSPLANTATION IN ACUTE LEUKEMIA PATIENTS

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Background: Beneficial effects of a Prophylaxis using Defibrotide after allogeneic transplantation has not clearly established yet.
Aims: To determine clinical results of a prophylaxis with Defibrotide in adult acute leukemia patients receiving allogeneic HSC transplantation after myeloablative conditioning.
Methods: 51 patients received prophylaxis with Defibrotide from commencement of conditioning to discharge. Controls were 56 patients who did not receive this prophylaxis. Defibrotide was used in all patients treated from year 2000 to 2008, while all patients treated from 2008 to 2013 did not receive prophylaxis with Defibrotide. The two groups were comparable for age (p=0.18), diagnosis of AML *vs* ALL (p=0.9), percentage having active disease at transplant (p=0.9), percentage having received a previous transplant (p=0.4) and bilirubin level at admission (p=0.20), BU-FLU was administered in 53% of "No Defibrotide" group *versus* 25% of "Defibrotide treated", (p=0.02). MUD transplants were more frequent in "No Defibrotide" group (42%) than in group treated with Defibrotide (20%), (p=0.01).
Results: Overall, cumulative incidence of TRM at 3 years was 20%. It was not influenced by Diagnosis (p=0.7), type of conditioning (p=0.6), age of patients (p=0.18) and disease activity at time of transplantation (p=0.7). TRM at 3 y was higher in MUD transplants (30%) compared to family donor transplants (10%), p=0.05. Taking competing risks into account, cumulative incidence of TRM at 3 y was 30% in "No Defibrotide" group, compared to 10% in "Defibrotide treated" group (log-rank p=0.02). When significant factors, such as MUD donor and Defibrotide use, were included in multivariate analysis "No Defibrotide" remained significant for an increased risk of TRM at 3y (OR: 2.822, p=0.05). Overall PFS at 3 y was 60%, with no difference between Defibrotide treated and untreated (PFS was 65% in "No Defibrotide" *versus* 55% in Defibrotide treated groups, p=0.15). OS was 50% at 3 years: 55% in "Defibrotide treated" and 45% in "No Defibrotide" groups (p=0.6). No patient suffered severe liver toxicity, clinically mild SOS was diagnosed in 5.3% of "No Defibrotide" group and in 3.9% of patients treated with Defibrotide (p=0.7); Bilirubin above 2.0 mg/dl was registered during the first 30 days in 21% of patients in "No Defibrotide" group and in 15% of "Defibrotide treated" group (p=0.4).

Table 1.

Summary and Conclusion: Prophylaxis using Defibrotide after allogeneic hematopoietic transplantation with myeloablative conditioning determined, in adults patients, a significant reduction in TRM. Reduction in liver toxicity was not statistically significant, thus effect on TRM is independent of any reduction in liver toxicity and may depend on modulation of allo-reactivity.

P1121

IMPACT OF NATURAL KILLER (NK) CELL KIR LIGAND MISMATCH AND B SCORE ON THE OUTCOME OF HAPLOIDENTICAL TRANSPLANTATION WITH POSTTRANSPLANTATION CYCLOPHOSPHAMIDE (PTCY) IN APLASTIC ANEMIA

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Background: NK Cell KIR Ligand Mismatch (KIRLMM) has been shown to reduce the risk of relapse in acute myeloid leukemia (AML) following myeloablative T cell depleted (TCD) Haploidentical BMT (HAPLO). However, there is no data on the impact of KIRLMM or KIR B-score based on centromeric and telomeric distribution of inhibitory and activating genes in HAPLO for Severe Aplastic Anemia (SAA) with or without TCD.
Aims: We prospectively evaluated the impact of KIRLMM and B-score in 10 consecutive patients undergoing HAPLO with PTCY for SAA following unmanipulated PBSC graft, and compared with 15 patients undergoing the same for AML.
Methods: Donors were not selected based on KIRLMM initially. The patients with High-Risk (HR) AML (n=15) received myeloablative conditioning with Flu-

IVBu6.4-Mel140 (n=10) and those with SAA received Flu-ATG-Cy- Mel120. In patients with AML, 11 were transplanted with active disease and 4 with HR cytogenetics were transplanted in CR1/CR2. The median age was 41 (6-46) years for SAA and 32 years (8-48 yrs) for AML. Patients with SAA were heavily transfused (median 35 *vs* 10 in AML).
Results: All but one engrafted in both groups within 2 weeks. aGVHD/cGVHD ocurred in 2/2 and 1/1 patient in AML and SAA groups respectively. The incidence of relapse was 10% in patients receiving transplant from a KIRLMM donor with a B-score >2 (n=8), *versus* 90% in patients without the same (n=7), with a DFS at 2 years of 80% *versus* 10% (p=0.04). In patients with SAA, all patients with KIRLMM donors with B-score>2 (n=4) had early alloreactivity and TRM within 100 days, whereas those without the same (n=6) had 100% DFS at 2yrs (n=0.007). We documented early lymphocyte proliferation with full donor chimerism in patients with SAA between days 2 and 5 post transplant, compared to muted proliferation in patients with AML with mixed chimerism (p=0.01).
Summary and Conclusion: This pilot study on the effect of NK cell on HAPLO PBSCT with PTCY highlights the adverse impact of KIRLMM donor with high B-score in patients with SAA, in contrast to the favourable impact in AML. Based on these findings, we hypothesise that the lack of previous cytotoxic therapy and less intense conditioning spares recipient dendritic cells which are matured rather than inhibited in a high DC:NK ratio, promoting proliferation of donor alloreactive T cells. This process is enhanced further by NK cell mediated killing of host T cells (more effective as there is no leukemic target) as demonstrated by complete donor chimerism of T lymphocytes before day 5 in patients with SAA, compared to mixed chimerism in patients with AML. This unique finding, we hope, will play a major role in donor selection and improving the outcome of patients with SAA undergoing Haploidentical HCT. We suggest, patients with SAA undergoing Haploidentical HCT with KIRLMM donor should receive a TCD graft, whereas those with nonKIRLMM donors can receive unmanipulated graft with PTCY with excellent outcome.

P1122

THE JOINT IMPACT OF HLA MATCH AND TOTAL NUCLEATED CELL DOSE ON OUTCOME OF SINGLE UNIT UNRELATED CORD BLOOD TRANSPLANTATION

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Background: It's accepted that higher total nucleated cell (TNC) dose and less human leukocyte antigen (HLA) mismatch(mm) may produce a satisfactory outcome in recipients conducted cord blood (CB) transplantation. However, there is no consensus that whether the higher TNC dose can compensate the adverse impact of HLA mm.We tried to explain the joint impact of HLA match and TNC dose on outcome of single unit unrelated cord blood.
Aims: To retrospectively study the joint impact of TNC dose and HLA match between donor and recipient on outcome of single unit unrelated cord blood transplantation (sUCBT).
Methods: 139 patients with hematological malignancies remedied with sUCBT in single center from May 2008 to August 2013 were analyzed. Of 139 patients at enrollment, 22 were 0 mm,69 were 1mm, 48 were 2mm by low-resolution HLA-A, -B, and high-resolution (HR) DRB1. All patients's conditioning regimen was myeloablative,and a combination of cyclosporine A(CsA) and mycophenolate mofetil (MMF) was given for graft-*versus*-host disease (GVHD) prophylaxis for all patients. The follow-up assessment was conducted before February 1,2014.
Results: Patients 0mm had a statistically significant higher cumulative incidence of neutrophil engraftment by day 42 than those 1 and 2mm(*P*=0.042 and 0.002),patients 0mm had a statistically significant higher cumulative incidence of neutrophil engraftment by day 42 than those 2mm with a higher prefreeze total nucleated cell(TNC) dose(>5×10^7/Kg) and lower dose(<5×10^7/Kg)(*P*=0.01 and 0.02). Patients 0mm had a statistically significant lower cumulative incidence of acute GVHD by day 100 than those 1 and 2mm(*P*=0.006 and 0.001).The difference of transplant-related mortality(TRM) by 1 year between 0 and 2mm patients was statistically significant(*P*=0.03). Patients 2mm given CB units with a TNC dose less than 5×10^7/Kg had a higher TRM by 1 year than 0mm patients(*P*=0.03). Patients 0mm had a statistically significant higher disease free survival (DFS)by 3 years than those 2mm (*P*=0.03), compared with patients 2mm given CB units with a TNC dose less than 5×10^7/Kg, 0mm patients and 1mm patients given CB units with a TNC dose greater than 4×10^7/Kg had a higher DFS rate (*P*=0.02 and 0.02).
Summary and Conclusion: The HLA typing mismatching between donor and recipient had a great impact on neutrophil engraftment and long term DFS after sUCBT,2mm cord blood unit with less TNC(<5×10^7/Kg) was not an optimum CB graft.

P1123

(90)Y IBRITUMOMAB TIUXETAN (ZEVALIN) FOLLOWED BY BEAM (Z-BEAM) AND AUTOLOGOUS TRANSPLANT (ASCT) IN POOR PROGNOSIS RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL): A SINGLE INSTITUTION EXPERIENCE

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Background: High dose chemotherapy (HDC) and ASCT is considered the most effective treatment for relapsed aggressive NHL. Radioimmunotherapy (RIT) with standard dose Zevalin (0.4 mCi/kg) combined with conventional BEAM (Z-BEAM) has been employed as conditioning regimen for the treatment of high risk relapsed/resistant NHL.
Aims: We performed a single institution retrospective study to determine the feasibility and to explore the possible implemented effect of the addition of standard dose Zevalin to a BEAM regimen in pts with high risk advanced stage NHL, who relapsed or failed to respond after previous line chemoimmunotherapy. A comparative analysis with an hystorical group of patients treated with standard BEAM without Zevalin was planned as secondary end point.
Methods: Patients were included into the study and considered at high risk of failure if showed: progression or early relapse (<1 year) from previous Rituximab containing chemotherapy or multiple relapses. Standard dose DHAP or ICE with Rituximab were used as salvage chemotherapy and mobilizing regimen. Between October 2006 and January 2013, 37 patients were treated with Zevalin (day –14) followed by standard dose BEAM (day –7 to –1) and ASCT.
Results: Clinical characteristics were: 19 refractory and 18 early or multiple relapse; 9 grade I-II follicular, 26 PML/DLBCL, 3 MCL and 2 indolent non follicular; 9 stage II and 28 stage III-IV; 16 patients received only one previous line of treatment and 21 were treated with 2 or more lines before Z-BEAM, all containing Rituximab. Response status after R-DHAP/R-ICE was: complete remission (CR) 16 (43%), partial remission (PR) 13 (35%) stable/progressive disease (SD/PD) 8 (22%). At the end of Z-BEAM response status was: CR 22 (59%), PR 10 (27%), PD 4 (11%), TD 1 (3%). Three-year progression free survival (PFS) from the start of salvage treatment was 68% and overall survival (OS) was 65%. Twelve patients relapsed or progressed after Z-BEAM and 15 pts died, 12 of them for lymphoma. One patient died during treatment due to infective toxicity (Aspergillosis+H1N1 pneumonia). Two patients in CR died because of progressive multifocal leukoencephalopathy (PML) and heart failure. We retrospectively compared this population with an hystorical matched group of 21 pts treated with BEAM alone. No significant differences in clinical presentation at relapse or in response rate were showed. A trend in favour of Z-BEAM was observed for 3 years-PFS (68% *vs* 48%, p=ns, figure 1) and a significant benefit with the addition of RIT was shown for patients with relapsed disease (72% Z-BEAM *vs* 22% BEAM p=0.01) and for those with CR at the end of salvage treatment prior ASCT (100% Z-BEAM *vs* 76% BEAM p=0.02).

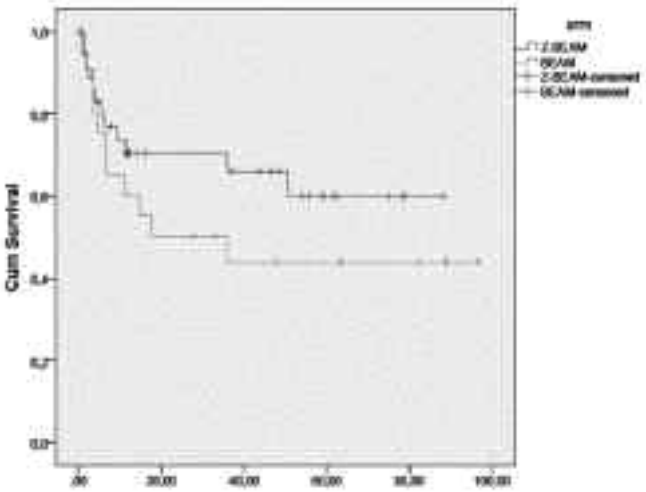

Figure 1.

Summary and Conclusion: In this group of patients with high risk relapsed/resistant NHL Z-BEAM+ASCT is able to achieve a high response and 3-yrs PFS rate. With the limit of a non randomized study, the comparison with the cohort of patients treated with BEAM suggests that the benefit of RIT was seen in patients with early or multiple relapses or with CR at the end of salvage treatment. Novel approaches are needed for patients with refractory disease to Rituximab-chemotherapy or with residual disease prior ASCT.

P1124

QUANTIFICATION OF THE TIME AND EFFORT ASSOCIATED WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL MOBILIZATION: A EUROPEAN PERSPECTIVE

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Background: Plerixafor is indicated in combination with G-CSF to enhance mobilization of hematopoietic stem cells to the peripheral blood (PBSC) for collection and subsequent autologous transplantation in patients with lymphoma (Non-Hodgkin's Lymphoma (NHL)) and multiple myeloma whose cells mobilize poorly. This agent may reduce the failure rate and/or the number of apheresis procedures required without increased toxicity, which may reduce total transplant costs.
Aims: With this background, the aim of this non-interventional study was to assess resource utilization to document provider costs associated with PBSC mobilization and apheresis. The study aimed to evaluate the impact on the time, effort and costs to the hospital when using plerixafor (P) with a primary focus to compare measures of time and effort of patients drawn from the Pre-P *versus* P eras.
Methods: The study population included patients aged ≥18 years, with a primary diagnosis of NHL, and who underwent PBSC mobilization in different European centers. Part I of the study is an ongoing retrospective medical record review of 200 NHL patients from 7 centers across France and Germany. Selected patients are evenly divided between two eras: 1) prior to approval of plerixafor (until June 1, 2009)="Pre-P era"; 2) after approval of plerixafor (July 1, 2010 and onwards)="P era". Patient characteristics will be reported. Outcome measures include number of visits for administration of mobilizing agents; duration (days) of administration of mobilizing agents; agents used as mobilizing agents; adverse events (AEs) detected during mobilization; number of apheresis sessions; hours of apheresis sessions; attainment of CD34+ target (yes, no) and days until CD 34+ target level was met. Part II of the analysis is an ongoing prospective study consisting of time and motion evaluation of actual apheresis, with 20 events recorded at each center. The actual apheresis events are being measured in consecutive patients scheduled to be candidates for PBSC mobilization and consent was obtained from these patients. The time-motion assessments will be obtained retrospectively (Part I) and prospectively (Part II) and will include the total time to prepare the patient, perform apheresis and manage AEs. Costs will be evaluated and quantified in terms of micro-costing group interviews with local hospital administration in each of the sites. The primary study end point is difference in mean time/effort to perform apheresis (including apheresis related AEs, if any) and total costs associated with mobilization to the hospital in terms of micro-costing per patient, between patients in the "Pre-P" *versus* "P eras".
Results: At time of abstract submission, data collection is still ongoing in all of the centers and final study results will be presented during the meeting. It is hypothesized that the key findings of this study will demonstrate the favorable impact of novel interventions on the number of apheresis procedures required to reach a target PBSC, and failure rate of mobilization, thus translating into reduced total transplant costs without increasing toxicity.
Summary and Conclusion: The financial implications for transplant centers could be significant and may lead to further studies aiming to optimize staff time and resource utilization related to apheresis in real-world practice. Funding provided by Sanofi.

P1125

PLERIXAFOR (MOZOBIL) FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ASCT) IN HARD TO MOBILIZE NHL AND HL PATIENTS: A PHASE IIB STUDY

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Background: Plerixafor (Mozobil®) is a novel CXCR4 antagonist. Upfront use of plerixafor may be indicated in patients with predicting factors for failing mobilization such as receiving multiple previous lines of chemotherapy, radiation or stem cell toxic drugs like Fludarabine, as well as advanced age.
Aims: We, therefore, initiated a prospective study assessing the use of plerixafor for mobilization of $CD34^+$ PB HSC in elderly or heavily pretreated patients with NHL or HL, eligible for ASCT (NCT01164345).
Methods: Twenty pts (M-9, F-11), median age 63 (range, 23-70 y) with NHL or HL eligible for ASCT were investigated. The predicting factors for failing mobilization were age above 60 (17/20), multiple courses of chemotherapy (3/20) or both (n=6). Pts received G-CSF (10µg/kg s.c.) x 4 days. Starting the evening of day 4, they received plerixafor 0.24mg/kg s.c. Apheresis was initiated in the morning, 12h after the dose of plerixafor. The target cumulative yield of CD34+ cells/kg was≥5 × 10^6 and the minimum number of cells required for transplant was≥2 × 10^6 CD34+ cells/kg. Seventeen out of the 20 pts underwent ASCT after BEAM protocol. Efficacy was evaluated by the number of PB CD34+ cells/µL, CD34+ cells/kg and myeloid and platelet engraftment.
Results: Mobilization and apheresis yields**:** The addition of Plerixafor to G-CSF resulted in a median 2.26-fold increase in PB CD34+ cell number from pre plerixafor (14.4cells/µL) to post plerixafor administration (32.6cells/µL). The median number of CD34+ cells collected by the first collection was 7.6 × 10^6cells/kg (range, 0.47-20.3). Fifteen of 20 pts met the target CD34+ cell collection of >5 × 10^6cells/kg in the first collection. Four additional pts collect >5 × 10^6CD34+ cells/kg after 2nd collection. Only 1 patient did not reach the minimum target of >2 × 10^6 after 3 days of stem cell collections.Transplant and engraftment: 17/20 pts underwent ASCT (3 were not transplanted due to disease progression-1;<2 × 10^6 CD34+ cells/kg -1; refused – 1). A median 5 × 10_6 CD34+ cells/kg (range, 2.08 -7) were infused at ASCT. Pts engrafted PMN and PLT within a median of 12 days (range, 9-18) and 14 days (range, 12-38), respectively. Safety: 8/20 pts suffered from AEs after Mozobil injections. These pts complained for headache (n=2), bone pain (n=2), abdominal pain (n=1), nausea (n=4), vomiting (n=1) and fever (n=3). Overall, the plerixafor related AEs were mild (grade I-II) and transient. The most common AEs post plerixafor were headache, bone and abdominal pain, fever, vomiting. The main transplant related toxicities were mucositis and infections (n-14). 10/17 pts (59%) suffered from mucositis usually grade II-III (grade II-III -9, grade IV-1). One patient developed acute coronary syndrome during BCNU treatment, 1 suffered from atrial fibrillation, 2 from fluid retention and other 2 from bacterial sepsis.
Summary and Conclusion: Our study indicates that upfront plerixafor is safe and efficient for HSCs mobilization in elderly and heavily pretreated NHL and HL pts predicted to fail mobilization with G-CSF. The vast majority of the pts (19/20 -95%) achieved the target CD34+ cell dose, 15 of them with 1 a pheresis procedure. All pts promptly engrafted post high dose chemotherapy using the plerixafor mobilized CD34+ cells. Plerixafor mobilization in these high risk heavily pre-treated pts was safe with only mild transient expected side effects.
Table: Efficacy of stem cell collection: Mean number of leukapheresis procedures - 1.3; PB CD34+ cells/µl prior first plerixafor - 14.4 (range, 0-54.17); PB CD34+ cells/µl post first plerixafor - 32.6 (range, 7.18-91); CD34+ cells/kg in the graft - 5 (range, 2.08 -7); CD34+ × 10^6 cells/kg in the first stems cell collection - 7.6 (range, 0.47-20.3).

P1126

COMPARISON OF UNRELATED CORD BLOOD WITH BONE MARROW AS A STEM CELL SOURCE FOR MYELOABLATIVE CONDITIONING TRANSPLANTATION IN PATIENTS WITH PEDIATRIC ACUTE LEUKEMIA: A RETROSPECTIVE COHORT STUDY

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Aims: To investigate the role of cord blood as an alternative stem cell source for hematopoietic stem cell transplantation (HSCT) in patients with pediatric leukemia.
Methods: We retrospectively analyzed the outcomes of unrelated cord blood transplantation (UCBT; n=35) and unrelated bone marrow transplantation (UBMT; n=56) with myeloablative conditioning for pediatric acute leukemia in our institute.
Results: The probability of overall survival (OS) (95% confidence interval) at 5 years was 49.8% (35.6%–62.4%) with UBMT and 53.8% (34.0%–70.1%) with UCBT (p=0.92). The non relapse mortality (NRM) at 5 years was 12.9% (5.6%–23.4%) with UBMT and 14.7% (5.3%–28.8%) with UCBT (p=0.56). The cumulative incidence of acute graft-*versus*-host disease (GVHD) (grade II to IV) was 48.2% (34.5%–60.6%) with UBMT and 43.4% (26.4%–59.2%) with UCBT (p=0.60). Engraftment probability was 90.7% (78.2–96.2) with UBMT and 93.9% (73.0–98.8) with UCBT (p=0.67). There was no statistically significant difference in OS, cumulative incidence of acute GVHD, NRM, and engraftment probability between the two groups. Median time from confirmation of transplant indication to day 0 was 208 days with UBMT and 161 days with UCBT (p=0.048). In cord blood grafts, the median number of nucleated cells was 7.4 (1.5–17) × 10^7/kg (n=35), while the median number of CD34-positive cells was 2.1 (0.3–7.6) × 10^5/kg (n=29). The cumulative incidence of engraftment was significantly higher, with the total cell number being ≥4 × 10^7/kg (n=28) and the CD34-positive cell number being ≥2 × 10^5 /kg (n=13).
Summary and Conclusion: Cord blood grafts are easily available because they have already been collected. UCBT can function as an important alternative to UBMT in patients with pediatric acute leukemia.

P1127

AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: CONTRIBUTION OF NEW PHARMACOLOGICAL HAEMATOPOIESIS SUPPORT

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Background: High-dose chemotherapy (HDCT) followed by autologous hematopoietic stem cell transplantation (HSCT) is the only curative therapy for some patients with recurrent Hodgkin's disease, non-Hodgkin's lymphoma, leukemia, and germ-cell tumors. HSCT has been shown to prolong survival in multiple myeloma (MM). The risks of HDCT include bleeding from thrombocytopenia followed by severe anemia. Thrombocytopenia occurring after HSCT may be isolated and prolonged. Blood product support can be complicated by iron overload, infectious disease transmission, and transfusion reactions. Romiplostim has been approved for the treatment of refractory idiopathic thrombocytopenic purpura, demonstrating a favorable safety profile and has been tested in delayed platelet recovery post-SCT. To address this question we have initiated a study to assess the efficacy and safety of romiplostim support at HSCT in MM patients.
Methods: Ten patients (median age, 53 years; range 42-63; 9 male and 1 female) with MM underwent HSCT with romiplostim support [romiplostim group]. The conditioning regimens were as follows: 200 mg/m^2 melphalan (n=8) and BEAM (n=2). The median dose of CD34+ cells infused was 2.4x10^6 cells/kg (range 1.3–3.6x10^6 CD34+ cells/kg). All the patients received 250 µg of the thrombopoietin receptor agonist romiplostim on day of HSC infusion. Thirty patients (median age, 51.4 years; range 26-63; 14 male and 16 female) with MM were assessed as a control group (without romiplostim support). The conditioning regimens were: MEL-200 (n=27) and BEAM (n=3). The mean of CD34+ cells infused was 2.7x10^6 CD34+/kg (range, 1.3–5.2x10^6 CD34+/kg). The endpoints of the study were: time to platelet count >25x10^9/l and >50x10^9/l, days (TPC); duration of grade 4 thrombocytopenia (<25x10^9/l) and grade 3 (<50x10^9/l), days (DT); patient-day, days (PD); platelet nadir (PN); and platelet transfusion volume, ml (PTV). Statistical analysis was conducted with nonparametric statistical methods (Mann-Whithey U test for continuous variables and Fisher exact and Chi-square tests for categorical variables).
Results: The DT grade 3 was 8.0 days in the romiplostim group in comparison with 10.2 days in the control group (*p=0.374*). DT grade 4 was 4.0 days for romiplostim group and 7.4 days for the control group (*p=0.003*), fig. 1. TPC >25x10^9/l was 10.4 days for romiplostim group and 13.0 days for the control group (*p=0.025*). TPC >50x10^9/l was 12.8 for romiplostim group and 14.5 days in control group (*p=0.594*). PD was 24.0 in the romiplostim group in comparison with 25.9 days in the control group (*p=1.0*). PN was 9.5x10^9/l and 10.9x10^9/l (*p=0.706*), respectively. PTV was 1022 ml for romiplostim group and 1071 ml for the control group (*p=0.257*). No significant adverse events of romiplostim administration were observed.

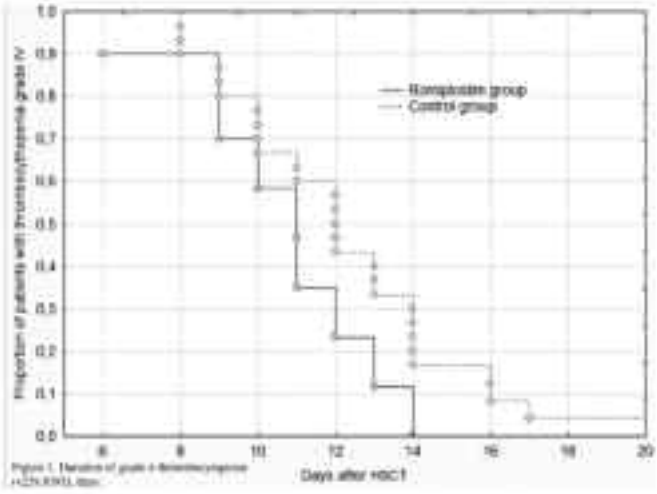

Figure 1.

Summary and Conclusion: The duration of grade 4 thrombocytopenia and the time to platelet count >25x10^9/l are significantly reduced when romiplostim is administered at HDCT. All another endpoints were favorable but not statistically significant in romiplostim group. It could be influenced by the limited number of patients. These tendencies confirm the rationale for further studies of romiplostim in setting of HSCT.

P1128

ANTI-THYMOCYTE GLOBULIN (ATG) COULD IMPROVE THE OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH AGGRESSIVE T CELL TUMORS

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Background: Aggressive T-cell lymphomas represent 10% to 15% of non-Hodgkin's lymphomas in adults. Patients withrelapsed or refractory disease are generally considered incurable with conventional therapies. ATG had been used in theconditioning regimen to reduce the incidence of GvHD for a long time especially in the matched unrelated donor HSCT.The early experiment result in our hospital showed that ATG inhibited the proliferation of lymphoid tumor cells in a dosedependentmanner especially in the T cell tumors.
Aims: We used the ATG as the part of the conditioning regimen in the allpatients and to evaluate the long-term anti-leukemia effect, the safety and complication in the patients with relapsed orhigh-risk T cell lymphomas.
Methods: 18 patients(male 9, female 9) were enrolled into this study. Median patient age atthe time of transplantation was 28 years (range, 7–55 years). At the time of transplant, 4 patients reached first orsubsequent complete response (CR) with conventional therapy or the salvage therapy, 4 patients had a partialremission(PR), 7 patients had relapsed disease not responding to salvage therapy or progressive disease, and 3 patientshad primary refractory disease. Donors were 10/10 HLA matched related (5), 10/10 matched unrelated (3), 8/10 matchedunrelated (5) and mismatched related (5). The median number of CD34+ cells within the allografts was 9.31/kg bodyweight (BW) (range,4.6–24.85/kg BW). Rabbit antithymocyte globulin (ATG 2.5 mg/kg×4 days) and total-body irradiation (10 Gy in five fractions) were used in all 18 patients. All patients but one also underwent cyclophosphamide (120 mg/kg).The only one who did not receive CTX had experienced with autologous transplantation. Fifteen high risk patients were inadditional use of VP 16 or VM26 30-40mg/kg. Two patients in CR1 and one patient who were 55 years old had notreceived VP16. Graft-*versus*-host disease (GVHD) prophylaxis was cyclosporine based, usually in combination withmethotrexate. Quantitative chimerism analyzes were performed using short-tandem-repeat-based polymerase chainreaction techniques at regular intervals for every 4 weeks after transplantation in bone marrow at the first six months.
Results: All patients but one achieved a complete remission in the first three months after allogeneic HSCT. One patientachieved PR on month 1 and soon died from progressive disease. The CR rate after transplantation was 94.4%. At amedian follow-up time of 13 months, fourteen(77.8%) patients are alive. OS at three years was 71%. Still four patientswere died after transplantation, two from relapse and two from treatment related complications. Acute GvHD grades II-IVoccurred in eight patients(50%) and grades III-IV in four patients. Two patients suffering from acute GvHD grade IV dieddue to treatment-related complications. The maximum cumulative incidence of cGVHD was 42.8%. The high levels ofCMV DNA were detected in seventeen patients in the first three months after transplant. Two of them gradually envolvedinto viral haemorrhagic cystitis. One patient suffered from aspergillus pneumonia while another two suffered frombacterial pneumonia. There was no case of venous occlusive disease.

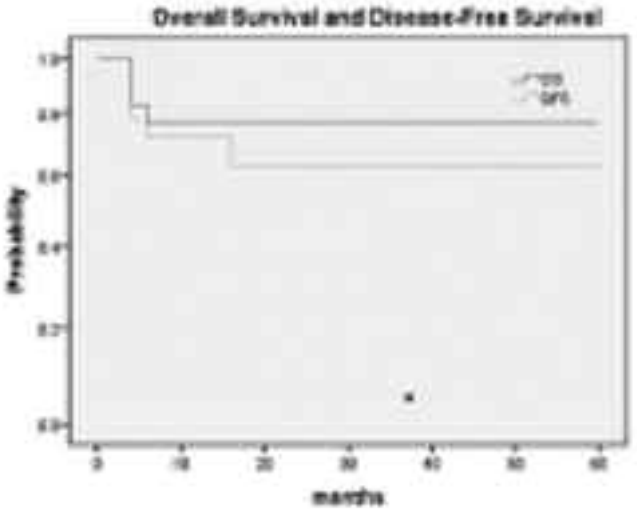

Figure 1.

Summary and Conclusion: Anti-thymocyte globulin (ATG) could improve the outcome of allogeneic hematopoietic stem celltransplantation in patients with aggressive T cell tumors.

P1129

SAFETY AND EFFICACY OF BRENTUXIMAB VEDOTIN (SGN-35) IN HODGKIN LYMPHOMA PATIENTS UNDERGOING REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANT FOLLOWING A RELAPSE AFTER AUTOLOGOUS TRANSPLANT

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Background: The prognosis of patients with Hodgkin lymphoma (HL) who relapse following autologous stem cell transplantation (ASCT) is generally poor, with long-term progression free-survival of about 20-30%. Allo-SCT continues to be recommended in this subset of patients because of the relatively young age and because it can induce some durable remissions; however the application of this procedure is actually limited by a difficulty to obtain an objective response before allotransplant. It was recently shown that Brentuximab vedotin, a new generation antibody-drug conjugate, is able to induce nearly 30% of complete remission in HL patients relapsing after autologous transplant; therefore this agent could effectively work in relapses after ASCT as a platform to allow a better disease control in order to improve the outcome of the allografting procedure.

Aims: Aim of this study was to analyze retrospectively the safety and the efficacy of brentuximab vedotin administered as single agent as a bridge to allogeneic transplant in HL patients relapsing after ASCT.

Methods: Between August 2011 and September 2013, 8 out of 12 patients, treated with allo-SCT at four hematologic Divisions of Northern Italy for HL relapse after ASCT, had received brentuximab vedotin as a bridge to the allografting procedure. Median age was 32 years (range 21-61) and median number of prior regimens was 5, including ASCT. Patients received a median of 6 cycles (range 4-7) of brentuximab vedotin administered every 3 weeks as a 30-minute outpatient IV infusion; median time between the last dose of brentuximab vedotin and the allo-SCT was 1 month (range 1-5 months). No patient experienced progression during treatment with brentuximab vedotin (2 complete remissions and 6 partial remission/stable disease). All but one patients did not have a HLA identical sibling, so they required a matched unrelated (4 patients) or haploidentical donor (3 patients); peripheral stem cells were the source in patients with HLA identical sibling or unrelated, whereas bone marrow was used in the haplo setting. Reduced-intensity was the conditioning regimen performed in all patients. Post transplant cyclophosphamide plus mycophenolate mofetil/tacrolimus was the graft-*versus*-host disease (GVHD) prophylaxis of the haplo setting, while Metotrexate/cyclosporine was administered in the other patients. Patients were monitored for engraftment, aGVHD, chimerism, and infectious complications per institutional standards.

Results: One patient had primary graft failure with autologous reconstitution; she was a patient with a high body-mass index, in whom the drug's doses of the conditioning regimen were underestimated in order to avoid excessive toxicity. All the other patients engrafted; median time to neutrophil recovery was 20 days (range 15-26). There was no delay of engraftment or increased incidence of CMV reactivations or other type of infections. No cases of worsening neuropathy or grade III-IV extrahematologic toxicities were reported after allo-SCT. Non-relapse mortality at 1 year was 0%. Only one patient had a relapse at 8 months after allo-SCT, whereas the others are alive and progression-free at a median follow up of 12 months.

Summary and Conclusion: These data suggest that brentuximab vedotin can be safely and effectively administered prior to RIC allo-HCT in HL patients relapsing after ASCT. It can provide an objective disease control in order to allow patients to undergo alloSCT. Furthermore we did not observe delays in engraftment, increase in non-relapse mortality, and post transplant infectious complications. Further clinical trials with larger number of patients and longer follow-up are recommended to confirm the promising role of brentuximab as a bridge to alloSCT.

P1130

VORICONAZOLE PROPHYLAXIS OF INVASIVE FUNGAL INFECTION IN HAPLOIDENTICAL ALLOGENIC STEM CELL TRANSPLANTATION

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Background: Haploidentical allogenic stem cell transplantation recipients are a heavily treated high risk population for infectious complications and the invasive fungal infection (IFI) represents one of the major causes of infectious-related deaths. An appropriate strategy of IFI prophylaxis must be applied in such context. Voriconazole, a third generation azole has already proved its large spectrum and good tolerance in the matched related and unrelated (MUD) stem cell transplant patients.

Aims: To evaluate the efficacy and tolerance of voriconazole primary and secondary prophylaxis for invasive fungal infection in haploidentical stem cell transplant recipients of our center.

Methods: From January 2008 to July 2010, voriconazole was used as primary prophylaxis of invasive fungal infection (IFI) during haploidentical transplant in 43 patients with a median age of 38 years [min 10, max 71] and as secondary prophylaxis in other 19 haploidentical transplant recipients in remission of a previous/probable/possible previous IFI, median age 51 years [min 25, max 61]. The main endpoint for the primary prophylaxis was defined as the ability to tolerate the fungal prophylaxis more than 100 days and the absence of probable/proven fungal infection at day 180. The principal objective for secondary prophylaxis was the incidence of proven/probable IFI.

Results: Primary prophylaxis: The median duration of voriconazole prophylaxis was 94 days (range: 13-356 days), adapted to individual infectious risk. Fungal infection free-survivals at day 100 and day 180 were 62% and 58%. 28% patients received treatment for a new IFI. In the context of concomitant triple graft *versus* host disease prophylaxis by sirolimus, mofetil mycophenolate and cyclosporine, 6 patients (14%) stopped the treatment for liver toxicity, only 2 (4.6%) of them before day 100. Overall cumulative survival was 67% at day 100, 60% at day 180 and 45% at 1 year. **Secondary prophylaxis:** In 68.4% of the patients (13 cases) voriconazole was the treatment for the previous infection. Median duration of voriconazole prophylaxis was 92 days (range: 18-359 days). Cumulative survival at one year 32%. IFI occurred in 7 cases post transplant (37%), all previously treated with voriconazole. Successful secondary prophylaxis at one year 20%. Only one patient out of nineteen (5.2%) had toxicity (hepatic) after 124 days of prophylaxis.

Summary and Conclusion: Voriconazole is efficacious and well tolerated in haploidentical SCT; simultaneously administration of enzymatic competitors as sirolimus is feasible although requires immunosuppressive drug monitoring.

P1131

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ELDERLY PATIENTS WITH MYELOID MALIGNANCY WITH A MYELOABLATIVE CONDITIONING CONSISTING OF TBI AND G-CSF-COMBINED CYTARABINE

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Background: We previously reported that allogeneic hematopoietic stem cell transplantation (HSCT) with a conditioning consisting of total body irradiation (TBI) and granulocyte colony-stimulating factor (G-CSF)-combined cytarabine for myeloid malignancy contributed to a favorable outcome due to a low transplant-related mortality and disease relapse (Bone Marrow Transplant 2007; Biol Blood Marrow Transplant 2008). However, efficacy and safety of this conditioning for elderly patients has not been evaluated.

Aims: To evaluate the efficacy and safety of the myeloablative conditioning consisting of TBI and G-CSF-combined cytarabine in allogeneic HSCT for elderly patients with myeloid malignancy.

Methods: We retrospectively evaluated the outcomes of patients aged 50 years or older who underwent allogeneic HSCT at Keio University Hospital (Tokyo, Japan) between January 1995 and May 2011 after being conditioned with TBI (12 Gy) followed by high-dose cytarabine (3 g/m^2) every 12 hours for 4 days in combination with the continuous administration of G-CSF. Cyclosporine or tacrolimus with short-term methotrexate was given for GVHD prophylaxis. Transplant outcomes were compared with those of patients aged 49 or younger who underwent the same transplant procedures during the study period.

Results: Thirty-five elderly patients (median age, 52 years; range, 50-58) were enrolled into analysis. Diseases were acute myeloid leukemia (n=16: CR1, n=5; CR2, n=6; not in CR, n=5), advanced myelodysplastic syndromes (n=16), and myeloproliferative neoplasms (n=3). Stem cell sources were bone marrow/peripheral blood stem cells from human leukocyte antigen (HLA)-identical siblings (n=11/3), bone marrow (n=17) and cord blood (n=4) from unrelated donors. Of the 34 evaluable patients, graft rejection occurred in one patient. Eighteen were alive at the date of analysis with a median follow-up of 9.4 years (range, 3.6-18.5). Causes of death were disease relapse in 7 patients, infection in 4, graft-*versus*-host disease (GVHD) in 3, and post-transplant lymphoproliferative disorder, liver failure and graft rejection each in 1. In all patients, 5-year overall survival (OS) and progression-free survival (PFS) rates were 51.4% (95%CI: 34.0-66.4) and 48.6% (95%CI: 31.4-63.7), respectively, which were comparable with those of 88 younger patients (67.7% (95%CI: 56.7-76.5, p=0.117) and 63.4% (95%CI: 52.3-72.5, p=0.140)).

Summary and Conclusion: These results suggest that TBI and G-CSF-combined cytarabine could be a tolerable and promising conditioning for allogeneic HSCT for elderly patients aged between 50 and 60 years with myeloid malignancy.

P1132

FIRST FRENCH EXPERIENCES OF TOTAL BODY IRRADIATION USING HELICAL TOMOTHERAPY

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Background: Total Body Irradiation (TBI) is widely used as a preparatory regime before BMT. Nevertheless, current accepted techniques of TBI are laborious, time-consuming and cumbersome. In addition, dose prescription and verification is usually limited to single-point measurements. The advent of

dynamic conformal radiotherapy in the form of helical tomotherapy (HT) offers a new and more quantitative paradigm for TBI dose planning and delivery.
Aims: Treatment planning, delivery, dose verification of the 6 first French experiences of TBI using HT are presented.
Methods: A review was performed on 6 TBI where HT was used. The conditioning regimen consisted of Fludarabine 30 mg / m² Day D1 to D3 followed by TBI 2 Gy D4 with the allograft D6. Patients were immobilized in a supine position using a whole body vacuum bag and a thermoplastic head mask (3 points). Two sets of planning kilovoltage CT with a 5 mm slice thickness were acquired: one for the upper body HFS oriented (from vertex to knees) and one for the lower body FFS oriented (from feet to pelvis). The target volume (PTV) was delineated with the external contours for lower and upper parts. The junction has been marked at mid-thighs. We prescribed 2 Gy to the PTV. In order to control the dose gradient in the junction region, we used five 2 cm transition volumes. In each transition volume, the sum dose prescribed of each plan (upper and lower) was 2 Gy. For the treatment planning we chose a 5 cm field width, a pitch of 0.43 and a Modulation Factor of 1.8. For quality insurance (QA), each plan was checked with delivery QA performed with cylindrical phantom, ionization chamber for absolute dose and film for relative dose. *in vivo* dosimetry was performed with thermoluminescent dosimeters and the junction region homogeneity was checked with radiochromic films.
Results: Five of the 6 patients planified received the treatment. We included all the 6 patients for the dosimetric analysis. A total dose of 2 Gy was delivered to the PTV in Helical Tomotherapy. ICRU criteria were achieved for 5 of 6 patients: V95% was covered by D95%, V2% did not exceed D107%. For 1 patient, $V_{95\%}$was 91.1% and $V_{107\%\%}$ was 6.4%. For each delivery QA, the difference between measured and calculated absolute dose was<2,5% (mean :1%) For relative dose, Gamma Index (3%; 3 mm) was<1 for at least 94% of the points (mean: 95.8%) Junction region inhomogeneity was less than ± 7.5% (mean: 5.8). Among 15 points, difference between *in vivo* measurements and calculated dose was >5% for 2 points with a maximum at 10% (mean: 0.42 %, standard deviation 1.2%). The total realization of TBI lasted 120 min with a beam on time of 17.2 min for the upper and 11.2 min for the lower part of the body. We needed 3 or 4 MVCT (mean 3.5) to replace the patient with accuracy: the deviation between expected and realized position was less than 1 mm in all directions.
Summary and Conclusion: Those were the first French experiences of TBI using HT. This technique guarantees high dose homogeneity throughout the body and dose verification is achievable, showing small difference between planed and delivered doses. The treatment was achievable in a confortable way for the patients and with accuracy. In view of these results, the use of HT should be a standard if TBI is recommended. Improved conformality with HT may offer new opportunities with regard to this therapy.

Gene therapy, cellular immunotherapy and vaccination

P1133

EFFECTIVE AND SUSTAINED CELLULAR IMMUNOTHERAPY TO ACUTE MYELOID LEUKEMIA USING T-CELL-RECEPTOR REDIRECTED EBV-SPECIFIC CD8+ T CELLS WITH MEMORY STEM-LIKE AND CENTRAL MEMORY PROPERTIES

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Background: Adoptive cellular therapy using donor-derived cytolytic T lymphocytes (CTL) directed to leukemia or herpesvirus has evolved as a promising strategy to confer adoptive antiviral immunity and improve graft-*versus*-leukemia (GvL) effects in immunocompromised patients following allogeneic hematopoietic stem cell transplantation (AHSCT). However, durable clinical responses are often hampered by detrimental graft-*versus*-host (GvH)-reactivity and limited capability of transferred, fully differentiated effector T cells (T_{EFF}) to establish persistent antileukemia immunity.
Aims: In the present study we therefore explored the memory formation and tumoricidal features of *in vitro* generated EBV-specific T memory stem cells (T_{SCM}) and central memory T cells (T_{CM}), T cell-receptor (TCR) redirected to acute myeloid leukemia (AML), for improved immunotherapy by adoptive transfer into a patient-tailored minimal residual leukemia NSG-mouse model.
Methods: EBV-specific T_{SCM} and T_{CM} were generated by stimulation of healthy donor derived, purified naïve $CD8^+CD45RA^+$ T cells with EBV-peptide mix loaded autologous stimulators or EBV-transformed B cells (B-LCL) derived from the HLA-class I-matched AML patient in the presence of Interleukin (IL)-12, -7, -15, -21, and inhibitors targeting glycogen synthase kinase-3β involved in the Wnt-signaling pathway. EBV-specific CTL from unselected $CD8^+$ T cells were established as controls. Phenotype, cytolytic activity as well as metabolic and migratory properties of unmodified and LV-transduced T_{SCM}/T_{CM} was analyzed by flow cytometry, IFN-γ ELISpot and ^{51}Cr-release assays, glucose uptake and transmigration analyses. αβ-TCR genes isolated from AML-reactive CTL (Albrecht *et al.* Cancer Immunol. Immunother. 2011) and lentiviral (LV) transduction of EBV-CTL was performed according to standard procedures. $eGFP^+$ $TCRV\beta7.1^+$ and $TCRV\beta21.3^+$ LV-transduced T_{SCM}/T_{CM} were monitored for AML-reactivity. To evaluate bioactivity *in vivo*, redirected Luciferase $(Luc)^+$ T cells were adoptively transferred into AML- and B-LCL-engrafted NSG mice, respectively, and monitored for engraftment, persistence and antileukemia reactivity.
Results: Following 3 to 4 weekly stimulations T_{SCM} and T_{CM} expressing a $CD8^+$ $CD45RA^+$ $CD45RO^-$ $CD95^+$ $CD27^+$ $CD28^+$ $CD62L^+$ $CCR7^+$ and $CD8^+$ $CD45RA^-$ $CD45RO^+$ $CD95^+$ $CD27^+$ $CD28^+$ $CD62L^+$ $CCR7^+$ phenotype elicited strong and to $CD8^+$ T_{EFF} EBV-specific cells comparable reactivity to B-LCL generated from the HLA class I-matched AML-patient. In line with recently described features of early differentiated memory T cells both unmodified and LV-transduced T_{SCM} and T_{CM} additionally showed reduced glucose uptake but enhanced migration properties in CCL-21 driven transmigration assays. Moreover, TCR-redirected EBV-specific T cells elicited comparable reactivity to AML blasts derived from the same patient as the B-LCL when compared with the original AML-reactive T cell clone. Adoptive transfer of redirected CTL into AML-engrafted NSG mice resulted in strong reduction of AML-burden. Moreover, these T cells persisted for more than 90 days in NSG mice. Additional studies on boosting antileukemia immunity by additional transfer of irradiated EBV-blasts into treated mice or supporting T cell homeostasis by weekly i.p. injection of a IL-15 superagonist are currently in progress.
Summary and Conclusion: In conclusion, this study demonstrates that stem-cell-like and central memory EBV-specific CTLs redirected towards AML-reactivity can induce sustained antileukemia immunity and thus might represent a promising tool to improve cellular immunotherapy within AHSCT

P1134

THE AB-DNTS IN LYMPH NODES, BONE MARROW AND PERIPHERAL BLOOD OF LYMPHOMA PATIENTS: POTENTIAL PROGNOSTIC FACTOR OF CLINICAL OUTCOME

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Background: The mutual interactions between the host immune system and lymphoma cells may either promote or control lymphomagenesis. The Double negative T cells (DNTs) have emerged as new subset of T cells that contribute specifically to anti-tumor immunity since they are involved in immune regulation

and tolerance acting as regulatory T cells (Treg) and/or cytotoxic T cells. DNTs express either αβ or γδ T-cell receptors (TCR) and lack CD4, CD8 and CD56. Functional studies showed that DNTs might have a direct *in vitro* anti-tumor activity against lymphoma cells. However no data are available on their role in human anti-Lymphoma immunity as well as their prognostic significance in lymphomas and how they are modulated during the therapy response.
Aims: The aim of this study was to evaluate the modulation of DNT expression during therapy response, from the diagnosis to the remission or relapse/progression, in lymph nodes (LN), bone marrow (BM) and peripheral blood(PB)of lymphoma patients in order to assess their role on clinical outcome and progression.
Methods: PB and BM samples of 72 Lymphoma patients (pts), with non-Hodgkin's Lymphomas and classical Hodgkin Lymphoma were collected at diagnosis and prospectively during the follow-up (1-6-12 months after chemo- or immuno-chemotherapy therapy) as well as at the relapse/progression. As control PB samples of 16 healthy donors were collected. Twenty fresh LN tissue from pts clinically suggestive of lymphoma was quickly processed. The ontogeny, functional attitude and TCR clonality of DNT subsets (TCRαβ+ and TCRγδ+) were evaluated in PB, BM and LN by following monoclonal antibodies: CD3, CD4, CD8, CD56, CD45, TCRαβ, CD45Ra, CD45Ro, CCR7, CD27, CD28, CD30, CD69, GITR, CD95, CD178, CD152, IFN-γ, TNF-α, perforin and granzymeB. For functional studies, DNTs were purified from PBMCs by LN and PB of pts through a negative selection and then cultured for 2 weeks. Other immune cells such as MDSCs and Treg was in parallel detected to evaluate the correlation with DNTs. 7 cases of LNs received a finally diagnosis of reactive follicular hyperplasia (RFH) so they was considered as LN controls. All pts provided their informed consent in accordance with the Declaration of Helsinki.
Results: The frequency of DNTs in BM of Lymphoma pts was less than in PB,but we observed a significant decrease (p=0.006) of αβ-DNTs in the PB of pts with untreated lymphoma (20.5 ± 4.8 SE) (Mean + SE) as compared with healthy controls (31.3 ± 3.4), and their number correlated with both disease relapse/progression or stage of therapy. (fig.1-2). In Hodgkin's Lymphoma pts the αβ-DNT frequencies were significantly increased as compared with other histotypes (p=0.005) (fig. 4) and if compared with subcategory of lymphoma such as indolent or aggressive (p=0.0001) (fig.3). The frequency of αβ-DNTs in 1-month post-cht or disease relapsed pts samples was significantly decreased (p=0.006) as compared with diagnosis.Interestingly, after *ex vivo* expansion, DNTs acquired an immunomodulatory cytokine profile, characterized by the secretion of IFN-γ and granzyme B which are known as central components of anti-tumor immune responses.More interesting the DNTs in pathologic LNs are significantly reduced compared to RFH-LNs (p=0.006) and are realated to the aggressiveness of the disease (fig.5-6).

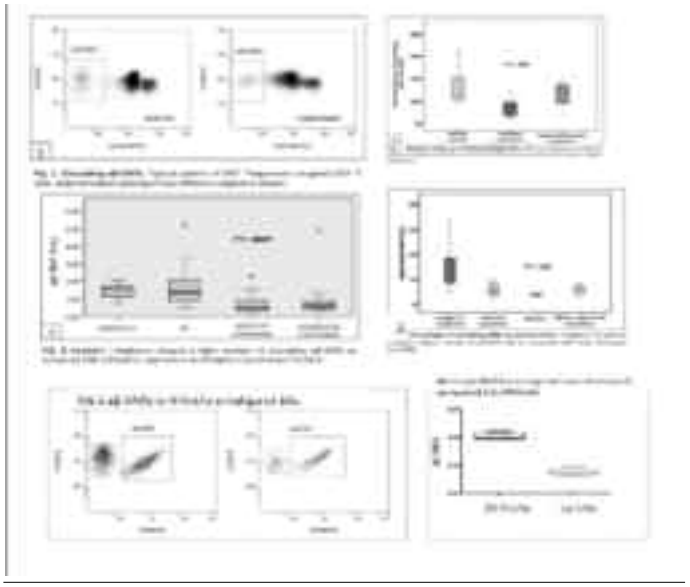

Figure 1.

Summary and Conclusion: Our study has demonstrated for the first time that αβ-DNTs could play an important role in both the development and the progression of lymphomas. This is the first study that evaluate the DNTs in LNs of Lymphoma patients,where the disease originates. More functional studies in malignant LN will help to more understanding the role of DNT in Lymphoma pathogenesis. In addition, based on our preliminary results, it is likely that ex-vivo expanded DNTs exert an anti-tumor activity thus suggesting their possible use as a new strategy for adoptive immune-therapy.

P1135

TOWARDS CLINICAL GAMMA DELTA TCR GENE TRANSFER: A BROADLY APPLICABLE T-CELL PRODUCT FOR CANCER PATIENTS

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Background: γδT cells are innate immune cells with strong anti-tumor activity and provide anti-tumor receptors that are interesting tools in immune therapy against cancer. The introduction of γδTCR genes into αβT-cells allows the adoptive transfer of T cells that are not limited by HLA-restriction, in any patient with any cancer (Marcu-Malina *et al.* Blood 2011, 50). Combinatorial γδTCR-exchange (CTE) has been applied to design a γ9δ2 TCR with strong and broad anti-tumor reactivity, referred to as TCR γ9-G115/δ2-cl5 (Gründer *et al.* Blood 2012, 5153).
Aims: Aim of the study is to design a clinical grade procedure which guarantees an efficient expression of the introduced γδTCR, an adequate enrichment of transduced cells, as well as functional efficacy.
Methods: Firstly, we introduced both γ and δ chains into the retroviral vector pMP71, separated by a 2A peptide sequence. Gene-engineered human αβT-cells expressed the γδTCR and recognized tumors of different hematological origin as well as solid tumors. Secondly, the orientation of the γ and δ chains and the particular 2A peptide sequence influenced TCR expression as well as anti-tumor function as previously demonstrated for αβTCR genes.
Results: Thirdly, introduction of the optimal γδTCR transgene cassette into αβT cells was followed by the depletion of untransduced αβTCR positive cells using a clinical grade anti-αβTCR antibody before rapid T cell expansion. This depletion procedure resulted in a highly pure population of γδTCR-engineered T cells with increased γδTCR expression, and improved anti-tumor function both *in vitro* but also *in vivo* in a humanized mouse model. Importantly, introduction of a γδTCR into αβT cells followed by clinical-grade depletion of non-transduced and transduced T-cells expressing higher levels of αβTCRs abolished residual allo-reactivity.
Summary and Conclusion: All together, we developed a clinical grade GMP vector which allowed a deliberate choice for the most potent clinical γδTCR receptor cassette. In addition, the clinical grade depletion of non-transduced and transduced T-cells expressing high levels of endogenous αβTCRs results in a potent γδTCR-engineered T cell product suitable for an autologous but also allogeneic clinical scenario.

P1136

ADOPTIVE TRANSFER OF RAPIDLY GENERATED EBV-CTLS FOR THE TREATMENT OF HIGH RISK ASSOCIATED EBV MALIGNANCIES

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Background: Adoptive immunotherapies with virus-specific T cells (VSTs) are showing increasing success for the treatment of viral-associated malignancies. We have previously demonstrated that the administration of Epstein-Barr Virus (EBV) -specific T-cells directed against the EBV latent cycle antigens, LMP1 and LMP2, resulted in over 50% of complete responses in patients with multiply relapsed or refractory EBV+ Hodgkin and non-Hodgkin lymphomas (HL and NHL). Unfortunately, the manufacturing procedures of those VSTs pose several impediments to the wider applicability of our strategy. These include the use of live virus (EBV) and adenoviral (Ad) vectors and the difficulty of generating EBV-transformed B-cell lines (EBV-LCL) for use as antigen-presenting cells (APCs) from patients with B-lymphoma who have received rituxan.
Aims: To simplify the manufacture and make our approach more broadly applicable, we have replaced EBV antigens expressed from the Ad vectors with overlapping 15mer peptide libraries (pepmixes) spanning LMP 1/2, as well as EBNA1 and BARF1, and used pepmix-pulsed dendritic cells for the first stimulation followed by pepmix-pulsed, autologous activated T cells (aATCs) and irradiated costimulatory cells (HLA-negative K562 cells expressing CD80, CD86, CD83 and 41BB-ligand [K562-CS] as transgenes) for further expansion. We hypothesized that the administration of VSTs generated using our new, pepmixes-based method, would be safely tolerated and control the progression of the disease in patients with high-risk EBV-associated malignancies.
Results: To date we have enrolled 17 patients in our IRB and FDA approved clinical trial (NCT01555892). We successfully expanded T-cells from 14/17 patients. In one case we did not isolate sufficient cells to start production, and

two VST lines are still under production. 8/14 VSTs lines showed EBV-specific activity against at least one of the four antigens used during the generation of the lines, as determined by ELISpot. The lines were highly enriched in CD3 (92% ± 11%), with a mix of CD4 and CD8 (55%% ± 15% and 34% ± 18%, respectively) and although the lines were predominantly composed of effector memory cells (45% ± 9%), we observed a persistence of a significant population expressing a central memory phenotype (CD45RO, CCR7 and CD62L) (33% ± 12%). Ten patients received 2 infusions of VSTs (range 0.2-1 x10e8/m2). We did not observe any toxicities related to the VSTs. Two patients are too early to evaluate response. Of 8 evaluable patients 3 patients treated while in complete remission remain in CR (follow up: 10, 6 and 2 months post infusion). Of five patients treated with active disease, 3 progressed while one had stable disease for 3 months and one had a partial response, which was sustained 5.5 months before showing signs of disease progression.
Summary and Conclusion: The administration of VSTs generated in the presence of viral pepmixes appears to be safe and well tolerated. Although we have seen activity in two patients, further data are needed to determine if rapidly generated and Ad/LCL-generated VSTs have similar anti-tumor efficacy.

P1137

GENERATION OF MULTIVIRUS-SPECIFIC T CELLS BY A SINGLE STIMULATION OF PBMCS WITH A PEPTIDE MIXTURE UTILIZING SERUM-FREE MEDIUM

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Background: Extension to donors other than HLA-matched siblings following advanced immunosuppressive treatment has resulted in the emergence of viral infections as major contributors to morbidity and mortality after hematopoietic stem cell transplantation (HSCT). While pharmacological agents are standard therapy for some, they have substantial toxicities, generate resistant variants, and are frequently ineffective. Moreover, immune reconstitution is necessary for long-term protection against pathogens after HSCT. Restoration of virus-specific immunity offers an attractive alternative to conventional drugs. Adoptive transfer of virus-specific CTLs from stem cell donors has been proved to be safe and effective in treatment of viral infection. Recently, the system for rapid generation of multivirus-specific T cells has been reported (Gerdemann, U, 2012). With this technique, polyclonal CTLs specific for multivirus antigens can be produced after single stimulation of PBMCs with a peptide mixture spanning the target antigens in the presence of IL4 and IL7.
Aims: We introduced and verified this system to apply for clinical use in Japan. To conform regulation by the Japanese FDA, we attempted to generate multivirus-specific T cells in serum-free medium. To meet the requirement for the viral infections after HSCT by broad virus species, we extended the target antigens to cytomegalovirus (CMV), EBV, adenovirus (AdV), HHV-6, BKV, JC virus and VZV.
Methods: 20×10^6 of PBMCs were stimulated with peptide mixture spanning the target antigens of 3 (CMV, EBV, AdV) or 7 (CMV, EBV, AdV, HHV-6, BKV, JC virus and VZV) viruses and cultured in serum-free medium with cocktail of IL4 and IL7 for 9-12 days.
Results: 20 x 10^6 PBMCs were stimulated with each antigen of CMV, EBV and AdV and cultured for 9-12 days in serum free medium or in RPMI1640 + 5% human serum (HS). We obtained average of 144.9×10^6 cells in RPMI with 5% HS and average of 92.0×10^6 cells in serum-free medium (n=4), which were statistically significant (p=0.025). Most of the prepared cells were positive for CD3, mainly consisted of CD4+ central memory cells in each condition. The average percentage of T cell subsets were as follows; CD3+ 97.7%, CD4+ 84.8%, CD8+ 11.7%, CD3+CD62L+CD45RO+ 89.0% in RPMI+5% HS and CD3+98.9%, CD4+ 74.4%, CD8+ 14.8%, CD3+CD62L+CD45RO+ 93.2% in serum-free medium. We observed no significant difference in specificity toward the CMV, EBV and AdV antigens between the cells cultured in medium with HS and in serum-free medium. IFNγ production was observed in average of 9.83% of the prepared cells (19.1 ×10^6 cells) in RPMI+5%HS and in average of 7.93% (10.0 ×10^6 cells) in serum-free medium when assessed by intracellular cytokine staining (ICS). We next tried to generate 7 viruses-specific T cells in the serum-free culture system. 20x 10^6 PBMCs were stimulated with each antigens of CMV, EBV, AdV, HHV-6, BKV, JC virus and VZV and cultured for 10-12 days yielding 151.9±39.6 ×10^6 cells (n=5). 95.6% of the cells after the expansion were CD3+ and contained both CD4+ and CD8+ cells. The average percentage of CD4 positivity was 74.1% and that for CD8 was 20.8%. 80% of CD3+ cells expressed central memory phenotype. These single stimulated and cultured cells contained average of 21.2% CD4+IFNγ+ cells and 5.1% CD8+IFNγ+ cells measured by ICS and showed specificity toward all the 7 virus antigens (n=4).
Summary and Conclusion: Our approach can be readily introduced to clinical practice and is expected to be introduced to countries in which the use of serum for cellular products are restricted as an alternative treatment/ prevention for viral infection after HSCT. We also seek a way to eventually establish a system to prepare multivirus-specific T cells by this rapid, easy and cost-effective method for the third party use.

P1138

GENETIC CORRECTION OF DIFFERENTLY MOBILIZED HEMATOPOIETIC STEM CELLS FROM PATIENTS WITH THALASSEMIA AND ENGRAFTMENT KINETICS AFTER XENOTRANSPLANTATION

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Background: Gene therapy is anticipated to render patients with beta-thalassemia transfusion-independent, through autologous transplantation of genetically-modified hematopoietic stem cells (CD34+).
Aims: In view of a forthcoming gene therapy clinical trial for thalassemia, we sought to determine, on the basis of CD34+cell yields, transduction efficiency, beta-globin expression and engraftment rates in xenografts, the optimal autologous graft source in thalassemic individuals mobilized with various strategies (G-CSF-alone, Hydroxyurea (HU)+G-CSF, Plerixafor-alone and Plerixafor+G-CSF).
Methods: The differently-mobilized CD34+ cells, obtained in two clinical trials, were transduced with research grade lots of the TNS9.3.55 beta-globin lentiviral vector and compared in terms of gene transfer rates, clonogenicity, restoration of the beta-globin expression *in vitro* and multilineage engrafting capacity after xenotransplantation of equal numbers of CD34+ cells into, partially myeloablated, IL2Rgamma^{null} mice. In addition and since for clinical gene therapy it will be necessary to freeze the CD34$^+$ cells at least once before administration, the effect of freezing/thawing courses on the transducibility and clonogenicity of hematopoietic stem/progenitor cells was tested.
Results: The combination of Plerixafor+G-CSF yielded the highest CD34+ cell doses per apheresis over the rest mobilization approaches (mean CD34+cells/apheresis: G-CSF 3.44±1.14 *vs* HU+G 3.31±1.00 *vs* Plerixafor 3.61±2.05 *vs* Plerixafor+G-CSF 8.85±2.88 X10^6/kg, p=0.0003). The differently mobilized CD34$^+$ cells generated similar numbers of colony forming units (CFU) after transduction. CD34$^+$cells transduced fresh or after one freeze/thaw cycle yielded similar clonogenic and gene transfer frequencies. Repeated freeze/thaw cycles of cultured, but not of unmanipulated cells, were marked by significant colony loss, with no impact on viability or transduction rates. Plerixafor-mobilized cells tended towards higher transduction rates, a feature probably associated with the higher content in cells at the G1 phase of cell cycle as compared to all other differently mobilized cells. Despite that Plerixafor+G-CSF cells displayed a significantly lower colony vector copy number (VCN)/cell (p<0.0001), they produced the highest β-globin output/VCN (p=0.04). All mobilized CD34$^+$ cells averaged 47.5%±2.9% vector-positive CFU and 1.92±0.04 VCN with expression approximating the one-copy normal β-globin output. *In vivo*, multilineage engraftment was encountered in all groups and the average VCN/cell measured in mouse blood ranged from 3.4 in the 1st month to 1.82 in the 5th month post transplantation. The Plerixafor+G-CSF-mobilized cells achieved superior short-term (p<0.004) and a trend towards superior long-term (p=0.05) engraftment rates over all other differently mobilized cells.
Summary and Conclusion: Overall, TNS9.3.55 gene transfer in variously-mobilized thalassemic CD34$^+$ cells was effective and stable.Plerixafor+G-CSF mobilization not only results in very high numbers of CD34+cells obtained by single apheresis but it also yields a CD34+cell population that, despite its lower susceptibility to transduction, provides increased β-globin expression/VCN and enhanced human chimerism in xenografts under a non-myeloablative setting. Consequently, Plerixafor+G-CSF-mobilized CD34+cells potentially represent the optimal autologous graft for thalassemia gene therapy and stem cell gene therapy applications in general.

P1139

NEXT-GENERATION DENDRITIC CELL VACCINATION AS POSTREMISSION THERAPY FOR AML PATIENTS WITH A NON-FAVORABLE RISK PROFILE

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Background: Post-remission therapy of patients with acute myeloid leukemia (AML) is critical for the elimination of minimal residual disease (MRD) and a prerequisite for achieving cure. Cellular immunotherapy is a highly effective treatment option as demonstrated by the low relapse rate after allogeneic stem cell transplantation (SCT). However, many patients are not eligible for this treatment. Therapeutic vaccination with autologous, antigen-loaded dendritic cells (DCs) is a promising strategy to induce cellular and humoral immune responses. Within recent years, we have developed a GMP-compliant protocol for the generation of *next-generation* DCs. A short, 3-day differentiation period is combined with a novel maturation cocktail that includes a TLR7/8 agonist. The resulting DCs are characterized by a positive costimulatory profile and a high production of bioactive IL-12p70. Both *in vitro* and *in vivo*, they have been shown to polarize $CD4^+$ T cells into Th1, to induce antigen-specific $CD8^+$ T cells and to activate NK cells.
Aims: Proof-of-concept phase I/II clinical trial evaluating *next-generation* DCs as postremission therapy for AML patients with a non-favorable risk profile.
Methods: Patients with AML and non-favorable risk profile in complete remission (CR) after intensive induction therapy are able to enroll. Standard exclusion criteria apply, and patients have to be ineligible for allogeneic SCT. DCs are generated from monocytes of the patients and then loaded with ivt-RNA encoding the leukemia-associated antigens WT1 and PRAME. Additionally, DCs transfected with RNA encoding CMV-pp65 are included as an adjuvant and surrogate antigen. Patients are vaccinated intradermally with one batch of $5x10^6$ DCs for each of the three antigens up to 10 times within 26 weeks, starting with weekly and continuing with monthly intervals. The primary endpoint of the trial is feasibility and safety. Secondary endpoints are immune responses and disease control, with particular focus on MRD conversion. Phase I will include 6 patients, and phase II another 14 patients.
Results: So far, two patients have been enrolled into the phase I of the trial. Pt. 1 was a 72 year-old man in CR with an adverse genetic risk profile (complex karyotype) and not eligible for allogeneic SCT. The differential blood count showed 11% monocytes of 7,6 G/l leukocytes (836 monocytes/µl). The leukapheresis yielded $3,4x10^9$ monocytes in total, and 14 vials of DCs per antigen were generated for clinical application. Pt. 2 was a 54 year-old man in CRi with an intermediate genetic risk group (cytogenetic abnormality not classified as favorable or adverse) and no HLA-matched donor. The differential blood count showed 7% monocytes of 5,9 G/l leukocytes (413 monocytes/µl). The leukapheresis yielded $2,2x10^9$ monocytes in total, and 6 vials of DCs per antigen were generated for clinical application. For both patients, the DCs fulfilled all quality criteria (cell count, viability, purity, sterility, phenotype). No adverse events have been observed so far except for slight erythema at the injection site.
Summary and Conclusion: We are currently conducting a proof-of-concept phase I/II clinical trial evaluating *next-generation* DCs as postremission therapy for AML patients with a non-favorable risk profile (NCT01734304). We report on the feasibility and safety of this protocol in phase I. Up-to-date clinical and immunomonitoring data will be presented.

P1140

FUNCTIONAL EVALUATION OF T-CELLS GENERATED FROM WT1-TCR TRANSDUCED HUMAN HEMATOPOIETIC STEM CELLS USING THE OP9-DL1 COCULTURE SYSTEM

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Background: Chemotherapy leads to cure of acute myeloid leukemia (AML) in less than half of the patients. Stem cell transplantation can be used as an immunotherapeutic treatment to cure the patient, but carries a high risk of toxicity and mortality, especially in older patients with comorbidities. Moreover, not all patients have a suitable donor. We have developed a novel immunotherapeutic treatment, in which we generate *in vitro*, starting from mobilized peripheral blood or cord blood hematopoietic precursor cells, T-cells that recognize WT1, a tumor antigen that is overexpressed on 70% of the AMLs.
Aims: In this study we have evaluated the functionality and specificity of the generated WT1-directed T-cells both *in vitro* and *in vivo*. The ultimate goal is to use these cells in patients, as this form of immunotherapy is promising and could be an option for cure in patients who should be treated with stem cell transplantation, but are not eligible. In contrast to the more widely used immunotherapy using TCR-transduced peripheral T-cells, our therapy is expected to be more effective and carry less risk of autoreactivity (due to the presence of only one TCR). Moreover, chimeric antigen receptor (CAR) T-cell therapy, which seems very promising in the treatment of lymphoid malignancies expressing a good, discriminating surface antigen, will probably not be able to be used for AML, because of the lack of a good surface antigen that is not present on normal myeloid cells, which coulld result in unacceptable toxicity.
Methods: $CD34^+$ cells isolated from cord blood and mobilized peripheral blood mononuclear cells were cultured on OP9-DL1 in the presence of the cytokines IL-7, Flt3-L and SCF, for 2 weeks, until T-cell commitment. Subsequently, they were transduced with a WT1-TCR, and again co-cultured until $CD4^+CD8^+$ double positive cells were abundantly present (generally after another 2-3 weeks). At that point, the agonist peptide WT1 was added to the culture together with IL-7, and 5 days later cells were harvested and expanded polyclonally or using agonist peptide,in the presence of IL-2, or IL-7+IL-15. *In vitro* evaluation: T-cells were evaluated using a ^{51}Chromium release assy, for cytotoxicity against WT1 and HLA-A2 positive and negative targets. Also,upon activation, production of IFN-g was evaluated using ELISA. *In vivo* evaluation: Immunodeficient 6-8 weeks old NSG mice were irradiated (200 cGy), and 24 hours later injected intravenously with a luciferase-positive, WT1, HLA-A2 transduced K562 cell line (R. Stripecke), together with $5x10^6$ or 10^7 WT1+ T-cells or CMV+ T-cells (negative control). Mice are evaluated using the IVIS bioluminescence assay.
Results: We observed that the highest number of WT1-TCR CD8+ T cells was generated if the cells were expanded after harvest from the coculture using the combination of the agonist peptide, IL-7 and IL-15. Using ^{51}Cr release assay and ELISA, we could show that upon activation, the T-cells showed specific cytokine production and efficient killing of tumor cells. Currently, experiments are ongoing evaluating the function of these cells on the expansion of WT1-HLA-A2+ K562 cells *in vivo* and survival of these mice.
Summary and Conclusion: We have shown that, using the OP9-DL1 model, we are able to generate large numbers of high-avidity tumor-specific naïve and resting T-cells, after a proicess similar to thymic positive selection. After expansion, polyclonal or antigen specific in the present of the agonist peptide) and activation, these cells show specificity and functionality *in vitro* and are currently evaluated in an *in vivo* immunodeficient mouse model.

P1141

OPTIMIZING TUMOR-REACTIVE SINGLE-CHAIN T CELL RECEPTORS FOR ADOPTIVE T CELL TRANSFER

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Background: Adoptive transfer of T cells modified by tumor antigen-specific T cell receptor (TCR) gene transfer is a promising strategy to induce tumor regression in cancer patients. The safety and efficiency of TCR gene transfer can be compromised by mispairing of the introduced TCRα or -β chains with the endogenously expressed TCR chains.
Aims: To reduce TCR mispairing and to increase expression and functionality of transferred TCR we aimed at the optimization of a human TCR specific for the melanoma antigen gp100.
Methods: We constructed a single chain (sc) TCR by covalently linking the variable domain of the TCRα-chain to the TCRβ-chain and co-expressed the constant domain of the TCRα-chain (Cα) to achieve stable scTCR expression. Since the human scTCR/Cα was not expressed at the cell surface due to instability, we substituted the constant TCR domains by mouse TCR domains (chim scTCR/Cα). As mouse-derived proteins could be immunogenic in patients, we minimally murinized the constant domains by replacing only selected amino acids in the human constant domains of the scTCR/Cα by their corresponding mouse amino acids (mm scTCR/Cα). To increase stability, additional disulfide bonds between scTCR and Cα were introduced. For further improvement, we linked the gene of the scTCR with the Cα by a 2A-element and expressed it on one retroviral vector.
Results: After retroviral transduction of human T cells with the scTCR/Cα, we detected a high cell surface expression for the chim scTCR/Cα and the mm scTCR/Cα. T cells transduced with the different TCR constructs were able to lyse melanoma cells and cell lines generated from melanoma metastases. T cell degranulation induced by incubation with gp100 peptide loaded cells was equal in T cells transduced with the chim scTCR/Cα and the mm scTCR/Cα. This assay also showed a degranulation of CD4 T cells, indicating that the TCR reacts CD8-independent. Affinity of both constructs as determined by K_D measurement with different tetramer concentrations was equivalent. Furthermore we are currently running an *in vivo* experiment to prove rejection of melanomas by the scTCR/Cα constructs in a mouse model. Therefore, melanoma cells were transduced with a luciferase gene to detect tumor growth and metastasis by *in vivo* imaging. A control of melanoma growth is especially seen in mice with a high frequency of transferred T cells in the blood. During the experiment, we plan also to scan for a panel of cytokines and chemokines to reveal potential immune escape mechanisms.
Summary and Conclusion: Our results show equal expression and functionality of mm and chim scTCR/Cα. These optimized gp100-specific TCRs

represent promising candidates for adoptive T cell transfer by providing effective antitumor responses. For the future it would be interesting to extend this TCR optimization technique to TCR recognizing antigens of leukemia or other hematologic malignancies.

P1142

COMBINING ADOPTIVE TCR IMMUNOTHERAPY AND ARGININE DEPLETION IN OSTEOSARCOMA XENOGRAFT MODEL

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Background: The adoptive transfer of TCR-engineered T cells is a promising approach in the treatment of human malignancies. However, a safety concern of TCR gene transfer is the risk of pairing between introduced and the naturally expressed endogenous TCR chains, resulting in the generation of self-reactive T cells. Moreover, after adoptive transfer of tumor-reactive T cell the anti-tumor effectiveness in cancer models and in patients remains very limited due to various tumor immune escape mechanisms. Targeted therapies against tumors and immunotherapy have recently proven clinical synergistic effect when combined together.
Aims: We aim at improving adoptive T cell-based tumor immunotherapy by (1) combining novel optimized specific TCR with enhanced tumor recognition as well as reduced potential for self-reactivity (2) pharmacological intervention to deplete arginine, an amino acid crucial for tumor cell metabolism and growth.
Methods: To overcome TCR mispairing formation, we designed a single chain (sc) TCR format by connecting the variable TCRa domain to the TCRb chain via a short peptide linker co-expressed with a truncated constant TCR a domain. To further enhance preferential TCR pairing, cell surface expression and TCR function, we introduced additional cysteine residues into the TCR α and β chain constant domains along with codon-optimization of the TCR sequences and cloning of the TCR constructs into one single 2A-based retroviral vector. This molecular design was applied to an HLA.A*02.01 (A2.1)-restricted p53(264-272)-specific TCR as promising antigen-driven immunotherapy for both solid tumors and hematologic malignancies. To deplete specifically arginine in tumor milieu, we used the pegylated form of arginine deiminase (ADI-PEG20), a drug successfully used in clinical trials. To study the *in vivo* efficacy of combined arginine depletion and scTCR gene transfer, we developed a NOD/SCID/γc^{null} (NSG) xenograft model of osteosarcoma.
Results: *in vitro* studies demonstrated that, scp53TCR-modified T cells display similar high-avidity compared to the full-length TCR and mediate specific lysis of $p53^{+}A2.1^{+}$ tumor cells. Importantly, scp53TCR-engineered T cells showed anti-tumor activity without inducing graft-*versus*-host disease (GVHD) in NSG mice inoculated with p53 mutant osteosarcoma cells. To enhance the anti-tumor response observed in the osteosarcoma model after transfer of T cells, tumor-engrafted NSG mice received repetitive injections of ADI-PEG20 and subsequently infused with TCR-redirected T cells. Unexpectedly, this therapeutic combination resulted in the abrogation of the anti-tumor response mediated by transferred T cells. Further analysis demonstrated that arginine depletion in the tumor micromilieu upon ADI-PEG20 treatment was associated with a downregulation of p53 tumor antigens. These results were confirmed *in vitro*. Preliminary results suggested that downregulation of tumor antigens upon arginine starvation is regulated at the transcriptional level.
Summary and Conclusion: Our study provided evidence that an optimized high-affinity scTCR-specific for the broadly expressed tumor-associated antigen p53(264-272) mediates anti-tumor response *in vivo* without inducing GVHD. Interestingly, arginine starvation affects the expression of tumor antigens in this oseosarcoma model. In the light of these new data, further studies are required to better understand the exact mechanisms.

P1143

HDAC6 INHIBITION AUGMENTS THE EFFICACY OF ANTI-CD20 MONOCLONAL ANTIBODIES BY UP-REGULATING CD20 LEVEL IN MALIGNANT B-CELLS

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Background: CD20, an integrate membrane protein expressed on the surface of normal and malignant B-cells is widely used as a molecular target for monoclonal antibodies (mAbs) in the therapy of non-Hodgkin's lymphomas and chronic lymphocytic leukemia (CLL). Accumulating evidence indicates that CD20 can be modulated at several levels, both transcriptional and posttranscriptional and its up-regulation would result in increased efficacy of anti-CD20 mAbs. CD20 antigen has been reported to be regulated epigenetically *e.g.* by histone deacetylases (HDACs).
Aims: The aim of our project was to check if specific inhibition of HDAC6 can influence CD20 level and improve the efficacy of anti-CD20 mAbs.
Results: The results of our preliminary experiments show that blocking the activity of a single HDAC isoform - HDAC6 with selective inhibitors (tubacin, ACY-1215 and tubastatin) leads to up-regulation of CD20 protein in B-cell lymphoma cell lines, EBV-transformed B-cells and primary cells from CLL patients but not in normal B-cells. The observed up-regulation of CD20 level correlates with increased efficacy of anti-CD20 mAbs - rituximab and ofatumumab in complement-dependent cytotoxicity (CDC) assays, but does not influence antibody-dependent cellular cytotoxicity (ADCC). We observed that HDAC6 silencing with shRNA also increases CD20 surface level, although overexpression of HDAC6 does not induce changes in CD20 level. HDAC6 inhibition and silencing lead to increase in total CD20 protein level assessed in Western blotting. Neither tubacin nor HDAC6 silencing with shRNA does alter the expression of complement inhibitors. In order to elucidate the mechanism by which HDAC6 inhibition increases CD20 level we performed qRT-PCR experiments using SyBR Green and hydrolysis probes. We observed that HDAC6 inhibition with tubacin and its silencing with shRNA do not alter CD20 mRNA. Accordingly, HDAC6 inhibition does not change CD20 promoter activity and leads to increase of CD20-tagged protein level expressed in Raji independently of CD20 promoter. HDAC6 is a unique member of HDAC family reported to be engaged mainly in the acetylation of non-histone substrates, protein degradation and transport. Since the effect of HDAC6 inhibition on CD20 molecule does not rely on transcriptional mechanisms we sought to determine HDAC6 role in CD20 protein transport. Using nocodazole – a microtubule disrupting agent we observed that the effect of HDAC6 on CD20 depends on the stability of microtubules. What is more, we observed that blocking protein anterograde protein transport with Golgistop abrogates tubacin effect on CD20 regulation. These results suggest that HDAC6 may be implicated in CD20 transport/degradation pathways. Nevertheless, extensive experiments aiming at elucidating this problem have to be performed.

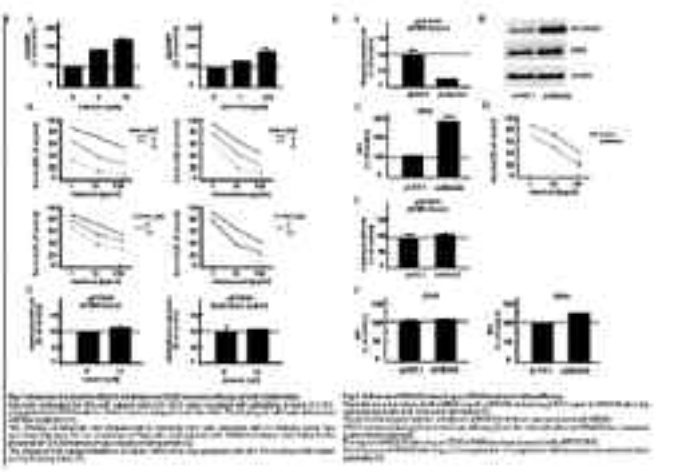

Figure 1.

Summary and Conclusion: The results of our study strongly suggest that combining HDACi with anti-CD20 antibodies can be a successful therapeutic modality for patients suffering from B-cell malignancies. The use of isoform-selective inhibitors of HDAC6 may be an effective strategy in enhancing the efficacy of anti-CD20 mAbs. Potentially these compounds would have less adverse effects than HDAC pan-inhibitors. However, their use in the therapy requires further investigation.

P1144

ADOPTIVE CIK CELLS IN PATIENTS WITH LEUKEMIA RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a curative treatment of many hematologic malignancies. Leukemia relapses imply failure of the transplantation and poor prognosis as none proved treatment was found. It is reported that allogeneic cytokines induced killer (CIK) cells have showed superiority in the effect of graft *versus* tumor/leukemia (GVT/GVL) with rare occurrence of graft-*versus*-host disease (GVHD).
Aims: We conducted a Phase I study to test the safety and feasibility of infusions of CIK cells to 11 patients who suffered leukemia relapse after allo-HSCT. Informed consent was obtained.
Methods: The median age of patients was 31.9 years (range 17-49) and their

diagnoses were acute myelogenous leukemia (AML, n=3), pre-B acute lymphoblastic leukemia (pre-B ALL, n=1), T acute lymphoblastic leukemia (T-ALL, n=2), chronic myeloid leukemia (CML-CP, n=1) and B acute lymphoblastic leukemia (B-ALL, n=4). 8/11 received allo-HSCT from HLA identical siblings, and the other 3 received it from unrelated donors. The mononuclear cells used for CIK induction were extracted from the patients themselves (n=9) and HSCT donor (n=2), respectively.
Results: The CIK cells were successfully amplified *in vitro* for all the 11 patients. For the patients with hematologic recurrence or extramedullary relapse of leukemia or loss of full donor chimerism, tapering of cyclosporine A(CsA) and the most appropriate chemotherapy treatment could be performed to achieve the best hematologic response and to allow the laboratory preparation of CIK cells. CsA were all withdrawn prior to infusion of CIK. The average amount of infused CIK cells was 1.56×10^5/site (*in situ* injection), or 3×10^9/d (intravenous injection) in two successive days. 1/11 patient performed as multiple subcutaneous mass in the head, which was not suitable for surgery and radiation. 1/11 patient was found increased thymic shadow in anterior mediastinum by PET-CT. 1/11 suffered both extramedullary and hematological relapse. The left 8 patients suffered from hematological relapse, with increased leukemia cells found in the bone marrow. The mass in the head turned flat and gradually disappeared after *in situ* injection of CIK cells. 10/11 patients received intravenous injection of CIK cells. For those 2 with anterior mediastinum, thymic shadow gradually disappeared after intravenous infusion of CIK cells. 10/10 patients achieved hematological remission after intravenous injection. The 2-year overall survival of patients after relapsed is 7/11. For the patients with intravenous injection, neither acute toxicity nor new-onset or worsening GVHD was observed. Acute GVHD was observed in 8 patients, after infusion of CIK, GVHD symptoms relieved.
Summary and Conclusion: In this study we have shown that large numbers of allogeneic CIK cells can be produced in a 12-days time period and safely given to patients who have relapsed after allogeneic transplantation. This is the first report, to our knowledge, of CIK cells *in situ* injection for extramedullary relapse. The results initially proved that CIK cell therapy for extramedullary relapse after allo-HSCT is not only feasible, but has a good security.

P1145

CYTOMEGALOVIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES RESTRICTED TO HLA-A2 OR HLA-A24 CAN BE EFFICIENTLY GENERATED BY USING GENE-ENGINEERED ARTIFICIAL ANTIGEN-PRESENTING CELLS

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Background: Reactivation of cytomegalovirus (CMV) is a major cause of morbidity after allogeneic hematopoietic stem cell transplantation. Adoptive transfer of CMV-specific cytotoxic T lymphocytes (CTLs) is a promising therapy to treat reactivation and prevent viral disease. However this noble modality has been hampered by the difficulty of consistently generating potent anti-CMV lymphocytes in a timely manner for every patient. To overcome this, we previously reported a culture system that can reproducibly generate antigen-specific CTL by using K562-based artificial antigen-presenting cells (aAPCs).
Aims: In the present study, we have applied the culture system to CMV and examined whether HLA-A*02:01 (A2) (a major HLA-A phenotype among Caucasian) or A*24:02 (A24) (a major HLA-A phenotype among Japanese)-restricted CMV-specific CTL can be generated using aAPCs.
Methods: HLA-A2 or A24-positive peripheral blood mononuclear cells were obtained from leukemia or cancer patients (n=11). To establish CMV-specific T cells, $CD8^+$ T cells were purified by positive selection using a magnetic beads method (Miltenyi Biotec). aAPCs expressing either HLA-A2 or HLA-A24 were pulsed with HLA-A2 or HLA-A24 restricted, CMV-pp65 immunodominant peptides (NLVPMVATV for HLA-A2 and QYDPVAALF for HLA-A24). aAPCs were then irradiated with 200 Gy and added to purified $CD8^+$ T cells at a ratio of 1:10 in 24-well plates in PRMI1640 supplemented with 10% human AB serum. Between stimulations, IL-2 (10 U/ml) and IL-15 (10 ng/ml) (both from Peprotec) were added to the cultures.
Results: Following 3 rounds of weekly stimulation with peptide-pulsed aAPCs, CMV-specific CTLs were evaluated by a tetramer staining. The percentage of HLA-A2 tetramer-positive cells was 0.05±0.05% before culture. It increased to 8.98±8.73% (176 fold increase) following the third stimulation. On the other hand, the percentage of HLA-A24 tetramer-positive cells before and after stimulation was 0.03±0.05% and 11.0±16.5% (367 fold increase), respectively. A kinetic study revealed that the percentage of tetramer-positive cells was 0.02±0.01% before stimulation. It increased to 2.10±1.72% (105 fold increase) and 9.14±8.13% (457 fold increase) following the second and the third stimulation, respectively, indicating a rapid increase in CMV tetramer-positive $CD8^+$ T cells. $CD8^+$ T cells stimulated with HLA-A2 CMV-peptide-pulsed aAPC were negative for both A24/CMV and A2/HIV tetramers, confirming HLA restriction and antigen specificity. And the opposite is also true for $CD8^+$ T cells stimulated with HLA-A24 CMV-peptide-pulsed aAPC. FACS analysis revealed that majority of CMV-specific CTLs expanded with CMV-peptide-pulsed aAPCs expressed a memory phenotype.
Summary and Conclusion: These results demonstrated that HLA-A2 or A24-restrited CMV-specific CTLs with a memory phenotype can be generated ex-vivo using peptide-pulsed gene-engineered aAPCs within a short period of time for clinical use.

P1146

DESIGNING TCR SPECIFIC FOR HUMAN MDM2 TUMOR-ASSOCIATED ANTIGEN FOR ADOPTIVE IMMUNOTHERAPY AGAINST MELANOMA AND MULTIPLE MYELOMA

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Background: Adoptive T cell immunotherapy is an attractive approach to treat cancer patients. However, isolating high-affinity tumor-associated antigen (TAA)-specific T cell receptors (TCRs) from the patient is often hampered by self tolerance mechanisms. We have already described that using HLA-A*0201 (A2.1) transgenic mice can circumvent self tolerance to TAAs. The TAA MDM2, the human homologous of the murine double-minute 2 protein, is abnormally up-regulated in soft tissue sarcoma (especially in liposarcoma), multiple myeloma (MM) and melanoma. We generated an HLA.A2-restricted CD8-dependent MDM2 (81-88)-specific TCR from a high-avidity murine CTL clone derived from CD8 x $A2K^b$ transgenic mice to redirect human T cells via retroviral gene transfer. TCR gene transfer however can result in the formation of mixed TCR heterodimers between introduced and native TCR chains leading to potentially self-reactive T cells.
Aims: Our aim is to establish a new approach in adoptive T cell immunotherapy where tumors overexpressing MDM2 can be targeted by human T cells engineered with MDM2 (81-88)-specific TCR. Therefore we aim to design the optimized TCR format.
Methods: For transduction of human T cells we used on the one hand the unmodified wild-type (wt) MDM2-TCR. On the other hand we optimized the wt MDM2-TCR-construct via several molecular modifications. To increase the expression level of transduced TCR we codon-optimized the sequence and cloned it in a bicistronic retroviral vector containing the self-cleaving 2A virus-derived peptide. The risk of formation of heterodimer TCR was reduced via addition of an inter-chain disulfide bond between TCR α and β constant domains. We analyzed the TCR expression level by flow cytometry. The affinity to bind HLA.A2 molecules loaded with the MDM2 (81-88)-peptide was measured by using labeled tetramers. The MDM2 expression of various tumor cell lines was determined by Western Blot. Finally we analyzed the capacity of recognition and killing of tumor cells in a standard cytolytic assay.
Results: We screened melanoma and MM cell lines for MDM2 and HLA.A2 expression. Cell lines which are highly HLA.A2 positive and also expressing MDM2 were tested for recognition by the wt MDM2 (81-88)-specific TCR in a ^{51}Chromium-release assay. Our data demonstrated that these cell lines were efficiently lysed by human T cells transduced with wt MDM2-TCR compared to melanoma or MM cell lines which do not express HLA.A2 or harbor weak MDM2 levels.
Summary and Conclusion: Our data show that the TAA MDM2 is a potential candidate to target melanoma and MM. Adoptive T cell therapy with T cell retrovirally transduced with MDM2 (81-88)-specific TCR may represent a novel approach for treating cancer cells overexpressing MDM2. To prevent the formation of mixed heterodimer TCRs and to improve consequently safety we are also planning to engineer an optimized single chain MDM2-TCR.

P1147

AN EFFICIENT AND COST EFFECTIVE METHOD FOR THE GENERATION OF CLINICALLY RELEVANT NUMBERS OF MYELOID CORD BLOOD-DERIVED DENDRITIC CELLS BY EMPLOYING CLINICALLY APPROVED AGENTS

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Background: The wide clinical application of T-cell based cancer therapy is hampered by the large number of dendritic cells (DCs) required to induce tumor- or virus – specific cytotoxic T lymphocytes and the cost and complexity of existing methods of DC production. New methods and sources are needed in order to obtain clinically relevant numbers of DCs (10^6-10^8) capable to prime naïve T-cells for cytotoxic activity.
Aims: We investigated the potential to generate myeloid DCs at large scale from CD34+cells derived from non-transplantable CB units - obtained under a signed informed consent from the parents.
Methods: Instead of the standard culture dishes or flasks, we used the G-Rex10 bioreactors. To address the ability of DCs expanded from CB-derived

CD34+cells to induce Th1 responses by employing clinically approved stimulating agents, we performed 24h- or 48h- DCs activation by commonly used vaccines [Act-HIV (bacterial form of influenza), Influvac (viral form of influenza), Typhim Vi and BCG] which contain Toll-Like Receptor ligands (TLR-Ls) as adjuvants, in comparison to TLR-L3+TLR-L7/8–stimulation. The mature phenotype of DCs was determined by flow cytometry (CD40, HLA-DR, CD83, and CD86) and the levels of IL-12p70, TNF-α, IL-6 and IL-10 were measured by ELISA. The endophagocytotic activity of DCs was assessed by a dead yeast engulfment assay.
Results: CD34+cells were enriched to more than 90% purity from CB units (n=5) after immunomagnetic separation and cultured in the presence of AB-serum (ABS) and media supplemented with SCF and GM-CSF for 4 weeks followed by IL-4 plus GM-CSF for 1 additional week. The cells were initially plated in a 6-well plate (10^5cells/well) and once they expanded up to $5x10^6$, half were transferred into a G-Rex10 bioreactor and half were cultured in conventional plates in the presence of conditioned medium. The cells were splited at confluency in the culture plates and every 3 days in the G-Rex. The median absolute number of myeloid DCs (CD33+/CD11+) obtained by a 5 week-G-REX10 culture was $1.5x10^9$ whereas the conventionally cultured DCs did not exceed a median number of $10x10^6$ cells. A strong potential for Th1 responses by the expanded myeloid DCs upon stimulation with vaccines was demonstrated by the increased levels of IL-12p70, TNF-α and IL-6 and low to undetectable IL-10 levels which was comparable to TLR-L-induced activation. Importantly, the peak response induced by Act-HIV vaccine was reached after 24h-stimulation, thus preserving the cells from exhaustion in culture. DCs, stimulated by either way, highly expressed the CD40, CD86 and CD83 surface markers and demonstrated significant phagocytotic activity within 1 minute upon stimulation.
Summary and Conclusion: In conclusion, we report for first time an efficient and cost-effective approach for over a 10^4 fold expansion of myeloid DCs from CB-derived CD34+cells displaying high potentiality of Th1 responses induced by clinically approved vaccines. This new method could contribute to a wide application of T-cell based cancer therapy.

P1148

HYPOMETHYLATING AGENT AZACITIDINE INDUCES FOXP3 NEGATIVE HLA-G EXPRESSING IMMUNOREGULATORY T CELLS
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Background: A major caveat of FOXP3+ T regulatory cells in immunotherapy against GVHD is their low numbers in circulation and the lack of specific cell surface markers for efficient purification. DNA methylation plays a key role in the regulation of T-cell effector function and cytokine gene expression, indicating a promising role of hypomethylating agents in immunomodulation. Recently it was shown that in-vitro treatment of conventional T-cells with the hypomethylating agent azacitidine (aza) induced FoxP3 expression and converted $CD4^+CD25^-$ cells into immunosuppressive T-cells, the suppressor function of which is independent of FoxP3 expression (Blood 2010;116:129-139), suggesting that aza induced suppressor function depends on the modification of other hypomethylated genes. Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule, shown to exert immunoregulatory functions, the expression of which is epigenetically regulated.
Aims: In this study we investigated whether aza can induce $HLA\text{-}G^+$ immunoregulatory T cells.
Methods: Negative selected T cells from peripheral blood of healthy individuals were stimulated with anti-CD3 plus anti-CD28 coated beads, then treated for 72 hours with aza (0.5-15 mM) in the presence of 50U/ml inteleukin-2 (IL-2). Phenotypical characterization of aza treated cells was performed with flow cytomentry. The effect of aza on HLA-G mRNA transcription and methylation of upstream regulatory HLA-G gene DNA sequence was quantitated with Real time PCR and bisulfite pyrosequencing respectively. Aza-induced $HLA\text{-}G^+$ T cells, were irradiated and then used as third party cells in CFSE based suppression assay. For the analysis of the in-vivo effect of aza on HLA-G expression, peripheral blood mononuclear cells (PBMC) of patients with myelodysplastic syndrome (MDS) were isolated at baseline and after Vidaza treatment and were analyzed for HLA-G expression.
Results: In-vitro treatment of $CD3^+$ T cells with aza induces, dose dependent, de-novo HLA-G expression on stimulated peripheral T cells of healthy individuals. The optimum aza concentration for maximum HLA-G induction with the lowest toxicity in CD3 T cells was determined at 5 mM (surface expressing HLA-G cells: 6,88±3,9%, p=0,0022, fold increase in HLA-G mRNA relevant expression: 18,32±2,33). Aza treatment of FACS-sorted $CD4^+CD25^{neg}HLA\text{-}G^{neg}$ cells induced $CD4^{low}$ $CD25^+$ HLA-G positive cells, revealing that aza induced $HLA\text{-}G^+$ cells are not the result of a selectively expanded preexisting $HLA\text{-}G^+$ population. Methylation analysis of upstream regulatory HLA-G gene DNA sequences and stability assays revealed that induced HLA-G expression is dependent on the presence of aza, and seems to be stabilized by progesterone. In-vitro Aza induced $HLA\text{-}G^+$ cells are immunosuppressive and HLA-G blocking experiments further confirmed that their suppressive function is HLA-G dependent. Phenotypic analysis revealed that aza induced $CD4^+HLA\text{-}G^+$ are FoxP3 negative $CD4^{low}CD25^{high}$. Data regarding surface HLA-G expression on T cells from peripheral blood samples of (MDS) patients under Vidaza treatment, show high intra-variability.
Summary and Conclusion: Conclusively, by using hypomethylating agent aza in-vitro, we generated a $CD4^{low}FoxP3^{neg}$ immunoregulatory population, which expresses extracellular HLA-G and therefore can be easily isolated for adaptive immunotherapies.

P1149

IMMUNOPHENOTYPIC PROFILE AND FUNCTIONAL CHARACTERISTICS OF NK CELLS EXPANDED FROM CYTOMEGALOVIRUS SEROPOSITIVE AND SERONEGATIVE DONORS: IMPLICATIONS FOR NEW STRATEGIES OF ADOPTIVE CELLULAR THERAPIES
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Background: Cytomegalovirus (CMV) infection represents one of the main causes of morbidity and mortality after an allogeneic stem cell transplantation (SCT). Natural killer (NK) cells are known to exert a protective role in CMV infection following SCT. During CMV viremia, proliferation of NK cells is characterized by an increased expression of specific activating receptors such as the NKG2C molecule in association with the CD57 marker; it appears that NKG2C+CD57+ NK cells preferentially expand and exert antiviral activity when isolated from CMV seropositive (+) donors, being therefore identified as memory-like NK cells.
Aims: Aim of our study is to compare the immunophenotypic profile, with particular attention to those antigens involved in anti-CMV response, of NK cells isolated from CMV seronegative (-) and CMV+ donors before and after *ex-vivo* expansion with a GMP-compliant method. The expansion capacity and cytotoxic activity of NK cells expanded from CMV- and CMV+ donors are also compared.
Methods: PBMCs were collected from 14 healthy donors (7 CMV- and 5 CMV+). For NK cell enrichment, $CD3^+$ cell depletion was followed by $CD56^+$ cell selection. Isolated NK cells were cultured for 14 days with autologous plasma, IL-2, IL-15 and irradiated autologous feeder cells. The phenotype of freshly-isolated and expanded NK cells was assessed using mAbs against the CD3, CD16 and CD56 antigens, activating and inhibitory receptors (CD158, CD158a, CD158e, NKp30, NKp44, NKp46, NKG2A, NKG2C, NKG2D and DNAM-1) and activation and maturation markers (CD25, CD69, CD62L, CD57). The cytolytic properties of expanded NK cells against the K562 cell line were tested in a ^{51}Cr release assay.
Results: Freshly-isolated NK cells from CMV- donors were characterized by a significantly reduced proportion of $NKG2C^+$ and $NKG2C^+CD57^+$ cells compared to CMV+ donors (12.9±9.6% *vs* 43.8±25.9%, p=.016 and 4.9±4.2 *vs* 23.5±17.2% p=.002, respectively). Both CMV- and CMV+ donor NK cells could be efficiently expanded *in vitro* under GMP conditions (expansion fold mean 19.9±9.2 and 22.2±11.8, respectively). After expansion, the percentages of $NKG2C^+$ and $NKG2C^+CD57^+$ cells increased significantly only in CMV- donors (p=.017 and p=.023, respectively), becoming comparable to the percentage observed in CMV+ donors (55.5±31.6% *vs* 67.2±36.9%, p=n.s. and 47.7±35.1% *vs* 64.2±34.5%, p=n.s, respectively). In addition, no relationship was observed between the serologic status for CMV and the expression of most of the NK receptors, activation and maturation markers. Finally, no differences were observed in the cytolytic activity against the K562 cell line of expanded NK cells from CMV- and CMV+ donors (mean cytotoxicity at a 50:1 E:T ratio 77±17% and 71±8%, respectively).
Summary and Conclusion: The results hereby presented confirm that a previous contact with CMV induces a specific antigen expression profile of memory-like NK cells active in controlling viral replication. Similar immunophenotypic characteristics, with an increment of $NKG2C^+$ and $NKG2C^+CD57^+$ cells, are also acquired by NK cells isolated from CMV- donors after expansion in GMP-compliant methods. NK cells expanded from CMV- and CMV+ donors present a similar expansion and cytotoxic capacity. Further studies are underway to define whether the acquisition of a specific antigen expression profile by NK cells expanded from CMV- donors corresponds to an increased capacity to control CMV replication. The ultimate goal is to verify whether NK cells expanded from CMV- and CMV+ donors are transplantable and exert clinically relevant antiviral activities.

P1150

GENERATION OF PATIENT-INDIVIDUALIZED AML-REACTIVE DONOR CD8+ T CELLS UNDER GMP-COMPLIANT CONDITIONS FOR ADOPTIVE TRANSFER IN LEUKEMIA PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Effective treatment of patients undergoing allogeneic hematopoietic stem cell transplantation (AHSCT) for chemo-refractory acute myeloid leukemia (AML) depends on the graft-*versus*-leukemia (GvL) effect mainly mediated by donor T cells. Unfortunately, this effect is frequently hampered by alloreactivity of donor lymphocytes resulting in graft-*versus*-host disease (GvHD) and insufficient GvL-reactivity to prevent relapse. Thus, protocols that include additional cellular therapy to improve antileukemia responses are warranted.
Aims: In the present study we therefore sought to establish a GMP-compliant manufacturing protocol for producing highly AML-reactive, patient-individualized donor CD8$^+$ T lymphocytes to confer sustained antileukemic immunity while reducing the risk of GvHD in leukemia patients post AHSCT.
Methods: Based on a reliable protocol published previously (Albrecht *et al.*, 2011, Cancer Immunol. Immunother.) naïve CD45RA$^+$CD8$^+$ T cells from healthy donors were immuno-magnetically isolated by the MACS^{TM} technology and stimulated with fully HLA class I-matched, primary AML blasts under optimized cytokine conditions comprising Interleukin (IL)-7/-12/-15 and -21 in allogeneic mixed lymphocyte/leukemia cultures (MLLCs) for 28 – 42 days (d). IL-12 was replaced by IL-2 after 14d of culture. In comparative studies MLLCs were then substituted with research-grade reagents or available GMP-grade components (IL-2, -7, -15, -21, TexMACS® medium, human serum) to define optimal GMP-culture conditions. Moreover, a newly developed 96/24 well micro structured cell culture system was tested in first studies to allow the setup of GMP-compliant MLLCs.
Results: So far, five different HLA class I-identical patient-donor MLLCs using naive CD8$^+$ T cells either isolated by the non-GMP-grade *Naïve CD8 T cell isolation Kit* or a GMP-guided *CD45RO depletion/CD8 isolation* procedure were performed and compared for purity of CD45RA$^+$CD8$^+$ cells, growth/expansion rates and numbers of individual AML-reactive T cell populations obtained from these MLLCs following culture in titrated standard research-grade *versus* (*vs*) GMP-grade components. In addition, we examined AML-reactivity of these individual populations by IFN-g ELISpot analyses. While purity was slightly higher (~100% *vs* ~90%) using the *Naïve CD8 T cell isolation Kit*, the frequency and AML-reactivity of T cells obtained upon culture in the presence of research-grade *vs* GMP-grade cytokines was overall comparable. Finally, generation of leukemia-reactive T cells required the presence of human serum despite the use of T cell compatible GMP-medium.
Summary and Conclusion: In summary, AML-reactive T cells can be generated *in vitro* under GMP-grade conditions with comparable frequency and reactivity when compared to antileukemic T cells cultured at standard research-grade level. Further studies to evaluate the biological activity of these GMP-compliant T cells in a patient-tailored preclinical AML xenograft model and to validate the upscaling procedure are in progress.

P1151

LOW-DOSE BORTEZOMIB SENSITIZES MULTIPLE MEYLOMA TO GAMMA-DELTA T CELLS LYSIS

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Background: Multiple myeloma (MM) is a plasma cell neoplasm that is currently incurable. There is increasing evidence that gamma-delta (γδ) T cells have a potential role in the control of MM. γδ T cells are potent effector lymphocytes of innate immunity involved in anti-tumor immune surveillance. Its lytic activity depends on expression of stress-induced ligands on tumor cells and endogenous pyrophosphate molecules that are overproduced in transformed cells. Low expression of stress-induced ligands, such as NKG2D ligands and TRAIL receptors on MM cells, is one of the mechanisms involved in the immune escape and reducing the response to γδT cells adoptive therapy in MM patients. An interesting therapeutic strategy for MM is the modification of MM cell sensitivity to killing.
Aims: Bortezomib is a proteasome inhibitor to be used in the treatment of MM as first line therapy or after relapse. It can not only induce apoptosis of MM cells, but also some studies have shown that it can enhance the immunogenicity of MM cells. The aim of this study was to investigate the immunomodulatory effects of bortezomib in sensitizing MM cells to the cytotoxicity of γδT cells.
Methods: The proliferation and apoptosis of U266 cells (MM cell line) treated with bortezomib was detected by MTT method and Annexin V in conjunction with propidium iodide (PI) staining method using flow cytometry (FCM) respectively. The expression of NKG2D ligands (ULBP1, ULBP2, ULBP3 and MICA/B) and TNF-related apoptosis-inducing ligand (TRAIL) receptors (DR4, DR5) on the surface of U266 cells pretreated with or without low-dose (10nM) bortezomib for 12h was detected by FCM. γδT cells were induced and expanded by zoledronate and IL-2 from peripheral blood mononuclear cells derived from one MM patient. The expression of Vγ9, CD3, CD69, NKG2D and TRAIL on γδT cells was detected by FCM. The cytotoxicity of γδT cells incubated with or without antibodies of NKG2D and TRAIL against U266 cells pretreated with or without bortezomib was measured by calcein-AM assay.
Results: Low-dose (10nM) bortezomib has little effects on the proliferation and apoptosis on U266 cells compared with those of higher doses of bortezomib. But the expression of ULBP1, ULBP2, ULBP3, MICA/B, DR4 and DR5 on U266 cells was increased after 12 hours' pre-incubation with low-dose bortezomib. γδT cells were successfully induced and expanded *in vitro*. After induction, the percentage of γδT cells was much higher than that prior to induction (89.62±7.51% *vs* 5.23±2.13%, P<0.05). The percentage of the induced γδT cells expressing NKG2D(80.26±6.76%), CD69 (60.36±8.16%) and TRAIL (30.21±5.36%), was also significantly increased. The cytotoxic effect of induced γδT cells on U266 cells pretreated with low-dose bortezomib was much higher than that in untreated group (49.93±4.11% *vs* 21.54±2.81%, P<0.05). The cytotoxicity of γδT cells blocked by NKG2D or TRAIL antibody, against U266 cells pretreated with low-dose bortezomib was much lower than that of γδT cells without being blocked by the antibodies (35.53±3.97% or 40.63±3.22% *vs* 49.93±4.11%, P<0.05), but it was higher than that of γδT cells against the untreated U266 cells (35.53±3.97% or 40.63±3.22% *vs* 21.54±2.81%, P<0.05).

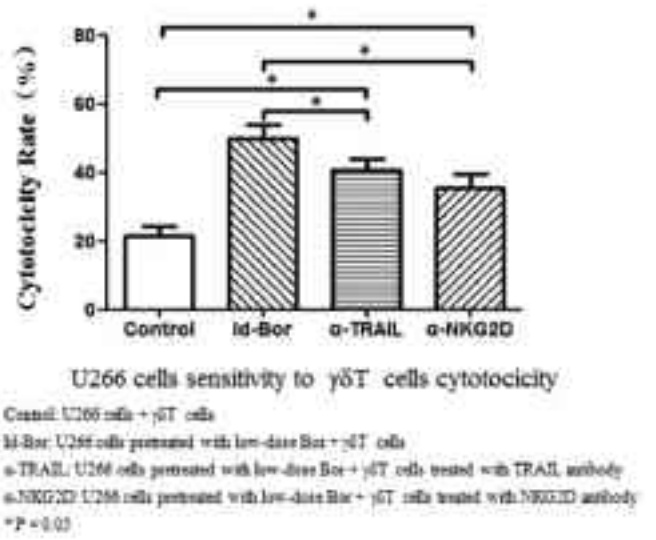

Figure 1.

Summary and Conclusion: These data demonstrated that low-dose bortezomib could increase the sensitivity of MM cells to γδT cells, partly by the upregulation of NKG2D and TRAIL ligands on U266 cells. Low-dose bortezomib might be rational to be used in this study to confirm its immunomodulatory effects without apoptotic effect on MM cells. It also implied that it could be practical and ensure the safety in the clinical application, because the dosage is much lower than that administered in the treatment protocol for MM. This study provides a novel approach for the treatment of MM, and it is worthwhile exploring this combination strategy in the clinical settings.

P1152

HUMAN MONOCYTE-DERIVED DENDRITIC CELLS EXPOSED TO HYPERTHERMIA SHOW A DISTINCT GENE EXPRESSION PROFILE AND SELECTIVE UPREGULATION OF IGFBP-6

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Background: Dendritic cells (DCs) have been used for immunotherapy of cancer but their immunostimulatory functions are still incompletely understood. Fever plays a role in activating innate immunity while its relevance in activating adaptive immunity is less clear. Even brief exposure to elevated temperatures significantly impact on the immunostimulatory capacity of DCs, but the molecular mechanisms underlying this effect and their consequences on immune response remain unclear.
Aims: We investigated whether exposure to 39°C induces a distinct gene expression profile program in monocyte-derived DCs and whether this allows identification of genes, not previously known to be part of response to hyperthermia.

Methods: We analyzed the gene expression profiles of normal human monocyte-derived DCs in paired samples from nine healthy adults subjected either to fever-like thermal conditions (39°C) or to normal temperature (37°C) for just 180 minutes.
Results: Short exposure of DCs to 39°C caused upregulation of 43 genes and downregulation of 24 genes. Functionally, the up/downregulated genes are involved in post-translational modification, protein folding, cell death and survival, and cellular movement. Notably, when compared to monocytes, DCs differentially upregulated transcription of the secreted protein IGFBP-6, not previously known to be specifically linked to hyperthermia. Indeed, DCs exposed to 39°C secreted IGFBP-6 which was found to induce chemotaxis of monocytes and T lymphocytes, but not of B lymphocytes.
Summary and Conclusion: Temperature regulates complex biological DCs' functions that most likely contribute to their ability to induce an efficient adaptive immune response. Our data indicate that IGFBP-6 may have relevance in cellular immunity, promoting the extravasation of monocytes and different lymphocyte subpopulations during the immune response.

P1153

ROLE OF LONG PENTRAXIN 3 (PTX3) IN WOUND CLOSURE INDUCED BY BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS
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Background: Although several studies have shown the capacity of mesenchymal stromal cells (MSCs) to repair and regenerate different tissues, the mechanisms underlying these processes are not understood. Long Pentraxin 3 (PTX3) is a multifunctional protein produced by MSCs and other cell subsets upon activation with inflammatory cytokines. PTX3 is involved in innate immunity, inflammation and extracellular matrix deposition.
Aims: In the present study we analyzed the potential role of PTX3 in wound repair process induced by MSCs.
Methods: PTX3 knockout MSCs (PTX3$^{-/-}$MSCs) were collected from bone marrow of PTX3$^{-/-}$ mice. After 3-5 culture passages the expression of surface markers was analyzed by flow cytometry and their osteogenic and adipogenic differentiation capacity was detected by alizarin red O and oil red S staining, respectively. The ability of PTX3$^{-/-}$MSCs to abrogate T cell proliferation was evaluated by co-culturing MSCs and PBMCs previously activated with Phytohaemagglutinin. Finally, equal number of both PTX3$^{-/-}$MSCs and wild type (WT) MSCs were implanted into excisional wounds created by a biopsy punch on the back of allogenic WT and PTX3$^{-/-}$ mice. Wound area was measured up to 14 day and calculated using an image analysis program. The wound specimens were collected at 2, 7 and 14 days and processed for histological analysis.
Results: We demonstrated that PTX3$^{-/-}$ MSCs, similarly to WT MSCs, displayed typical fibroblastoid morphology and expressed common MSC markers such as CD29, CD90, CD44 and ScaI. Moreover, no contamination by hematopoietic and myeloid components (*i.e.* CD45, CD117, CD11b) was evidenced in both samples. When cultured in adipogenic and osteogenic medium PTX3-deficient MSCs confirmed their ability to differentiate into adipocytes and osteoblasts as well as control cells In addition, they drastically decreased the mitogen-induced proliferation of lymphocyte. Importantly, in a mouse model of wound healing, PTX3$^{-/-}$ MSCs showed a highly significant defect in wound closure compared to WT MSCs, at each time point. Histological evaluation of skin samples treated with PTX3$^{-/-}$ MSCs displayed a reduction of the granulation tissue and an excessive accumulation of fibrin at the 2nd day after injury. Accordingly, PTX3$^{-/-}$ MSCs showed a defective ability to degrade the fibrin matrix *in vitro*. Finally, PTX3$^{-/-}$ MSCs failed to close the ulcers in PTX3$^{-/-}$ mice.
Summary and Conclusion: In conclusion**,** we demonstrated that PTX3 deficiency does not alter the phenotype and the capacity of MSCs to differentiate into mesengetic lineages; however, the production of PTX3 represents an essential requirement for MSC ability of enhancing tissue repair.

Thalassemia major

P1154

CHANGES IN ALTERNATIVE IRON OVERLOAD PARAMETERS FOLLOWING 1 YEAR OF DEFERASIROX THERAPY IN PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA: A LONGITUDINAL ANALYSIS FROM THE THALASSA STUDY
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Background: Patients with non-transfusion-dependent thalassemia (NTDT) can develop iron overload, primarily through a combination of ineffective erythropoiesis, anemia and hypoxia, which increases intestinal iron absorption. The effect of iron chelation therapy on iron overload parameters associated with the NTDT phenotype other than liver iron concentration (LIC) and serum ferritin (SF) has not been extensively studied. THALASSA was a 1-year randomized, placebo-controlled Phase II clinical trial of deferasirox that enrolled 166 NTDT patients with iron overload (LIC ≥5 mg Fe/g dw) (Taher *et al. Blood* 2012). Deferasirox significantly reduced iron overload compared with placebo as shown by dose-dependent reductions in LIC and SF. The same large, controlled dataset has now been used to study the effects of deferasirox on other iron overload parameters.
Aims: To assess the effects of deferasirox over 1 year on alternative iron overload parameters associated with the NTDT phenotype.
Methods: Detailed study design and inclusion/exclusion criteria for THALASSA have previously been described. Briefly, patients aged ≥10 years with NTDT and iron overload (LIC ≥5 mg Fe/g dw) and SF levels >300 ng/mL were enrolled. Patients had not received transfusions within 6 months or chelation therapy within 1 month of study entry. Unplanned transfusions during the study were allowed. Data were summarized descriptively from patients with a value at both baseline and Month 12 of deferasirox or placebo and Pearson's correlation analyses were performed between parameter changes at Month 12 from baseline.
Results: 166 patients with β-thalassemia intermedia (n=95), HbE/β-thalassemia (n=49) or αthalassemia (n=22) were randomized to starting deferasirox doses 5 mg/kg/day (n=55) or 10 mg/kg/day (n=55) with matching placebo at 5 or 10 mg/kg/day (n=56). Alternative iron overload parameters assessed at baseline and Month 12, including absolute change from baseline, are summarized in the Table. Mean change from baseline in the deferasirox 5 or 10 mg/kg/day and placebo groups for pre-dose labile plasma iron (LPI; –0.11, –0.11 and 0.03 Units, respectively), non-transferrin-bound iron (NTBI; –1.17, –0.69, –0.11 µmol/L) and transferrin saturation (TfSat; –6.0, –3.0, 3.0%) indicate greater decreases with deferasirox *versus* placebo. There was also an apparent dose-dependent reduction in hepcidin with deferasirox *versus* placebo, in parallel with reductions in LIC and SF; however, change in hepcidin did not correlate linearly with the change in LIC or SF in the deferasirox 5, 10 mg/kg/day or placebo group (LIC: r=–0.0637, r=–0.0340 and r=–0.2239, respectively; SF: r=–0.0506, r=–0.0574 and r=–0.0388, respectively).

Table 1. Summary of mean (SD) alternative iron parameters at baseline and month 12 of deferasirox treatment or placebo

	Deferasirox		Placebo
	5 mg/kg/day (n=55)	10 mg/kg/day (n=55)	5 and 10 mg/kg/day (n=56)
LPI pre-dose (LPI unit)			
Baseline	0.15 (0.54)	0.15 (0.34)	0.30 (0.61)
Month 12	0.12 (0.31)	0.06 (0.13)	0.33 (0.60)
Change from baseline	–0.11 (0.48)	–0.11 (0.30)	0.03 (0.46)
NTBI pre-dose (µmol/L)			
Baseline	1.76 (2.54)	1.74 (2.14)	1.63 (2.32)
Month 12	0.60 (2.10)	1.06 (3.08)	1.54 (2.34)
Change from baseline	–1.17 (1.88)	–0.69 (2.55)	–0.11 (1.66)
TfSat measured (%)			
Baseline	81.2 (16.6)	83.1 (20.8)	81.6 (21.3)
Month 12	75.3 (24.3)	79.3 (23.7)	83.2 (24.0)
Change from baseline	–6.0 (18.0)	–3.0 (27.0)	3.0 (15.0)
Plasma hepcidin (ng/mL)			
Baseline	6.8 (6.3)	7.0 (9.4)	5.4 (3.6)
Month 12	8.1 (5.2)	4.4 (3.1)	5.8 (7.5)
Change from baseline	–0.3 (9.8)	–2.8 (9.1)	–0.03 (9.7)

SD, standard deviation; LPI, labile plasma iron; NTBI, non-transferrin-bound iron; TfSat, transferrin saturation

Summary and Conclusion: Treatment with deferasirox compared with placebo appeared to reduce TfSat and NTBI as well as LPI, a subfraction of NTBI that is redox-active. The observed reductions in TfSat, NTBI and LPI reflect a range

of actions of deferasirox on cellular and plasma iron pools. Correlations between hepcidin and LIC or SF changes were not robust enough to suggest the clinical utility of hepcidin as a predictor of chelation response; the observed changes in hepcidin require further investigation. Changes in the alternative iron parameters studied here over 1 year in patients treated with deferasirox or placebo were modest and subject to considerable variability. While they provide mechanistic insights into the effects of iron chelation with deferasirox, their clinical utility for monitoring patients is not demonstrated in this analysis. Based on these data, LIC and SF remain the most robust markers of response to chelation therapy in NTDT patients.

P1155

QUANTITATIVE T2* MRI FOR BONE MARROW IRON OVERLOAD ASSESSMENT IN A LARGE COHORT OF THALASSEMIA MAJOR PATIENTS

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Background: Multiecho T2* MRI is a well-established technique for cardiac and hepatic iron overload assessment, but there are limited data on its potential to quantify iron in organs other than the liver and heart.
Aims: The aims of this study were to describe for the first time the T2* values of the bone marrow in patients with thalassemia major (TM) and to investigate the correlation between bone marrow T2* and iron deposition in myocardium, liver and spleen.
Methods: 283 TM patients (139 men and 144 women, 8-57 years old, mean age 32.25±8.28 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network underwent MRI (1.5T). For the measurement of iron overload, fast-gradient-echo multiecho T2* sequences were used. Bone marrow T2* values were obtained on a circular regions of interest (ROI) located in the visible body of the first or second lumbar vertebra. The left ventricle was segmented into a 16-segment standardized model and the T2* value on each segment was calculated as well as the global value. In the liver the T2* value was assessed in a single ROI defined in a homogeneous area of the parenchyma and it was converted into liver iron concentration (LIC). Splenic T2* was estimated in a circular ROI located at the periphery of the posterior segment of splenic parenchyma.
Results: Mean bone marrow T2* was 8.42 ± 6.53 ms. Bone marrow T2* values increased with age in a significant manner (R=0.343, P<0.0001) and were significantly lower in females than in males (Figure 1, left). A weak positive association was found between bone marrow and global heart T2* values (R=0.143, P=0.016). Bone marrow T2* values were negatively correlated with LIC values (R=-0.439, P<0.0001) and mean serum ferritin levels (R=-0.582, P<0.0001). 166 patients (58.7%) were splenectomised and splenectomised patients showed significantly higher bone marrow T2* values than non splenectomised patients (9.78± 6.78 ms *vs* 4.61±3.85 ms, P<0.0001; Figure 1, right). The difference remained significant also correcting for the age, significantly higher in splenectomised patients. For the 87/117 patients with the spleen, the splenic T2* value was assessed (mean value: 17.54±11.76 ms). A significant correlation was detected between bone marrow and spleen T2* values (R=0.448, P<0.0001).

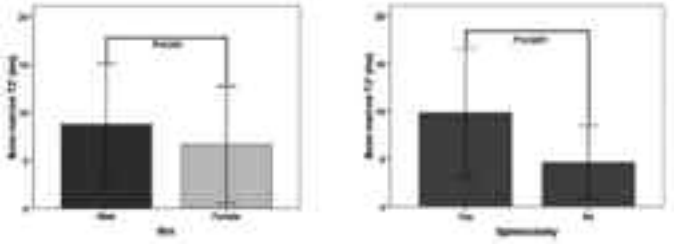

Figure 1.

Summary and Conclusion: In TM patients bone marrow T2* values increased with age. Males showed significantly higher T2* values. Gender-differences of iron deposition were have not been found in other organs, so this difference may be due to the fact that the male sex is associated with severely low bone mass, which can influence the T2* values. However further studies are needed to better characterize the relationship between bone marrow T2* values and bone mineral density (BMD). Bone marrow T2* values were associated with heart, liver and spleen T2* values. Our results are in agreement with that ones of Papakonstantinou et al, who found positive correlations between the degree of hepatic, splenic, and bone marrow siderosis, as expressed by respective R2 values. Splenectomised TM patients showed higher bone marrow T2* values. The bone marrow contains reticuloendothelial cells and, of consequence, it is among the first organs to be affected by iron overload. Splenectomy is normally performed in TM patients with hypersplenism to reduce transfusion requirements.

P1156

BONE MINERAL DENSITY IMPROVEMENT IN PATIENTS WITH THALASSEMIA MAJOR ON LONG-TERM CHELATION THERAPY WITH DEFERASIROX

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Background: Osteopenia and osteoporosis represent significant causes of morbidity among patients with thalassemia major (TM). Abnormalities in bone mineral density (BMD) result in high risk for fractures, which can greatly impact patients' quality of life. To date, despite preventative measures, the progressive loss of bone mass, as part of the natural history of TM, cannot be halted. The once-daily oral iron chelator deferasirox (DFX) was shown to be effective in chelating cardiac and hepatic iron with associated clinical benefits. Furthermore, we observed a low prevalence and progression of endocrine diseases in TM patients on long term therapy with DFX, thus suggesting a possible role of this drug in bone metabolism.
Aims: Our aim was to investigate the effect of long-term therapy with DFX on BMD change in patients with TM.
Methods: This was a retrospective cohort study of TM patients followed up in 4 Italian centers. Inclusion criteria were: continuous treatment with DFX for at least 3 years and ≥ 2 DEXA scans (one at the baseline and at least one post baseline) during the study period. Absolute values in g/cm^2 were used for comparative analyses of femoral and lumbar BMD at the baseline and at the end of study in addition to BMD T- score and laboratory values for calcium and phosphorus. The diagnosis of bone disease was based on the indications of the International Committee for Standards in Bone Measurements (ICSBM).
Results: Forty-nine patients (mean age 39 ± 7.3 years) were enrolled. The mean exposure time to DFX and the mean dose were 4.4 ±1.6 years (range: 3-10) and 25.4 ± 5.2 mg/kg/day (range: 16-33), respectively. The mean calcium value remained stable (from 9.3 ± 0.5 mg/dL to 9.2 ± 0.43 mg/dL, p=0.8) and a significant reduction in mean phosphorus (from 4.5 ± 0.7 mg/dL to 4.1 ± 0.6 mg/dL, p<0.001) occurred. A significant increase in mean lumbar spine BMD g/cm^2 was observed (0.895 ± 0.160 g/cm^2 *versus* 0.972 ± 0.150 g/cm^2 – P=<0.001) and the number of patients with diagnosis of osteoporosis in lumbar spine significantly decreased (22 *versus* 13 patients [P=0.02], OR 0.443). With regard to femoral neck assessment, the mean BMD value (0.777 ± 0.155 g/cm^2 at baseline to 0.794 ± 0.124 g/cm^2 at end of study- P=0.190) and the number of osteoporotic patients (17 *versus* 12 patients [P=0.227], OR 0.611) remained stable with a trend towards improvement in both analyses (Table 1). There was no association between bisphosphonate therapy, calcium or vitamin D supplementation and BMD improvement (P>0.100).

Table 1.

Value	Baseline	End of Study	p
Lumbar spine BMD g/cm²	0.895 ± 0.160	0.972 ± 0.150	<0.001
No. of patients with lumbar osteoporosis	22	13	0.02
Femoral neck BMD g/cm²	0.777 ± 0.155	0.794 ± 0.124	0.19
No. of patients with femoral osteoporosis	17	12	0.22

Summary and Conclusion: This is the first study suggesting a beneficial role of long-term chelation with DFX on bone metabolism in TM. These results are clinically relevant, especially considering the available data on the progressive and relentless "aging" of bone, beginning in early childhood. The maintenance of bone mass, as resulted at femoral neck, appears indeed as a success. Furthermore the significant rise in BMD at lumbar spine without association with bisphosphonate therapy achieves an exciting result. As the pathogenesis of TM osteopathy is multifactorial, it is unclear the exact mechanism by which DFX acts on the bone. It probably works through several factors, such as the

maintenance of a good endocrine function and a better compliance with long-life chelation therapy. A specific effect of DFX on bone metabolism is likely, and it needs to be investigated further.

P1157

DEFERASIROX IN PATIENTS WITH TRANSFUSION-DEPENDENT THALASSEMIA RECEIVING LOW TRANSFUSION INTENSITIES

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Background: Within the broad spectrum of thalassemia disease severity, transfusion requirement varies substantially, due to splenectomy or local transfusion policy, for example. Blood intake can be relatively low even in some patients with transfusion-dependent thalassemia (TDT). Thus, it is important to assess the safety and efficacy of iron chelation therapy with deferasirox in patients with low blood intake in relation to the rest of the TDT population. The threshold of<7 mL packed red blood cells (pRBC)/kg/month (<7 mL) can be used to define low blood intake, based on the transfusion intensity definition in the deferasirox summary of product characteristics.
Aims: To review the safety and efficacy of deferasirox in TDT patients with low transfusion intensity in parallel to the rest of the study population, as assessed by monthly blood intake.
Methods: Patient data from 6 previously published studies of 1- to 5-year duration in TDT patients treated with deferasirox were pooled; dose regimen varied across studies. Blood intake was calculated and categorized for each year at the threshold of 7 mL pRBC/kg/month. Endpoints were adverse events (AEs; year of onset) and selected laboratory parameters (end of each year). Data are summarized descriptively by year, blood intake of that year and dose. Data are presented for low blood intake patients (<7 mL) in parallel to the rest of the population receiving ≥7 mL pRBC/kg/month (≥7 mL).
Results: Among 2102 included β-thalassemia patients, 10–15% had low blood intake (<7 mL) in each year up to 5 years. Mean age was 22.4 years (≥7 mL patients: 16.9 years). Distribution of these patients in Year 1 by deferasirox dose categories (mg/kg/day) was: ≤10: 3.2%; >10–20: 29.4%; >20–30: 53.5%; and >30: 14%, which was similar to the rest of the study population. Serum ferritin (SF) decreased after 5 years of treatment; from 3171 ng/mL (range 322–18,614; n=306) at baseline to 1188 ng/mL (231–11,307; n=45) (≥7 mL patients: 2728 ng/mL [252–32,068; n=1744] to 1147 ng/mL [107–8531; n=205]). Overall, a dose-response effect was apparent (Figure). Most common (≥5%) AEs suspected related to deferasirox in Year 1 were 7.8% increased blood creatinine (≥7 mL: 6.1%), 7.8% diarrhea (6.1%), 5.2% nausea (4.8%) and 6.5% rash (9.0%). In Year 1, 6/11 patients (54.5%) on ≤10 mg/kg/day had a drug-related AE *vs* 11/48 patients (22.9%) on >30 mg/kg/day (≥7 mL: 31.5 *vs* 43.4%, respectively). Serious AEs (SAEs; ≥0.5%) regardless of causality in Year 1 were 1.6% pyrexia (≥7 mL: 0.6%), 1.0% cholelithiasis (0.3%), 0.7% gastroenteritis (0.5%), 0.7% headache (0.1%), 0.7% gastric ulcer (0.1%) and 0.3% abdominal pain (0.5%). Frequency of AEs and SAEs decreased yearly up to 5 years. Creatinine clearance (CrCl) after 1 year was 152.8±47.7 mL/min (–23.1±37.7) (≥7 mL: 146.4±49.0 mL/min [–21.9±37.3]); this initial reduction was followed by increases in subsequent years. After 1 year, absolute CrCl for patients on ≤10 mg/kg/day was 143.8±57.3 mL/min (≥7 mL: 138.4±46.9 mL/min) and 149.1±37.6 mL/min (≥7 mL: 143.8±46.6 mL/min) for patients on >30 mg/kg/day. The greatest alanine aminotransferase (ALT) improvement of –37.4±73.0 to 55.9±33.8 U/L was seen at doses >30 mg/kg/day after 1 year (≥7 mL: –9.7±51.2 to 53.9±52.6 U/L). After 5 years, the mean ALT was 40.4±40.4 U/L (≥7 mL: 30.2±30.6 U/L).

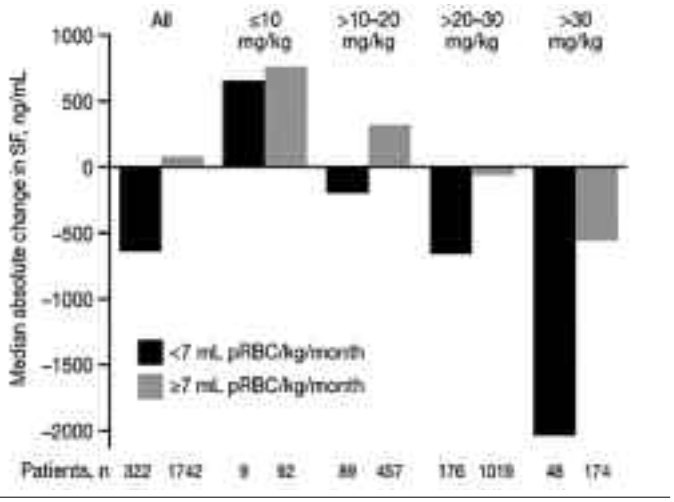

Figure 1.

Summary and Conclusion: Baseline iron burden in patients with low blood intake was substantial and similar to patients receiving ≥7 mL pRBC/kg/month, with the majority of patients receiving >20–30 mg/kg/day deferasirox. High iron burden in low blood intake patients may be due to older age or suboptimal prior chelation. Deferasirox was effective for iron removal in all patients over the long term, with dose adjustment to trends in iron load likely contributing to the degree of iron reduction. While this analysis is limited by heterogeneity of included studies and retrospective categorization of patients, results indicate that when patients are dosed according to the range of iron burden observed here, the 5-year safety profile of deferasirox is consistent across the spectrum of transfusion intensity.

P1158

18 MONTHS DATA OF A RANDOMIZED CONTROLLED TRIAL OF COMBINED DEFERIPRONE (DFP) AND DEFERASIROX (DFX) VERSUS COMBINED DEFERIPRONE AND DEFEROXAMINE (DFO), IN YOUNG B-THALASSEMIA MAJOR

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Background: B-Thalassemia major (B-TM) patients with severe iron overload may require a more efficient therapy for reduction of iron burden. Combined chelation using deferoxamine (DFO) and deferiprone (DFP) is widely used, but the inconvenience of parenteral administration of DFO reduces the effectiveness of this regimen. Minimal data are available on safety and efficacy of the combined two orally active chelators.
Aims: Prospective randomized comparison of safety, efficacy, compliance, treatment satisfaction, and quality of life (QoL) associated with two combination chelation regimens: DFP and DFO *versus* DFP and deferasirox (DFX).
Methods: An open-label trial registered as (NCT01511848) conducted at 2 treatment centers in 96 patients aged ≤18 years with β-TM and severe iron overload (serum ferritin (SF)>2500 µg/ L on chelation monotherapy, with 50% uptrend over last year). Patients were randomly allocated to one of two 18-month treatment regimens. All patients received DFP on 75 mg/kg/day. Those in Arm 1 additionally received DFO on 40 mg/kg/d, while those in Arm 2 additionally received DFX on 20 mg/kg/d. The primary efficacy endpoints were the difference between treatment groups in the change from baseline to 18 months of SF, liver iron concentration (LIC), and cardiac MRI. Safety endpoint was the occurrence of serious adverse events (SAEs). Secondary efficacy endpoints were the changes in QoL, compliance and patient-reported outcomes (PROs).
Results: All the studied patients were comparable regarding baseline clinical and hematological parameters. Observed changes in each arm during therapy were compared using two-way ANOVA for repeated measures. **In DFP and DFO** patients mean SF at 18 months were significantly lower compared to 6, 12 months (P=0.002) and baseline (P=0.01) with percentage of changes of -11.46% and -24.05% respectively. Mean LIC levels at 18 months were significantly lower compared to 6, 12 months (P=0.01) and baseline(P=0.01) with percentage of changes of -18.65% and -39% respectively. Significantly higher mean cardiac T2* at 18 months compared to 6,12 months (P=0.003) and baseline (P=0.002) with percentage of changes + 12.08% and +19.52% respectively. **In DFP and DFX** patients mean serum SF at 18 months were significantly lower compared to 6 and 12 months (P=0.01) and baseline (P=0.002) with percentage of changes of - 17.8% and -36.59% respectively. Mean LIC at 18 months were significantly lower compared to 6 months (P=0.001) and baseline (P=0.003) with percentage of changes of -13.24% and -27.98% respectively. Significantly higher mean cardiac T2* at 18 months

compared to 6 months (P=0.01) and baseline (P=0.004) with percentage of changes + 17.8% and +39.5% respectively. Patients receiving DFP+DFX had significantly lower SF and higher global heart T2* at study end compared to DFP+DFO patients(P=0.001,p=0.002). QoL was significantly improved in both arms while treatment compliance and patients satisfaction were significantly higher in arm 2 compared to arm 1(p<0.001). No SAEs necessitating discontinuation or interruption of therapy in both arms.
Summary and Conclusion: No mortality was reported in this randomized trial. Both forms of combination therapy were effective in reducing iron overload, DFP and DFX patients showed a higher decline in SF, greater improvement in cardiac T2*,higher treatment satisfaction and better compliance than did DFP and DFO patients, with no increased toxicity.

P1159

HIGH PREVALENCE, EXTENT AND SEVERITY OF SPINAL DEGENERATIVE CHANGES IN ADULT THALASSAEMIA PATIENTS PRESENTING WITH BACK PAIN

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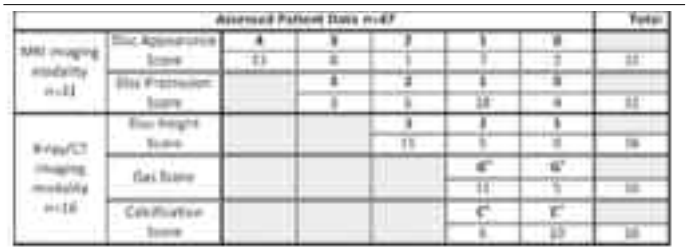
Background: Increasing numbers of adult thalassaemia major (TM) patients present with chronic back or joint pain. Imaging for this shows an increased frequency of severe degenerative changes in spine and a resulting reduction in the quality of life in the most severely affected patients The degenerative changes are thought to be multifactorial with iron chelation, osteoporosis, vitamin D deficiency all thought to play a role.
Aims: To assess the severity of the degenerative changes on imaging and assess factors that may predispose to these in comparison to patients who had not warranted imaging.
Methods: An audit was conducted on the number of adult TM patients routinely transfused at Whittington Hospital who had undergone spinal imaging for back pain. All images were rescored with a standardised scoring system (1) and Table 1. Data on chelation history and co-morbidities including hypogonadism, hypoparathyroidism, vitamin D deficiency and osteoporosis were also collected from medical notes and pathology reports.
Results: Of 106 patient records reviewed, 47 had undergone imaging of the spine because of back pain at a median age of 45 years. The median age of the remaining 59 patients who had not required investigation was 35 years. Of the imaged cohort, 31 had been assessed using MRI and 16 by means of CT or plain x-rays. Of the MRI patients, 13/31 (42%) were found to have the most severe degree of disc degeneration with respect to disc appearance (grade 4) often at multiple levels. In terms of disc displacement, 18/31 had grade 1 change (annular disc bulge) and 9/31 patients had focal disc protrusion or extrusion (grade 2 and 3, respectively). Moderate to marked reduction in disc height (grade 2 and 3) was demonstrated in all 16 patients who had undergone plain x-rays or CT. The prevalence of degenerative spinal changes among the entire cohort of 106 patients was highest in patients >40 years, with 60% of this age group being affected; however, these changes were also evident in 35% of patients aged<30 years and 35% of those aged between 31-40 years. 32% of affected patients received physiotherapy, 19% received spinal and/or nerve root injections and 9% underwent surgery. All patients with signs of degenerative disease were previously chelated with desferrioxamine and 87% had also received deferiprone. 11% had documented skeletal complications with desferrioxamine, and 19% had a history of knee pain with deferiprone.
When comparing imaged patients to those who were not imaged, imaged cohort 79% had osteoporosis *versus* 70% not imaged; 79% were hypogonadal *versus* 66%, 83% were vitamin D deficient *versus* 95% and 21% had hypoparathyroidism *versus* 15%. 81% were on bisphosphonates *versus* 66%. Surprisingly, 17% of these patients had concurrent extramedullary haematopoiesis despite optimal transfusion.

Table 1.



Summary and Conclusion: These findings show that multi-level degenerative disc disease was present in 94% of the patients imaged and was present in all age groups with increasing frequency in those over the age of 40 years. These changes are more common in patients with osteoporosis, hypogonadism and hypoparathyroidism, suggesting that these may be contributory factors. These along with iron chelation and bone protection therapy may have a role in the early onset and increased prevalence of degenerative joint disease. 1. Desigan S, Hall-Craggs MA, Ho CP, Eliahoo J, Porter JB. Degenerative disc disease as a cause of back pain in the thalassaemic population: a case-control study using MRI and plain radiographs. Skeletal Radiol. 2006;35(2):95-102.

P1160

PEDIATRIC THALASSEMIA MAJOR PATIENTS : PROSPECTIVE COMPARISON OF CHELATION TREATMENTS

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Background: Pathophisiology and timing of body iron burden in pediatric thalassemia major (TM) patients is so far not well known. Moreover, there are no prospective studies comparing the effectiveness of the three iron chelators commercially available in preventing or decreasing iron overloading in the heart and liver.
Aims: The aim of this study was to evaluate prospectively the effectiveness of 3 iron chelators in monotherapy in pediatric TM patients by quantitative magnetic resonance imaging (MRI) in the heart and in the liver over a follow up at 18 months.
Methods: Among the first 1545 TM patients consecutively enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network, we evaluated the fifty-two pediatric patients (31 male, mean age 14.06 ± 2.77–range 7.1-17.8 years) who had been received only one chelator in monotherapies between the two MRI scans. Cardiac and liver iron overload was determined as variation of the MRI signal in multiecho T2* sequences. Biventricular function parameters were quantitatively evaluated by SSFP cine images. Due to the low sample size no inter-treatment comparisons were performed and intra-treatment comparison was performed only in the DFX group.
Results: Three groups of patients were identified: 6 patients (mean age 10.02 ± 2.17 years) treated with desferioxamine (DFO – mean dosage 43.67± 6,77 mg/kg/die**),** 7 patients (mean age 15.51 ± 1.73 years) treated with deferiprone (DFP– mean dosage 75± 9,23 mg/kg/die) and 34 patients (mean age 14.49 ± 2.66) treated with deferasirox (DFX – mean dosage 27,17 ± 6,55 mg/kg/die). Compliance was excellent/good in all three groups. At baseline in DFO and in DFP group no patient showed a global heart T2* value lower than 20 ms. In all 3 groups all patients who showed no cardiac iron overload at baseline maintained at follow-up (FU) the same status. At baseline in DFX group 6 patients (17.6%) had pathological heart T2* values; at FU 4 pts (66.7%) showed normal global heart T2* values (≥ 20 ms), while 2 pts (33.3%) maintained pathological global heart T2* values. Patients in DFP had higher global heart T2* values at baseline (DFP 35.25 ± 4.98 ms >DFX 33.18 ± 5.47 ms >DFO 31.86 ± 10.53 ms) and at follow-up (DFP 39.45 ± 6.07 ms >DFX 36.46 ± 5.84 ms >DFO 33.65 ± 7.88 ms). In the DFO group at baseline 1 patient showed pathological LVEF and he recovered at the follow up. In the DFP group at baseline 2 patients showed pathological LVEF and both recovered at the follow up. In the DFX group at baseline 3 patients showed pathological LVEF and 2 recovered at the follow up, conversely 7 patients with normal LVEF at baseline showed pathological LVEF at the follow up. In the DFO group the percentage of patients with MRI LIC >3 mg/g/dw went up from 83% to 100%. In the DFP group all patients showed MRI LIC >3 mg/g/dw at baseline and they maintained this status at the FU. In the DFX group the percentage of patients with MRI LIC >3 mg/g/dw went down from 70% to 50%. The MRI LIC mean difference was -2.00 ± 4.53 mg/g/dw (P=0.004).

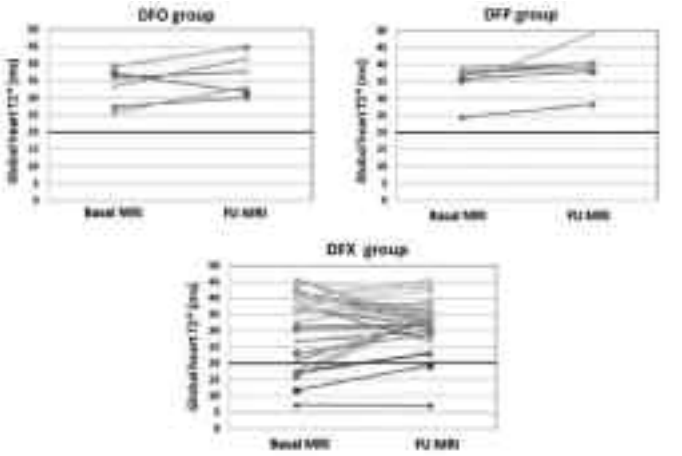

Figure 1.

Summary and Conclusion: This longitudinal analysis confirms significant rate of iron overload even in very young TM population, in particular in the liver. In this population, DFP seems to be more effective in heart with a concordant positive effect on the global systolic function. Conversely, DFX seems to be more effective in the liver. However, further prospective trials are needed on larger study population to confirm the data.

P1161

TISSUE IRON DEPOSITION AND MARKERS OF GLUCOSE DYSREGULATION IN THALASSEMIA MAJOR

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Background: Impaired glucose tolerance (IGT) and diabetes mellitus (DM) remain a significant problem for thalassemia major (TM). Iron toxicity induced insulin resistance and decreased insulin secretion contribute to glucose dysregulation (GD) in TM.
Aims: The aim of this study is to evaluate somatic iron depositions and biochemical markers of glucose metabolism in TM for having an insight for early identification of patients with increased risk for GD.
Methods: TM patients aged ≥8 years and without insulin dependent DM were invited to participate to the study. Homeostatic model assessment and oral glucose tolerance test (OGTT) were performed. MRI was used to assess hepatic, pancreatic and cardiac iron burdens. Cardiac T2*<20 msec and pancreas R2*>50 Hz was considered as cardiac and pancreatic iron loading. Glucose metabolism assessed by OGTT classified according to WHO criteria. HOMA-IR ≥2.5, insulinogenic index (II)<0.4 and disposition index (DI)<1 was associated with insulin resistance, decline in insulin secretion and impaired beta cell function respectively. Relationship between iron stores and markers of glucose metabolism was analyzed by using correlation and sample T test procedures. Differences were considered statistically significant at a level of p<0.05.
Results: The patient cohort (24 male, 18 female) ranged from 10–33 years of age (19.7±6.4) and was on regular transfusion for 18±7.5 years and on chelation therapy. Family history indicated DM in 42% of the cohort. Patients had ferritin (SF) of 1972±1968 ng/ml (291-8757), liver iron (LIC) of 6.8 ±9.1 mg/g (0.4-38.7) and cardiac T2* of 23±6.7 msec (6.2-33.7). Normal liver (<3.2mg/g) and cardiac (T2*>20 msec) iron was observed in 50% and 70% of patients, respectively. LIC was 3.2-7mg/g in 17.5%, 7-15 mg/g in 20% and >15 mg/g in 12.5% of the cohort. All patients had normal cardiac function and none had chronic liver disease. The patient cohort had BMI of 19.9 ±3.2 kg/m^2, only 2 patients were overweight. OGTT revealed IGT in 8 (18.5%) and DM in 3 (7%) patients. The patients with GD were significantly older and had a higher prevalence for family history of DM than those of normal glucose metabolism. Most patients with GD (8/11, 73%) had decline in insulin secretion and the rest of them (3/11, 27%) had insulin resistance. All patients with IGT and DM had a pancreas R2*>50Hz. Although, no patient with normal OGTT had insulin resistance, 13/25 (50%) had decline in insulin secretion in whom only 3 had a pancreas R2*>50Hz. In this cohort, 6 of 25 (25%) with normal insulin secretion had a pancreas R2*>50Hz. The cohort with abnormal OGTT showed a significantly higher pancreas R2* (p=0.001) than those of normal OGTT. Mean SF, LIC and cardiac T2* did not differ significantly between patients with abnormal and normal OGTT. None of the patients with pancreas R2*<50 Hz had a cardiac T2*<20ms. Although, LIC showed a negative correlation with cardiac T2* (r=-0.47, p<0.005), there was no significant correlation between LIC and pancreas R2*. Patients' age showed a positive correlation with pancreas R2* (r=0.43, p<0.01) but no correlation with LIC or cardiac T2*.
Summary and Conclusion: Age and family history in TM have an impact on GD. This study confirmed that impaired insulin secretion was more prevalent than insulin resistance and pancreas R2* provide useful information for predicting risk for GD in TM. Patients with pancreas R2*>50 Hz but still preserve normal insulin secretion may have benefit from intensive iron chelation for reserving pancreatic function. Prospective follow up is required in patients with impaired insulin secretion while pancreas R2*<50 Hz to determine its functional significance on developing GD.

P1162

PRESENCE OF THE IVS1-6 MUTATED ALLELE PREDISPOSES PATIENTS WITH MAJOR B THALASSAEMIA TO EXTRAMEDULLARY HAEMATOPOISES

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Background: Since the initiation of regular transfusion programmes from an early age for all major thalassaemic patients, extramedullary haematopoises (EMH) has not been considered a clinical issue any more. In contrast, haematopoises at sites other than the bone marrow is sporadically described only within the intermedia thalassaemia cohort.
Aims: To record the incidence of EMH in chronically transfused major thalassaemic patients followed at our institution and investigate for possible risk factors associated with its occurrence.
Methods: We retrospectively analysed the medical notes and images of 104 major thalassaemic patients (45 male /59 female) with a median age of 32(19-47) years followed in our comprehensive care unit for a period of 30(18-45) years. Time point for the evaluation of all relevant demographic and biological factors was either the first positive or the last negative MRI scan for patients with or without EMH respectively. Age at first transfusion was 1.3(0.1-6.2) years and are regularly transfused (2 units RBC/15days) in keeping with current recommendations and targeting pre-transfusion Hb above 9-10mg/dl. 35/104 underwent splenectomy at the age of 17(8-27) years and 66 had cleaved HCV with no active HCV case in the present. In terms of ABO/Rh blood group 45 were A, 16 O, 8 B, 21 AB while 95 were Rh/D positive with 11/104 being alloimmunized against RBC antigens. We applied MRI T2* for the evaluation of liver and cardiac iron accumulation and patients were grouped in terms of severity based on readings. Chelation therapy was deferasirox for 58 patients, 14 received Desferrioxamine (DFO), 9 Ferriprox and 23 combination of DFO+Ferriprox. Ferritin levels were 1580(195-6320) ng/ml. Within the female group 13/59 had at least one successful pregnancy. With regard to genotype patients were categorized as follows: 19 IVS 1-110/IVS1-110, 12 IVS 1-110/cd 39, 8 cd39/cd39, 34 IVS 1-110/other, 22 IVS 1-6/other, 24 various and 7 unknown. We used the annual routine MRI scanning for the detection of possible EMH sites.
Results: EMH was revealed in 15/104(14%) patients, 10 female/5 male, aged 30(23-39) years. In all cases EMH was an incidental finding across the spinal cord without any clinical signs of compression or inadequacy to retain pre-transfusion Hb levels. The median period from last negative to first positive MRI scan was 2.6 (1-5) years and the follow up period since the first EMH detection is 2(0.2-2.7) years without signs of radiological or clinical progression. In a univariate analysis we found that the presence of IVS 1-6 either in homozygosity or compound heterozygosity was significantly related with the development of EMH (p<0.05). None of all the other demographic and biological factors was found to be related with the presence of EMH. Of note, splenectomy despite what was expected was not correlated with EMH.
Summary and Conclusion: To date, we first record a profound incidence of asymptomatic extramedullary haematopoiesis in well transfused major thalassaemic patients, detected mainly within the last 5 years. Whether this finding is related to amelioration in imaging techniques or truly constitutes a new emerging phenomenon genetically determined, particularly for patients carrying the IVS1-6 mutation, merits further investigation.

P1163

BONE MINERALIZATION AND ENDOCRINE DISEASE IN PATIENTS WITH BETA-THALASSEMIA INTERMEDIA (TI)

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Background: Osteopathy and endocrine disease are well-studied complications in β-thalassemia Major (β-TM), while prevalence in β-thalassemia intermedia (β-TI), a non-transfusion dependent thalassemia (NTDT), is less known.
Aims: To retrospectively evaluate bone mineralization, endocrine function and thalassemia-related factors in a series of TI patients (pts).
Methods: Seventy TI patients (37 M, 33 F; 41±12 years) were evaluated.

Among them, 37 (52%) had never been transfused, 33 (66%) had been transfused (24 [34.2%] occasionally, 9 [12.8%] regularly every 90 to 120 days); 34 (48.5%) had undergone splenectomy, 39 (55.7%) were on chelating drugs, and 11 (15.7%) were on hydroxycarbamide. The patients were scanned by dual energy X-ray absorptiometry at vertebral and femoral sites; in addition, blood and urine tests were performed to evaluate the following endocrine glands: pituitary, thyroid, parathyroids, gonads, adrenals and pancreas. Univariate and multivariate statistical analysis were used to analyze the relationships between bone mineralization and the following variables: demographic, clinical, hematological, and endocrine-metabolic data; iron status with liver iron concentration (LIC from T2*); and treatment.
Results: The prevalence of bone demineralization was high in the β-TI patients studied; conversely, the prevalence of endocrine defects was no different from that expected in the general population.

Table 1.

T-score V*†	Z-score V§+	T-score FT*	Z-score FT§	T-score FC†	Z-score FC+
-2.0±1.3	-1.7±1.3	-1.0±1.0	-0.8±1.0	-1.1±1.0	-0.7±1.0

***** *p<0.01;* † *p<0.01;* § *p<0.01,* + *p<0.01*

As shown in the table, bone mineralization indices were significantly lower in the lumbar spine than at femoral sites; there were no gender differences. Osteopenia was found in 29 (41%) pts and osteoporosis in 25 (36%), with selective vertebral localization in 19 (27%), and femoral localization in 1 (1%); both sites were affected in 5 pts (7%). The most significant correlation found with multivariate analysis was between bone mineralization and splenectomy, with splenectomized pts showing a worse bone status at all sites. A significant correlation was also found between bone mineral density indices and iron chelation therapy, irrespective of the drug used. The presence of a more favourable bone condition in non-chelated pts may be due to better iron status. Among the pts with LIC >3, significant differences were found in spine mineralization, BMD, T-score and Z-score lower in the group with LIC >7 *vs* those with LIC between 3 and 7. By contrast, modulation of fetal haemoglobin production with hydroxycarbamide appeared uncorrelated to bone condition.
Summary and Conclusion: from the present study, osteopathy appears to be an important complication of β-TI, with major involvement of axial bone. On the other hand, the high prevalence of endocrinopathies well described in β-TM was not confirmed in our pts with β-TI. As both the severity of anemia and the clinical sequelae span a broad spectrum in this disease, the main factors related to the risk of developing osteopathy and/or endocrinopathy should be better clarified through clinical studies. Close surveillance, preventive intervention and early management are essential to secure bone health and improve quality of life in this NTDT.

P1164

INVESTIGATION OF RENAL FUNCTION DISORDERS IN PATIENTS WITH THALASSEMIA AND THEIR RELATION WITH IRON OVERLOAD

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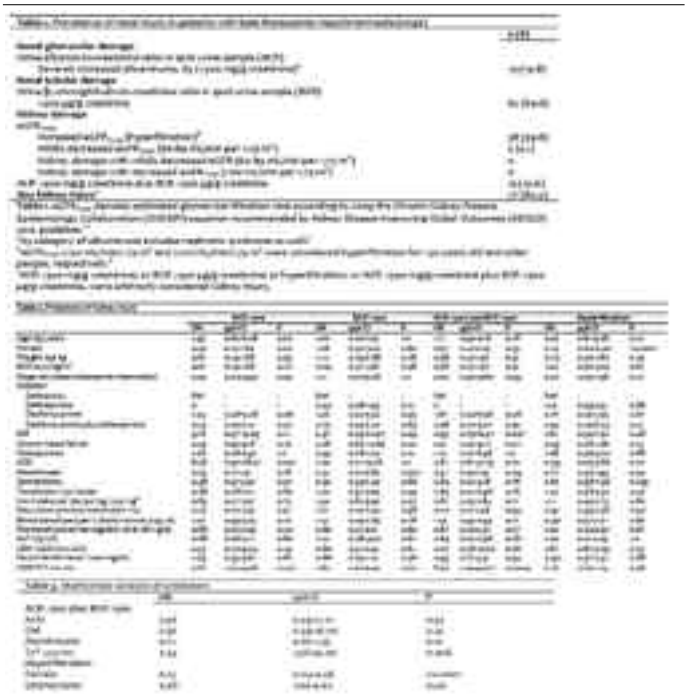
Background: Thalassemia major is an inherited disease that is characterised with defective globulin chains of hemoglobin molecule and it causes tissue and organ damage by ineffective erythropoiesis, chronical hypoxia, transfusion dependency and oxidative stress.
Aims: We planned to evaluate the organ involvement in thalassemia, primarily kidney.
Methods: 96 individuals with diagnosis of thalassemia major and transfusion dependent thalassemia intermedia were included. All of the patients were adults and duration of transfusion dependency was over 10 years. In addition to routine examination, spot urine beta-2 microglobulin levels were measured.
Results: Among 96 thalassemic patients prevalence of any kidney injury was 80,2% (Table 1). When kidney injury is evaluated as, Renal glomerular damage, Renal tubular damage and Kidney damage; Renal tubular damage is the overriding form between them (%64,6) (Table 1). According to our study, predictors of Renal glomerular damage, Renal tubular damage and Kidney damage and hyperfiltration were; Cardiac T2* value ≤20 ms [Odds ratio (OR): 3.87, 95% Confidence Interval (95% CI): 1.10-13.46, p=0.013]; alendronate medication [OR: 0.31, 95% CI: 0.12-0.80, p=0.007]; female gender [OR: 0.13, 95% CI: 0.04-0.38, p<0.0001] and splenectomy [OR: 3.08, 95% CI: 1.04-9.93, p=0.04] respectively (Table 2-3).

Table 1.



Summary and Conclusion: Our study is one of the few studies which evaluates renal involvement in thalassemia in adults. It is concluded that, renal damage prevalence is higher in thalassemic patients. It was observed that, degree of cardiac iron overload, male gender, splenectomy and not to use alendronate increase the risk of renal involvement. At the end of this study prevalence of kidney injury was found higher than previous studies but exact interpretation could not be made about physiopathology of possible causes. Therefore in thalassemia, new studies with large groups of patients are needed to evaluate kidney injury with other physiopathological mechanisms aside from iron overload such as oxidative stress.
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P1165

ASSOCIATION OF OR51B2 SEQUENCE VARIATIONS WITH RESPONSE TO HYDROXYUREA THERAPY IN IRANIAN PATIENTS AFFECTED WITH BETA-THALASSEMIA INTERMEDIA

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Background: Enhanced fetal hemoglobin (HbF) production in β-globin gene disorders improves the clinical symptoms of the underlying disease such as β-thalassemia intermedia (β-TI). Hydroxyurea (HU) activates γ-globin gene, results in increasing of HbF synthesis. The olfactory receptors (ORs) are members of a G-protein-coupled receptors (GPCRs) large family, which arising from single coding-exon genes. Elements of the ORs gene cluster might play a regulatory role in γ-globin gene expression.
Aims: We aimed to assess the possible relationship between response to HU and *OR51B2* gene sequence variations in Iranian patients affected with β-TI.
Methods: In this cross sectional study 100 patients affected with β-TI from Southern Iran were randomly enrolled between February 2012 and October 2013. All patients were treated with HU. Based on the need to blood transfusion and hemoglobin level, our patients were divided into two groups: good responder and poor responder.
Results: We compared demographic and clinical variables between good and poor responders. Only Nucleated Red Blood Cells showed a significant association with response to HU (P=0.045). From all evaluated SNPs, only rs10837814 SNP in *OR51B2* gene was significantly associated with response

to HU, as a significantly higher frequency of CT genotype (89.3%) was observed in patients with good response compared with CC (67.3%) and TT genotype (88.2%) (P=0.038). We also find three novel nucleotide variations (-665 (A→C), -1301 (T→G),-1199 delA) who had good response to HU therapy in our study.
Summary and Conclusion: It seems that rs10837814 SNP in OR51B2 gene probably can be used as a marker for response to HU in β-TI patients, but further studies by larger study groups is needed for more evaluation in future.

P1166

LEFT VENTRICULAR GLOBAL FUNCTION INDEX BY CMR IS MORE STRONGLY ASSOCIATED TO DIFFERENT PATTERNS OF MYOCARDIAL IRON OVERLOAD THAN THE GLOBAL SYSTOLIC FUNCTION

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Background: The Cardiovascular Magnetic Resonance by the multislice multiecho T2* technique allows to detect different patterns of myocardial iron overload (MIO). Moreover, the analysis of cine images allows the quantification of the left ventricular global function index (LVGFI) that combines the LV stroke volume, endsystolic and end-diastolic volumes, as well as LV mass. A LVGFI<37% was shown to be strongly predictive of cardiovascular events.
Aims: We aimed to verify the association between different patterns of MIO and the LVGFI *vs* the LV ejection fraction (EF) in thalassemia major (TM) patients.
Methods: We considered 812 TM patients (391 M, 30.4±8.6 years), consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network. The T2* value in all the 16 cardiac segments was evaluated. LVGFI and LVEF were quantitatively evaluated by SSFP cine images. Heart dysfunction was diagnosed in presence of LVEF<2 standard deviations (SD) from the mean value normalized to age and gender.
Results: We identified 4 groups of patients: 138 with homogeneous MIO (all segments with T2*<20 ms), 97 with heterogeneous MIO (some segments with T2*<20 ms, others with T2*≥20 ms) and significant global heart iron (global heart T2*<20 ms), 238 with heterogeneous MIO and no significant global heart iron, and 339 with no MIO (all segments with T2*≥20 ms). The mean LVFGI was significantly different among the 4 groups (Figure, up). Compared to the group with no MIO, all the other 3 groups were significantly more likely to have a LVGFI<37%, conversely, only the groups with homogeneous MIO and with heterogeneous MIO and significant global heart iron showed a significant higher risk to have LV dysfunction. For all groups the association between different patterns of MIO with a LVGFI<37%, was stronger than the association with a LV dysfunction (Figure, bottom).

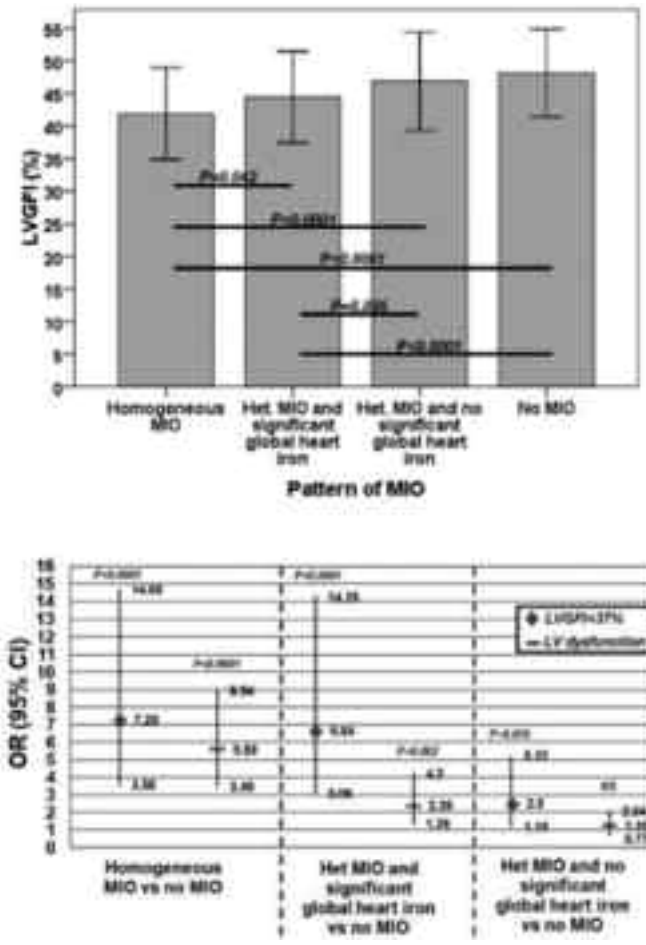

Figure 1.

Summary and Conclusion: LVGFI is a functional parameter integrating structural as well as mechanical behaviour more strongly associated to different patterns of MIO than the LVEF. Thus, a LVGFI<37% could better identify a significant higher risk of adverse cardiovascular events beyond heart failure in iron loaded patients.

P1167

PROSPECTIVE MRI STUDY IN PEDIATRIC THALASSEMIA MAJOR (TM) PATIENTS IN THE MIOT NETWORK: A TOOL TO STRENGTHEN MEDICAL DECISIONS

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Background: Iron overload, due to regular blood transfusions, leads to significant morbidity and mortality in TM patients. Recent MRI studies have demonstrated significant hepatic, cardiac and pancreatic iron overload even in TM patients less than 2 years, contradicting historical belief that children younger than 10 years would be at low risk for developing systemic iron loading. MRI deployment alongside appropriate modification of patients' and doctors' approach to chelation therapy were shown to be the most likely cause for reduction in morbidity and mortality in adult TM patients but to date very little is known about the evolution of iron overload in children with TM and the impact of MRI implementation on pediatric hematologists' behavior.
Aims: Our aim was to investigate the effect of MRI deployment on the longitudinal evolution of iron overload and on the alteration in management of chelation therapy in a large cohort of TM children.
Methods: This was a prospective MRI survey of TM children (<18 years) enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) Network who performed a baseline and a follow-up (18 +/- 3 months) MRI scan. Myocardial

and hepatic iron burdens were quantified by the T2* technique and liver T2* was converted into liver iron concentration (LIC). Clinical and laboratory details were collected in a web based database for each patient included into the study.
Results: At the baseline mean age of the 68 TM children (42 M) was 13.74 ± 3.09 years. Patients started regular transfusions and chelation therapy at a mean age of 1.57 ± 0.82 and 2.95 ± 1.08 years, respectively. Table 1 shows the evolution of different iron overload risk classes between the baseline and the FU. The percentage of liver iron overloaded patients who achieved a normal LIC value was 18.5% *versus* 35.7% of patients with baseline normal LIC who switched to pathological LIC at the FU. Regarding cardiac iron burden, 25% of overloaded patients achieved a normal T2* value at the FU *versus* 1.9% of patients who newly developed cardiac iron overload. Adjustments in chelation therapy were made only in 28% of patients with hepatic iron overload but in 56% of patients with cardiac iron overload at baseline (P=0.105 and P<0.001, respectively).

Table 1.

		FU LIC			
		<3 mg/g dw	3-7 mg/g dw	7-15 mg/g dw	≥15 mg/g dw
Baseline LIC	<3 mg/g dw (N=14)	9	4	1	0
	3-7 mg/g dw (N=21)	6	10	4	1
	7-15 mg/g dw (N=15)	3	5	6	1
	≥15 mg/g dw (N=18)	0	3	2	13
Total at the FU		18	22	13	15
		FU global heart T2*			
		≥20 ms	10-20 ms	<10 ms	
Baseline global heart T2*	≥ 20 (N=52)	51	1	0	
	10-20 ms (N=12)	4	7	1	
	< 10 ms (N=4)	0	3	1	
Total at the FU		55	11	2	

Summary and Conclusion: No longitudinal study on liver and heart iron in TM children are available in literature. This analysis confirms significant rate of liver and cardiac iron overload even in very young children. This could reflect a conservative approach to chelation therapy in pediatric population. Clinicians tend to be more worried for cardiac than hepatic iron overload and they adjust chelation therapy more frequently if cardiac iron accumulation is present. The data can support a chelation therapy approach timely tailored to iron burden in order to achieve a safe level and avoid detrimental evolution.

P1168

THE ROLE OF LEFT VENTRICULAR GLOBAL FUNCTION INDEX FOR THE PREDICTION OF CARDIAC COMPLICATIONS IN THALASSEMIA MAJOR

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Background: Cardiac complications are one of the main causes of death in thalassemia major (TM) patients. Recently, the MESA study showed the strong predictive value of the left ventricular global function index (LVGFI) evaluated by cardiovascular magnetic resonance (CMR) in the prediction of the of cardiovascular events. LVGFI is a functional parameter integrating structural as well as mechanical behaviour derived from the analysis of cine SSFP images.
Aims: We evaluated the predictive value of LVGFI and other CMR parameters for cardiac complications in thalassemia major (TM).
Methods: We followed prospectively 537 white TM patients enrolled in the MIOT network. Fifty patients were excluded from the analysis because a cardiac complication was present at the time of the first CMR. All prognostic variables associated with the outcome at the univariate Cox model were placed in the multivariate model and were ruled out if they did not significantly improve the adjustment.
Results: At baseline the mean age was 29.5±9.0 years and 222 patients were males. The mean follow-up time was 58±18 months. After the first CMR only the 37.8% of the patients did not change the chelation regimen or the frequency/dosage. We recorded 40 cardiac complications: 19 episodes of HF, 19 arrhythmias, all supraventicular hyperhyperkinetic, and 2 pulmonary hypertensions. A LVGFI<37% was a significant univariate prognosticator of cardiac complications (HR=3.42, 95%CI=1.56-7.52, P=0.002). The other significant univariate prognosticators were heart iron, atrial dilatation, ventricular dysfunction evaluated by the left ventricular ejection fraction (LVEF), and myocardial fibrosis. Serum ferritin and liver iron by T2* MR were not predictive factors for cardiac complications. In the multivariate analysis the independent predictive factors were a LVGFI<37% (HR=3.08, 95%CI=1.32-7.20, P=0.010), an homogeneous pattern of MIO (compared to no MIO) (HR=3.95, 95%CI=1.56-10.04, P=0.001), and myocardial fibrosis (HR=3.45, 95%CI=1.68-7.09, P=0.001).
Summary and Conclusion: We detected few cardiac events thanks to a CMR-guided, patient-specific adjustment of the chelation therapy. A LVGFI<37%, severe and homogeneous MIO, and myocardial fibrosis identify patients at high risk of cardiac complications globally considered. Importantly, the dysfunction evaluated by the LVEF lose its predictive value for cardiac complications when included in a multivariate model.

P1169

RIGHT VENTRICULAR WALL MOTION ABNORMALITIES IN PATIENTS WITH THALASSEMIA MAJOR AND INTERMEDIA

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Background: The role of the right ventricle (RV) is gaining ground in thalassemia patients and the magnetic resonance imaging (MRI) is the gold-standard for the study of its anatomy and function.
Aims: In this study we investigated for the first time the relationship between RV motion abnormalities, volumes and function in both thalassemia major (TM) and thalassemia intermedia (TI) patients.
Methods: CMR was performed in 1369 TM patients (537 males; 30.9±8.9 years) and 266 TI patients (38.5±11.5 years) enrolled in the Myocardial Iron Overload in Thalassemia Network. Cine images were acquired to evaluate wall motion and to quantify RV volumes and ejection fraction (EF).
Results: The presence of RV motion abnormalities was comparable between TM and TI patients (3.0% *vs* 4.5%; P=0.201). Out of the 41 TM patients with abnormal RV motion, 35 were hypokinetic, 5 were dyskinetic and 1 was akynetic. Out of the 12 TI patients with abnormal RV motion, 8 were hypokinetic and 4 were dyskinetic. Table 1 and Table 2 show the comparison between TM patients with normal and abnormal RV motion and between TI patients with normal and abnormal RV motion, respectively. TM patients with abnormal RV motion were older and they were more frequently males. Regarless by the form of thalassemia, right volumes were significantly higher in patients with abnormal RV motion while the EF was significantly lower.

Table 1. Comparison between TM patients with abnormal and normal RV motion

	Abnormal RV motion	Normal RV motion	P
Age	34.7 ± 5.8	30.9 ± 8.9	0.005
Sex (M/F)	31.10	633/695	<0.0001
RV EDVI (ml/m2)	109.0 ± 45.9	82.7 ± 16.9	<0.0001
RV ESVI (ml/m2)	60.7 ± 28.9	32.1 ± 11.2	<0.0001
RV EF (%)	45.2 ± 10.1	61.5 ± 7.7	<0.0001

Table 2. Comparison between TI patients with abnormal and normal RV motion

	Abnormal RV motion	Normal RV motion	P
Age (years)	41.3 ± 9.2	38.4 ± 11.6	0.307
Sex (M/F)	7/5	128/126	0.591
RV EDVI (ml/m2)	111.6 ± 35.3	83.4 ± 19.2	0.012
RV ESVI (ml/m2)	55.7 ± 32.6	31.5 ± 10.1	<0.0001
RV EF (%)	51.0 ± 14.8	63.9 ± 7.3	0.001

Summary and Conclusion: Conclusions. Movement abormalities of the right ventricle are not common in thalassemia and have a comparable frequency between TM and TI patients. In both the forms of thalasemia, movement

abormalities of the right ventricle were associated with RV dilation and dysfunction.

P1170

TROPONINE T AS A MARKER OF CARDIAC IMPAIRMENT IN PATIENTS SUFFERING THALASSEMIA

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Background: Heart failure (HF) resulting from myocardial iron loading is the leading cause of death in β-thalassemia major (β-TM) patients. A prompt diagnosis of HF is crucial in determining the survival of these patients.
Aims: We aimed to evaluate myocardial impairment dosing the levels of troponine-T (TnT) in peripheral blood of (β-TM) patients relating these findings with iron overload indices and echocardiographic measurements.
Methods: This pilot cross-sectional study recruited 50 consecutive β-TM patients from June 2013 to January 2014 recruited at Our Institution. Male/Female ratio was 1:1; Median age was 32 years (ranged between 16 and 48 years). All of them were affected by β-TM, except 2 male patients affected by Severe Intermedia Thalassemia. All echocardiographic measurements were carried out according to the recommendations of the American Society of Echocardiography by an external investigator, who was kept blind to the biochemical results. Left ventricular mass (LVM) was calculated according to the Devereux formula and indexed to height. Left Ventricular Hypertrophy (LVH) was defined by a LVM index >47 g/m in women or >50 g/m in men. HF was defined if clinically evident signs or symptoms were present. Informed consent was given to participants.
Results: Information on LVM was available for 46 out of 50 patients. All of them presented a LVH, except 2 female patients, both presenting normal levels of TnT. Median value of TnT was 3,925 ng/L, ranged between 3 and 60,3. Overall, 5 patients presented levels of Tnt greater than 14. Troponine-T concentrations was related with the level of LVM (Left Ventricular Mass) (Correlation coefficient r: 0,36; p=0,01; 95% CI for r: 0,07-0,59) while no relationship was found with iron overload markers (Ferritin; Saturation Index; T2* of the Liver) neither with the average Haemoglobin Level of the patient and the Ejection Fraction at Echography. The best cutoff for LVH was at 3 ng/L (sensitivity: 60.47%, specificity: 100%; Area Under the Curve: 0,802; p=0,0001). Additionally, the presence of an HF was related with higher levels of TnT with a cutoff value of 5,21 ng/L (sensitivity: 100%, specificity: 66,7%; AUC: 0,81; p=0,0002).
Summary and Conclusion: Taken together, our findings suggest that in (β-TM) patients TnT appeared to be a sensitive index of myocardial dysfunction.

P1171

Abstract withdrawn

Anemia not related to hemoglobinopathies

P1172

THE ANTI-HEPCIDIN SPIEGELMER® LEXAPTEPID PEGOL (NOX-H94) AS TREATMENT OF ANEMIA OF CHRONIC DISEASE IN PATIENTS WITH MULTIPLE MYELOMA, LOW GRADE LYMPHOMA, AND CLL: A PHASE II PILOT STUDY

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Background: Lexaptepid pegol (LP) is a PEGylated L-stereoisomer RNA aptamer that binds and neutralizes hepcidin. Hepcidin, a 25 amino acid peptide induced by inflammatory stimuli is a pivotal regulator of iron resorption and iron release from intracellular stores, which are severely impaired in anemia of chronic disease. Disturbances in iron metabolism resulting in functional iron deficiency are a key component of anemia of chronic disease which frequently complicates malignant disease.
Aims: We evaluated the pharmacokinetics, pharmacodynamics, safety and efficacy of hepcidin blockade by LP as sole treatment of anemia of chronic disease in patients with multiple myeloma, low grade lymphoma, and CLL.
Methods: Twelve patients with functional iron deficiency anemia with the following baseline characteristics, presented as median (range), were enrolled: age 64 years (35-77), hemoglobin (Hb) 9.6 g/dL (8.0-10.7), serum ferritin 317 µg/L (193-2805), serum iron 29 µg/dL (18-97), and transferrin saturation 12% (6-46). LP was injected i.v. at a dose of 1.2 mg/mg, TIW for 4 weeks. Blood counts, serum biochemistry, and iron status were evaluated weekly until two weeks post treatment and at week four after the end of therapy. The primary endpoint was increase in Hb by ≥1 g/dL at any time between start of therapy until 1 week after end of treatment. The study has the clinicaltrials.gov identifier NCT 01691040.
Results: Five of the 12 patients reached the target Hb increase of ≥1 g/dL, 3 patients achieved this goal within 2 weeks. Four of the 5 responding patients had hypochromic red cells (MCH 22-26 pg) and moderately increased baseline ferritin levels (200-350 µg/L). Median serum ferritin decreased from 317 to 232 µg/L (p=0.014) in the entire cohort of patients, and from 253 to 203 µg/L in responders (p=n.s). Reticulocyte hemoglobin (Chr) increased from 22.0 to 25.2 pg (p=0.019) in responding patients, while in non-responders no increase was noted (30.0 to 30.1 pg). Similarly, a tendency towards increased reticulocyte indices was observed in the responding patients (0.9 to 1.3, p=n.s.) only. Soluble transferrin receptor levels (sTfR) did not vary significantly in the entire group and in the responders as well (10.6 to 10.3 mg/L, p=n.s.). Baseline hepcidin levels of 12.7 nM (4.9-54.5) were increased in patients in comparison to those obtained in healthy volunteers in former clinical trials (1.78 nM (0.37-7.26). Patients with mean corpuscular volume (MCV) or Chr baseline levels below the lower limit of the normal range (MCV<82fL, Chr<28pg) represent 75% of the responders, respectively, and all patients with high baseline sTfR levels (>8 mg/L) reached the primary endpoint. Treatment with LP was well tolerated without major adverse reactions.

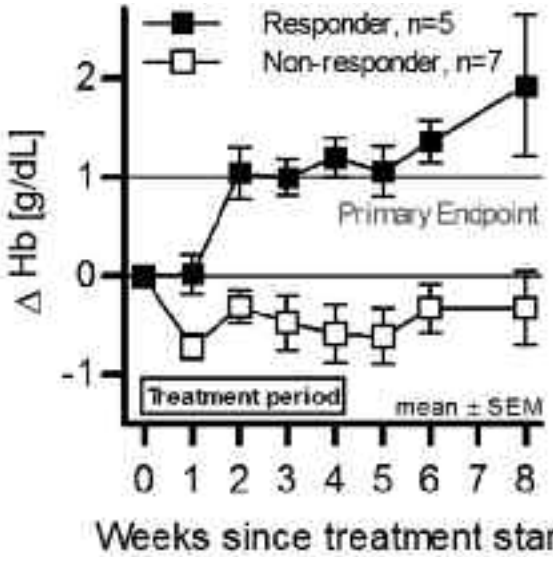

Figure 1.

Summary and Conclusion: Treatment with LP resulted in a significant increase in Hb levels (≥1 g/dL) in 5 of 12 patients supporting the concept of hepcidin inhibition as valuable treatment of chronic anemia of cancer. Responding patients showed an increase in reticulocyte hemoglobin. Baseline levels of MCV, Chr and sTfR were identified as potential diagnostic predictors that could support the selection of patients with an increased susceptibility for this therapy. After confirmation in additional clinical trials these predictive markers could help to increase response rates in this indication.

P1173

CLINICAL HETEROGENEITY OF AUTOIMMUNE HEMOLYTIC ANEMIA: A GIMEMA STUDY OF 308 PATIENTS

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Background: Autoimmune haemolytic anaemia (AIHA), usually classified as warm (WAIHA), cold (CHD), mixed, and atypical forms, is a greatly heterogeneous condition both in terms of clinical presentation and response to treatment.
Aims: To correlate serological characteristics and severity of anaemia with type and response to treatments, clinical outcome, and occurrence of acute complications.
Methods: 308 patients from 8 Italian Centres, diagnosed between 1978 and 2013, were included in this retrospective study, and classified according to DAT-positivity and Hb levels at onset (≤6, 6.1-8, 8.1-10, >10 g/dL). The following therapy lines were recorded: steroids±IvIg, immunosuppressors, rituximab, splenectomy, plasma-exchange, and erythropoietin. Clinical outcome (recovery, relapse, death) and occurrence of infections, thrombosis, and renal failure were evaluated.
Results: Patients (112 M and 196 F, median age at diagnosis 63, range 1-97) had been followed-up for a median of 33 months (range 12-372); 49% of cases were WAIHA (DAT+ for IgG), 27% CHD (DAT+ for C), 19% mixed (DAT+ for IgG and C), and 5% atypical (14 DAT- and 1 DAT+ for IgA only). Considering the degree of anemia at onset, 27% of cases had Hb levels ≤6, 37% Hb 6.1-8, 24% Hb 8.1-10, and 12% Hb>10 g/dL; mixed and atypical forms (mean Hb 6.6±1.9 and 6.5±1.7 g/dL, respectively) were more severe than WAIHA and CHD (mean Hb 7.3±2.3 and 8.6±2.2g/dL, respectively, P=0.0001). The mean reticulocytes progressively increased with the worsening of anemia (144±95, 160±122, and 242±156x10^9/L for Hb>10, Hb 8.1-10, and Hb 6.1-8g/dL, respectively), but not in cases with Hb<6 g/dL (192±128 x10^9/L, p=0.0001), possibly contributing to the clinical severity. Concerning therapy, steroids were administered in 273 cases (88%), mostly WAIHA (N=144), mixed (N=56) and atypical (N=14) (CR 43-50% and PR 27-36%); 59 CHD were treated with steroids, with lower CR (27%). As regards second line therapy, splenectomy was performed in 33 cases (11%), mostly severe warm or mixed forms (p=0.027), with a response rate of 71%; splenectomy had been performed in 3 CHD and was ineffective in 2. Cytotoxic drugs were administered in 77 patients (25%) (azathioprine 29% CR, 41% PR; cyclophosphamide 35% CR, 37% PR; cyclosporine 30% CR, 20% PR). Rituximab was administered in 55 cases (18%) (45% CR, 35% PR), more frequently in cold and mixed severe forms. Plasma-exchange was performed in 4 cases, and erythropoietin administered in 14, mostly severe forms, their efficacy being not evaluable because of concomitant therapies. Transfusions were given to 38% of patients.

Table 1.

N° Therapy lines	Serological AIHA type			
	Warm	Cold	Mixed	Atypical
0	8/153 (5%)	20/83 (24%)	1/57 (2%)	3/15 (7%)
1	90/153 (59%)	28/83 (34%)	18/57 (32%)	8/13 (60%)
2	35/153 (23%)	19/83 (22%)	25/57 (44%)	2/15 (13%)
3	15/153 (10%)	13/83 (16%)	11/57 (19%)	2/23 (13%)
4	5/153 (3%)	4/83 (4%)	2/57 (3%)	1/15 (7%)
	Hb levels at onset			
	>10 g/dL	8.1-10 g/dL	6.1-8 g/dL	<6 g/dL
0	15/38 (39%)	9/73 (12%)	5/112 (4%)	0
1	15/38 (39%)	39/73 (53%)	51/112 (46%)	40/82 (49%)
2	6/38 (16%)	15/73 (21%)	32/112 (29%)	23/82 (30%)
3	2/38 (5%)	10/73 (14%)	19/112 (17%)	10/82 (12%)
4	0	0	5/112 (4%)	7/82 (9%)

Summary and Conclusion: AIHAs showed a marked clinical heterogeneity, 1/3 of cases with a severe onset and 1/10 with life threatening complications. These cases are frequently mixed or atypical forms and refractory to different therapies.

P1174

REDUCED 25-OH VITAMIN D LEVELS IN PATIENTS WITH AUTOIMMUNE CITOPENIAS: CORRELATION WITH HAEMATOLOGICAL PARAMETERS AND CLINICAL SEVERITY

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Background: 25-OH vitamin D plays a crucial role in calcium and bone homeostasis, but it has also been shown to regulate several immune functions by acting on Th1/Th2 balance, T-reg activation and cytokine production. Vitamin D has been reported to be decreased in various autoimmune diseases such as multiple sclerosis and type 1 diabetes mellitus and to correlate with clinical outcomes.
Aims: To evaluate 25-OH vitamin D values in patients with autoimmune cytopenias, namely primary immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA) and chronic idiopathic neutropenia (CIN), and to relate them with clinical severity and hematologic parameters.
Methods: Clinical history, physical examination, complete blood count, serum samples and informed consent were collected at the time of enrolment, starting from January 2013. Hematologic parameters were also recorded at the time of the diagnosis. The number of immunosuppressive therapy lines (steroids, immunosuppressors, rituximab, splenectomy, and thrombopoietin agonists for ITP) and infectious episodes were retrospectively collected. Bleeding history was recorded for ITP. 25-OH vitamin D levels were evaluated in all patients and in 40 age and sex matched healthy controls, using an ELISA kit. Statistical analysis was performed using Student't test for continuous variables and chi-square test for categorical variables.
Results: Ninety-eight patients were enrolled, median age 59 years (range 17-86), 32 males and 71 females. As regard hematologic parameters at enrollment, ITP patients (N=44) showed median platelets values of 73x10^3/mmc (7-349x10^3/mmc) and vitamin D levels of 1.42 ng/mmc (0.21-8.69 ng/mmc), AIHA patients (N=35) displayed median Hb values of 11.6 g/dL (7.1-16.8 g/dL) and vitamin D levels of 1.94 ng/mL (0.09-6.71 ng/mL), and CIN cases (N=19) showed median ANCs values of 1.2x10^3/mmc (0.25-7x10^3/mmc) and vitamin D levels of 1.55 ng/mL (0.25-4.28ng/mL). As shown in figure 1, serum 25-OH vitamin D levels were significantly lower in patients than in controls (2.3±1.8 *vs* 6±6 ng/mL, mean values±SD, p<0.001), particularly in AIHA cases, regardless sex and age; considering the number of therapy lines vitamin D levels were reduced in AIHA patients who underwent two or more lines compared to cases with 0 or 1 line of therapy (1.7+1 ng/mL *vs* 2.8+1.8 ng/mL, p=0.04). As regards hematologic values at the time of diagnosis, all patients with vitamin D levels lower than 4 ng/mL showed significantly reduced Hb levels (9.3±3.5 *vs* 13.1±1.9 g/dL, mean values±SD, p<0.001) and absolute neutrophil counts (2.9±1.9 x10^3/mmc *vs* 4.3±2.1 x10^3/mmc, mean values±SD, p=0.001); the latter was particularly evident in CIN patients (0.57±0.3 x10^3/mmc *vs* 1.3±1.9 x10^3/mmc, mean values±SD, p<0.001). No relationship was found between hematologic

parameters at diagnosis or at enrollment and vitamin D levels in patients with ITP. Considering infections, 15 grade 2 episodes occurred during the study time, of whom 6 in CIN patients, without relationship with hematologic parameters or with 25-OH vitamin D levels.

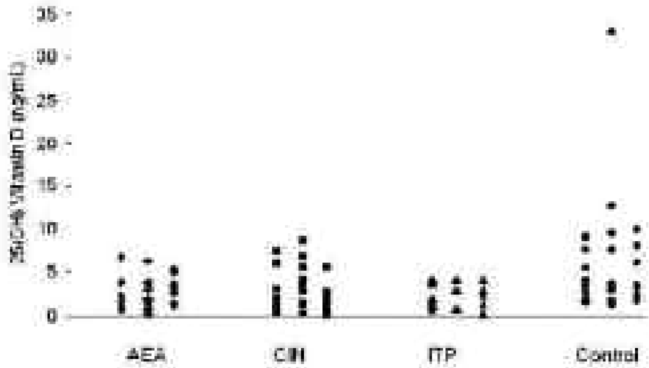

Figure 1.

Summary and Conclusion: This is the first demonstration of reduced values of vitamin D in autoimmune hematologic cytopenias. The reduction is particularly evident in AIHA cases with severe clinical picture and/or refractory to first line therapy and in CIN patients with more marked neutropenia. These data suggest a possible pathogenic role of reduced vitamin D in immune disregulation and provide hints for therapeutic options.

P1175

ASSESSMENT OF LIVER AND CARDIAC IRON OVERLOAD USING MAGNETIC RESONANCE IMAGING (MRI) IN PATIENTS WITH CHRONIC ANEMIAS IN LATIN AMERICAN COUNTRIES: RESULTS FROM ASIMILA STUDY

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Background: Patients receiving regular transfusions are at risk of developing iron overload (IOL) which can lead to increased morbidity and mortality.Therefore, accurate assessment of IOL is required for diagnoses and management with iron chelation therapy. Traditionally, IOL has been evaluated by serum ferritin (SF) levels or direct measurement of liver iron concentration (LIC) using biopsy (Taher *et al. Am J Hematol* 2013). Use of magnetic resonance imaging (MRI) has been extensively evaluated in many clinical trials (Pennell *et al. Haematologica* 2012) and has enabled accurate and non-invasive monitoring of IOL of liver and heart (Wood, *Hematology* 2011).
Aims: In this trial, MRI R2 and T2* techniques complemented SF to determine the prevalence and severity of liver and cardiac IOL, in transfusion-dependent patients with chronic anemias, excluding thalassemia.
Methods: This multicenter, observational study conducted in Latin America, enrolled male or female patients, aged >10 years, with sickle cell disease (SCD), low risk and intermediate-I myelodysplastic syndromes (MDS), aplastic anemia (AA), Diamond Blackfan anemia (DBA), congenital sideroblastic anemia or other rare anemias, with a lifetime history of >20 units of RBC transfusions and/or SF level >2000 ng/mL. All patients underwent liver MRI at Day 15 of study entry. Cardiac MRI was to be performed based on physician's criteria and cardiac MRI availability across all diseases except SCD, for which it was not required. The primary endpoint, incidence of IOL, was defined as percentage of patients with liver R2 MRI >2 mg Fe/g dw and/or cardiac T2* MRI<20 ms among patients who underwent a liver and/or cardiac IOL assessment as per protocol, respectively. Secondary endpoints included correlation between SF, LIC, and cardiac T2*.
Results: Of the 212 patients screened, 175 were considered eligible for the present analysis, who met the inclusion / exclusion criteria and performed cardiac and / or liver MRI per protocol. The mean age was 34.6 ± 17.0 years (range: 11 to 81 years) and consisted of Caucasians (31.4%), Hispanics (31.4%), and Africans (29.1%). Majority of patients had SCD (52%), followed by AA (17.7%), MDS (8.6%), DBA (4%), pure red cell aplasia (1.1%), and others (16.6%). Prior to study entry, the most common method for IOL assessment was SF (89.1%), followed by transferrin saturation (66.9%), echocardiogram (29.7%), liver MRI (24%), and cardiac MRI (8.6%).
In the current study, liver IOL (>2 mg Fe/g dw) was observed in 76.4% (n=133/174; mean liver R2 MRI, 14 ± 14 mg Fe/g dw) patients, while cardiac IOL (T2*<20 ms) was seen in 19.2% (n=14/73; mean T2*, 27.3 ± 10.7 ms). The incidence of liver IOL in patients by disease was: SCD, 80.2%; MDS, 73.3%; AA, 77.4%; pure red cell aplasia, 100%; DBA, 71.4%; and other transfusion disorders, 65.5%. Overall, patients demonstrated high SF, high LIC, and normal cardiac T2*, with notable variability within and across diseases (Table 1). Treatment for IOL was reported by 103 (58.9%) patients that included deferasirox (50.3%), deferoxamine (8.6%), deferiprone (4.0%), and other treatments (4.6%). A moderate correlation between LIC and SF was observed in SCD and MDS patients (r=0.47 [95%CI: 0.29, 0.61] and r=0.61 [95%CI: 0.14, 0.86], respectively). However, no correlation was observed between cardiac T2* and SF. There were no significant safety findings during the 15-day study period.

Table 1.

	All patients	Sickle – Cell disease (homozygous)	Myelodysplastic syndrome	Aplastic anemia	Pure red cell aplasia	Diamond-Blackfan anemia	Other transfusion dependent disorder
Serum Ferritin (ng/mL)							
n	175	91	15	31	2	7	29
Mean ± SD	2099.7 ± 2221.8	2612.6 ± 2279.1	2023.8 ± 1212.5	1731.4 ± 2264.4	1026 ± 1050	1144.3 ± 1042.9	1891.7 ± 1912.6
Median	1801	[illegible]	1818	1076	1026	661	111
LIC (mg Fe/g dw)							
n	174	91	15	31	2	7	28
Mean ± SD	14 ± 14	14.3 ± 13.6	19 ± 14.6	16.5 ± 15.8	13.8 ± 12.2	5.6 ± 4.8	10.2 ± 12.8
Median	9	9	18	10	13	6	4
Cardiac T2* (ms)							
n	73	3	12	24	0	7	27
Mean ± SD	27.3 ± 10.7	18.4 ± 9.8	21.9 ± 10.9	27.8 ± 10.1	-	25.1 ± 6.7	29.4 ± 5.2
Median	28	13	23	28	-	29	30

Summary and Conclusion: Assessment of IOL by MRI in regularly transfused patients from the Latin American region showed a high prevalence of liver IOL. Notably, high LIC values were observed across all diseases, most evident in MDS, SCD, and AA patients. Interestingly, unlike thalassemia patients, most patients across diseases had normal cardiac T2*. The use of chelation therapy in only some patients may have contributed to the range of IOL observed. Overall, the assessment of IOL by MRI of the liver and heart continues to demonstrate value in complementing SF measurements, both in patients with or without iron chelation therapy.

P1176

SIROLIMUS AS TREATMENT OF STEROID DEPENDENT/RESISTANT AUTOIMMUNE HAEMOLYTIC ANEMIA/PURE RED CELL ANEMIA IN CHILDREN

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Background: Autoimmune Haemolytic Anaemia (AIHA) is a rare disease in children. In most cases, it is secondary to infections, immunologic diseases or malignancies whereas about 40% of children suffer from an idiopathic/primitive disease. Although steroid treatment routinely used as first line approach is successful in about 80% of cases, relapsed/resistant disease may became a burning issue due to the absence of controlled studies on second and further line treatments to manage a potential life-threatening disease. Moreover, in children who experience a chronic clinical evolution of the disease, a long term treatment with medium/low dosage of steroid is often needed. The possibility to use a light and tolerable immunosuppressive therapy as maintenance in this setting could help to manage a steroid-dependent disease and the steroid-associated side-effects. Sirolimus has been reported to be useful in several cases of post-transplant AIHA, in few patients with immune-mediated cytopaenia secondary to Autoimmune Lymphoproliphrative Syndrome (ALPS), and in one case of multi-resistant Pure Red Cell Anaemia/AIHA.
Aims: The aim of this report is to retrospectively evaluate the fficacy and safety of Sirolimus given as maintenance treatment for steroid-dependent/resistant AIHA/PRCA in paediatric patients.
Methods: We evaluated the records of 4 children with AIHA/PRCA who were administered Sirolimus at the starting dose of 2mg/m^2 (then modulated according to blood levels) as maintenance treatment in steroid-dependent/resistant disease. The drug was given off-label and informed consent was obtained from parents. All patients were screened to identify any infective or immunological underlying disease including ALPS. Response to the treatment was defined as Hb and Reticulocyte within normal range for age.

Results: Four children (aged 5-25 months) afftected with and AIHA (3) and PRCA (1) received Sirolimus for refractory (1) or steroid-dependent (3) disease. All patients but one affected with ALPS, had idiopathic disease. Hb at diagnosis ranged between 2 and 6.4 gr/dl. First line treatment included Prednisone (PDN) in all cases. One patient with AIHA received Sirolimus as second line treatment for steroid dependent disease. The patient affected with PRCA received it as third line treatment for refractory disease after failure of PDN and mycofenolate. The remaining 2 children were administered Sirolimus as maintainance treatment in a steroid dependent disease after failure of PDN and rituximab associated to Cyclosporine in one case. Complete remission was achieved in all cases after 1-3 months of treatment and the drug was well tolerated in all patients. At last follow-up 2 patients had just stopped Sirolimus treatment given for 24 months at full dose and 6 months tapering. One patient was still receiving treatment after 9 months and the child with ALPS was receiving Sirolimus for 40 months.
Summary and Conclusion: Prospective trials on treatment of AIHA are lacking and will be hardly built up. Anecdotal data are available on the use of Sirolimus AIHA/PRCA. This experience contributes further on the usefulness of its use in both ALPS and non-ALPS children with AIHA and PRCA who may benefit of a tool to overcome steroid-dependency and chronic disease. This study also outlines the need of trial in very rare disease aimed to optimize different line treatments.

P1177

ANALYSIS OF CAUSES OF ANAEMIA FOUND IN A GENERAL PRACTICE POPULATION: PREVALENCE AND SURVIVAL

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Background: Information on the causes of anaemia found in general practice is limited. A prospective cohort study with general practice patients diagnosed with anaemia was set up to increase knowledge and improve quality of care for anaemia patients.
Aims: An evaluation of the causes of anaemia found in a cohort of general practice patients.
Methods: Between the 1st of February 2007 and the 1st of February 2013 patients presenting to one of the 63 participating general practitioners with a newly diagnosed anaemia (*i.e.* no anaemia in the preceding two years) were included in the study. Men were included when 18 years or older and women were included when 50 years or older to prevent an overabundance of hypermenorrhea as a cause of the anaemia.
A wide range of parameters was analysed for each patient to aid diagnosis. Two experts independently reviewed the laboratory results of all patients included and established the underlying cause of anaemia. In case of discordance, the experts deliberated until a consensus was reached.
Survival in months after entry into the study was determined per gender and for patients with microcytic, normocytic and macrocytic anaemia.
Results: A total number of 2738 patients were included in the study. A single cause of anaemia was found in 2493 patients (91.1 %). Anaemia of chronic disease was established in 899 patients (29.8 %), haemoglobinopathy in 24 patients (0.8 %), renal anaemia in 342 patients (11.3 %), haemolysis in 18 patients (0.6 %), possible bone marrow disease in 117 patients (3.9 %) and other causes were found in 119 patients (4.0 %). Iron deficiency was established in 552 patients (18.3 %), vitamin B12 deficiency in 122 patients (4.1 %) and folic acid deficiency in 24 patients (0.8 %). When no cause could be established it was classified as unknown, which was found in 797 patients (26.4 %). Overall survival of the cohort was 66.2 months (95% CI 65.2-67.3) after entry into the study. Men (N=1463, mean age at entry 66.8 years) demonstrated an overall survival of 66.5 months (95% CI 65.1-67.9) and women (N=1275, mean age at entry 75.1 years) a survival of 65.2 months (95% CI 63.6-66.7) (p=0.637). The survival for patients with microcytic (N=335, mean age at entry 65.1 years), normocytic (N=2213, mean age at entry 71.2 years) and macrocytic anaemia (N=190, mean age at entry 74.1 years) was 65.5 (95% CI 62.6-68.4), 66.9 (95% CI 65.7-68.0) and 57.9 (95% CI 53.4-62.5) months respectively. The survival of both microcytic and normocytic patients was significantly longer than the survival of patients with a macrocytic anaemia (p=0.005 and p<0.001 respectively).
Summary and Conclusion: Over a six year time period an extensive database of general practice patients diagnosed with anaemia was created. Besides supplying information on the prevalence of different causes of anaemia in this population, the data will also be used to develop a new guideline for the diagnosis of anaemia.

P1178

RENAL FUNCTIONAL AND STRUCTURAL INTEGRITY IN CHILDREN WITH IRON DEFICIENCY ANEMIA: RESPONSE TO IRON THERAPY

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Background: Background: Iron deficiency anemia (IDA) is the most common hematological disease of infancy and childhood. Researchers have investigated the deleterious effects of IDA on the cardiovascular and nervous systems, but the impact of this condition on renal function has not been examined in depth.
Aims: This study aimed to assess renal functional and structural integrity in 50 children with IDA compared to 50 age- and sex-matched healthy controls and evaluate their response to oral iron therapy.
Methods: IDA patients were studied stressing on symptoms and signs of anemia, anthropometric measures, evidence of renal, hepatic or cardiac disease, hematological profile, serum iron studies, urinary sodium and creatinine with calculating the fractional excretion of sodium (FeNa), urinary albumin excretion, leucine aminopeptidase (LAP), urinary excretion of trace elements (iron, copper, zinc, calcium and magnesium). Patients received oral iron therapy and were followed-up both clinical and laboratory for 3 months.
Results: IDA patients had significantly lower hemoglobin, red blood cell indices, iron, ferritin and transferrin saturation (p<0.001) with higher white blood cells (p=0.014) and platelets (p=0.006) than healthy controls. All the studied urinary markers including urinary trace elements were markedly increased among IDA patients compared to controls (p<0.001). Upon comparing the studied hematological parameters among IDA patients pre- and post-iron therapy, a significant increase in hemoglobin (p=0.001), mean corpuscular hemoglobin (p=0.011) and ferritin levels (p=0.005) was found. As regards urinary markers, a significant decrease in FeNa (p=0.017), urinary microalbumin (p=0.048), LAP (p=0.023), iron (p=0.007), copper (p=0.023), zinc (p=0.006) and calcium (p=0.042) was also found after therapy. Although urinary magnesium was decreased post- therapy (median, 246.3 with a range of 141.2-368.1) compared to baseline levels (median, 290.7 with a range of 168.9-834.9), the difference did not reach a significant level (p=0.156). No significant correlation was found between the studied urinary parameters and baseline levels of baseline levels of serum iron, Total iron binding capacity (TIBC), ferritin or transferrin saturation (p>0.05) except for a positive correlation between TIBC and urinary calcium (r=0.457, p=0.026). A significant positive correlation was found between urinary microalbumin and urinary LAP (r=0.437, p=0.025). Baseline urinary LAP was also positively correlated to all the studied trace elements (p<0.001 for all). Urinary copper levels were positively correlated to urinary microalbumin (r=0.437, p=0.029) while urinary magnesium levels was positively correlated to FeNa (r=0.426, p=0.034). Moreover, significant positive correlations were found between all the studied urinary trace elements (p<0.001 for all).
Summary and Conclusion: We suggest that IDA wields an adverse influence on renal functional and structural integrity. Improvement of IDA could protect against glomerular and tubular damage. Urinary excretion of albumin and LAP in IDA can be used as potential biological markers for renal dysfunction and for monitoring the response to therapy. Increased loss of trace elements in urine of IDA patients denotes that these metals could be reliable indicators and risk predictors of renal damage. Further longitudinal studies are needed to verify the effect of iron therapy on renal functions.

P1179

MULTIFACTORIAL PATHOGENESIS OF ANEMIA IN PREGNANCY

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Background: It is known that iron deficiency is one of the main pathogenic factors of anemia in pregnancy (AP). But own investigations results and some other researchers' data in field of AP give the bases to believe that AP has more complex (multifactorial) pathogenesis than ineffective erythropoiesis, caused by iron or folate deficiency. Recombinant human erythropoietin (rHuEPO) is very effective (up to 85%) for the treatment of AP which often are resistant to an iron therapy alone. If AP relate to iron deficiency anemia (IDA) only, then rHuEPO therapy of the anemia has not been such beneficial indeed. Blunt erythropoiesis is one of some AP causes. But of data about blunt erythropoiesis prevalence in AP insufficiently.
Aims: In the present study, we investigated the adequacy of the EPO production for the degree of anemia in pregnancy. We evaluated role of estrogens and hepcidin in multifactorial pathogenesis of AP.
Methods: To investigate the adequacy of the EPO production for the degree of anemia in pregnant women total 133 anemic pregnant women (105 - iron deficiency anemia (IDA) and 28 - non-IDA) and 47 normal Hb level pregnants were tested. Control group consisted of 22 non-pregnant women with iron-deficiency anemia (IDA). EPO values were measured immunoenzymometrically by using ELISA-EPO kits. Serum levels of estradiol and hepcidin were determined in anemic pregnants and in 47 healthy non-pregnant women in addition. The adequacy of EPO level in relation to the degree of anemia was evaluated individually by computing the ratio between the logarithm of the observed value and the logarithm of the expected result [O/E log (EPO) ratio] according to the regression line constructed with the control group.

Results: The ratio of observed/predicted (O/P) serum EPO was inadequate (<0.8) for the degree of anemia in 51 (48.6%) of 105 IDA pregnants and in 11 (39.3%) of 28 non-IDA pregnants. The significant elevated serum levels of estradiol were observed at all anemic pregnant women *versus* healthy pregnant women with normal Hb (Table). Serum hepcidin concentration in non-IDA pregnant women was significant higher than in IDA pregnants group: 34.8±6.1 μg/L and 3.9±1.2 μg/L respectively (p<.001). Serum INF-γ concentration in IDA pregnant women was significant higher than in non-IDA pregnants: 240.2±80.37 ng/L and 84.2±30.59 ng/L respectively (p<.05).

Table 1. Serum levels of iron deficiency indices and anemia of inflammation markers

Anemia in pregnancy	Serum ferritin (SF), mcg/l	Transferrin saturation, %	sTfR/ log SF	Hepcidin μg/l	Estradiol nmol/l	INF-γ ng/l (previously published data)
IDA (n=105)	8,3±0,3*	17,4±1,4*	3,9	3,9±1,2*	16,1±1,0*	240,2*
non-IDA (n=28)	78,1±16	37,6±4,6	1,4	34,8±6,1	14,7±1,3	84,2
Healthy pregnants (normal Hb level) (n=47)	–	–	–	12,3±1,5	7,8±1,0	70,3

*: p < .05

Summary and Conclusion: Thus inadequately low production of EPO for the degree of the anemia is important mechanism in pathogenesis of AP. Some AP (non-IDA) relate to systemic proinflammatory response developing during pregnancy and associate with increased production of hepcidin. Blunted erythropoiesis in IDA pregnants seems has relation to elevated production of and other cytokines with placenta in hypoxia. T-lymphocytes stimulated with estrogens can be sources of INF-γ too. That is why rHuEPO therapy of AP is high effective. Complex algorithm is necessary to provide optimal guidance in management of AP. Main therapeutic options are oral iron, intravenous iron and ESA. Response to iron therapy of AP (oral or intravenous) isn't above 50% usually. In our opinion so resistance to iron relate to inadequately low production of EPO to degree of anemia. RHuEPO combined with intravenous iron is more effective therapeutic option in this case.

P1180

THE IMPACT OF LIFESTYLE FACTORS ON RAISED RED CELL MEAN CORPUSCULAR VOLUME IN AN IRISH POPULATION-NOT JUST A 'HAEMATOLOGY' PROBLEM

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Background: The prevalence of macrocytosis in adults is estimated between 1.7% and 8%. Its diagnosis may warrant an extensive work-up for vitamin deficiency, haematological malignancy, heavy alcohol intake, liver disease, myelodysplasia and myeloma. The degree to which lifestyle factors contribute to clinically significant macrocytosis (MCV≥99fl) has not been extensively reported in the literature. An independent link between smoking and macrocytosis has been previously observed (McNamee *et al.* Haematinic deficiency and macrocytosis in middle aged and older adults. PLoS One. 2013; 8(11): e77743).
Aims: To study the determinants of clinically significant macrocytosis with particular reference to the independent effects of lifestyle factors in a cohort 2,047 Irish patients aged 50-69 years sampled from a primary care centre in southern Ireland.
Methods: Details of the Mitchelstown Cohort study have been previously described (Kearney *et al.* Int. J. Epidemiol. (2012) doi: 10.1093/ije/dys131). The study is based in a primary care centre serving a defined population in Southern Ireland. Vitamin B12 and folate, fasting plasma glucose (FPG) and lipid profiles, HbA1c, liver function and full blood counts were measured using standard laboratory methods. Smoking status and alcohol intake were recorded using a validated questionnaire. The metabolic syndrome (MS) was defined using the International Diabetes Federation (IDF) 2006 criteria. Blood pressure, body mass index (BMI) and waist circumferences were measured. Statistical analysis was performed using Stata©. Multivariate logistic regression was used to estimate prevalence odds ratios with 95% Confidence Intervals (OR, 95%CI) for macrocytosis and its potential determinants. Population attributable fractions (PAF) were estimated for variables that were significantly associated with macrocytosis in multivariate analyses.
Results: The prevalence of MCV≥99fl in this sample of 2,047 patients was 1.6%. The prevalence of B12 deficiency was 2.4%, folate deficiency, 1.5%, elevated gamma-glutamyltransferase (GGT), 18%, elevated alanine aminotransferase (ALT), 8%, elevated aspartate aminotransferase (AST), 4.7%, current smoking, 15% and MS, 31%. In multivariate logistic regression analysis with adjustment for age and gender the following variables were significantly associated with MCV ≥99fl; folate deficiency OR 8.2 (95% CI 2.3-29.0), elevated AST OR 8.0 (95% CI 3.5-18.6), B12 deficiency OR 6.1 (95% CI: 2.0-18.4), current smoker OR 6.0 (95% CI 2.8-12.5), MS OR 3.4 (95% CI 1.6-6.9), elevated GGT OR 2.3 (95% CI 1.0-4.9) and elevated TG OR 2.3 (95%CI 1.1-4.7).. Central obesity, BMI, elevated FPG, low HDL and self-reported alcohol intake did not reach significance. In further analyses adjusted for all significant variables, the association of macrocytosis with both smoking and MS was essentially unchanged, OR 5.7 (95%CI 2.6-12.7) and OR 3.0 (95%CI 1.3-6.9) respectively. PAF for smoking was 38.4%, followed by elevated AST, 22.9%, MS, 13.8%, vitamin B12, 11.7% and folate deficiency, 7.0%.
Summary and Conclusion: Attention should be given to the impact of lifestyle factors on MCV. Our findings suggest that smoking is an important cause of macrocytosis. Potential mechanisms include the direct toxic effect on erythrocytes of acetaldehyde in tobacco smoke and the response to reduced oxygen-carrying capacity. We also observed an independent association between macrocytosis and the MS. Non-alcoholic fatty liver disease is strongly linked to the MS. However we have demonstrated an association independent of abnormal liver indices. As the obesity epidemic escalates worldwide, GPs, physicians and haematologists should consider its potential impact on red cell mean corpuscular volume before embarking on haematological work-up.

P1181

THROMBOEMBOLISM OCCURRED REGARDLESS OF HEMOLYSIS IN PATIENTS WITH PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA– A PROSPECTIVE STUDY OF 464 PATIENTS IN A MULTICENTER CHINESE REGISTRY

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Background: Thromboembolism (TE) has been well recognized as the most common cause of death in patients with paroxysmal nocturnal haemoglobinuria (PNH). Recent studies showed that PNH patients with elevated hemolysis indicated by lactate dehydrogenase levels (LDH) ≥ 1.5 times the upper limit of normal (ULN) at diagnosis were at significantly high risk for TE than patients with LDH<1.5×ULN. However, the pathogenesis of thrombosis in PNH patients tends to be multifactorial, not only through intravascular hemolysis.
Aims: To better undstand the natural history of PNH and the correlation between TE and hemolysis in Chinese population, a prospective, non-interventional PNH registry was conducted in 8 centers.
Methods: PNH diagnosis was confirmed using a consistent flow cytometry protocol based on 2010 ICCS guideline: fluorescent aerolysin (FLAER) and one more reagent against GPI-anchored proteins were used in white blood cell testing; CD59 was used in erythrocyte testing. Patients with a detectable PNH clone irrespective of treatment were enrolled in the study and would be followed every six months. Clinical characteristics, quality of life, complications including TE events were recorded and analyzed.
Results: Total 464 PNH patients were enrolled in the study from Nov 2011 to Dec 2013. The median age at diagnosis was 37 years (range 6-84), male to female ratio was 1.07:1, median duration of PNH was 3 months (range 0.03-307 months); 86% patients's PNH history was less than 3 years. 50.8% patients were classical PNH, 42.5% patients were PNH with bone marrow failure and 6.6% were subclinical PNH. All of the patients received conventional therapy (67% patients used corticosteroids). The average PNH clone size was 53% in granulocytes and 32% in erythrocytes. The mean LDH level was 992 U/L at average 4.0 folds of ULN. 60 thrombosis events (TE) were reported in 38 patients, of whom 42% had multiple TE. The TE event rate with conventional therapy was 7.19 events/100 patient- years. TE events accounted for 36.4% (4/11) of deaths during the study period. TE occurred at both venous (70%) and arterial (30%) sites. Deep vein thrombosis of the limbs was most common at 32%, hepatic/portal vein was 23%, cerebral artery was 15%. There was no statistical difference in the prevalence of TE events between patients with granulocyte clone size ≥50% and patients with 10-50% clone (9.4% *vs* 8.5%, p=0.788). Median age at first TE was 42.9 years. There was no significant difference in age and gender between the patients with or without TE. TE events occurred in PNH patients at any age, but most commonly were seen in patients at age 31-40 years (26%). TE occurred regardless of hemolysis status. 10 TE events (19.6%) occurred in 5/38 (13.2%) patients with LDH<1.5×ULN; these patients' median granulocyte clone size was 40.3% and mean LDH was 0.86×ULN. These 10 TE events were myocoardia infaction (1), DVT of the lower limbs (3), portal/hepatic vein (3), pulmonary embolism (1), stroke (1) and unstable angina (1). ROC analysis showed that LDH ≥1.2×ULN represented the most sensitive threshold to detect 90.6% of the patients with TE; LDH ≥1.5×ULN or ≥3.0×ULN as cut-off only detected 84.4% or 56.3% of the population with TE. Univariate analysis showed patients with LDH ≥1.2×ULN had a significantly increased incidence of TE compared with patients with LDH<1.2×ULN (OR 4.17; 95% CI 1.24-13.97, P=0.013)
Summary and Conclusion: TE is a major contributor to the morbidity and mortality in Chinese PNH patients on conventional therapies. TE occurrence

was not correlated with patients' age or gender. TE could happen when PNH patients didn't have significant hemolysis. Attention need to be paid to these PNH patients without elevated LDH because other mechanisms are involved in the pathogenesis of thrombosis.

P1182

NEW RED BLOOD CELL AUTOMATED PARAMETERS FOR IRON DEFICIENCY SCREENING IN CHRONIC INFLAMMATORY DISEASE

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Background: New red blood cell parameters are available on Beckman-Coulter Unicel DxH800 instrument, *i.e.* LHD% (Low Haemoglobin Density %), RSf (Red cell Size factor) and MAF (Microcytic Anaemia Factor). Previous studies showed that those parameters might be useful for iron deficiency diagnosis, especially in order to detect iron depletion in the context of chronic inflammation.
Aims: The aim of our study was to evaluate those parameters for assessing latent iron deficiency and iron deficiency anaemia associated with chronic inflammatory disease.
Methods: During six weeks, adult patients' samples (n=182) from the daily laboratory workflow, with a dosage of haemoglobin (Hb), ferritin (FET), C-reactive protein (CRP) and with a normal renal function (creatinine<1mg/dL or<1.2mg/dL for women and men, respectively) were included. A control group (n=267), with all parameters within the reference range values, was also constituted. Subjects were separated into five groups based on iron deficiency (ferritin<30 µg/L or<40 µg/L in presence of chronic disease), anaemia (women, Hb<120 g/L and men, Hb<130 g/L), and inflammation (CRP >10 mg/L):
• IDA Iron Deficient Anaemia without inflammation (n=49);
• LID Latent Iron Deficiency without anaemia nor inflammation (n=69);
• ACD-ID Anaemia of Chronic Disease–Iron Deficient (n=21);
• ACD-PID Anaemia of Chronic Disease–Possibly Iron Deficient (n=30);
• ACD Anaemia of Chronic Disease without iron deficiency (n=13).
Soluble transferrin receptor concentration (sTfR, Roche Diagnostics, Vilvoorde, Belgium) was measured for the groups with anaemia of chronic disease *i.e.* ACD-ID, ACD-PID and ACD. Furthermore, some 81 patients with a diagnosis of inflammatory bowel disease (IBD) were included in the study and were separated into seven groups: IDA (n=3); LID (n=14); ACD-ID (n=1); ACD-PID (n=5), ACD (n=4), non-anaemic patients with ferritin>40µg/L, CRP<10mg/L (n=42), and non-anaemic patients with ferritin>40µg/L, CRP>10mg/L (n=15). Mann-Whitney test, unpaired t test and one-way ANOVA were used for statistical analysis.
Results: The patients (n=64) from the first part of the study with ACD-ID, ACD-PID, and ACD were further characterized by sTfR; statistically significant differences ($p<0.05$) were found between ACD-ID and ACD-PID, and between ACD-ID and ACD. Concerning the three automated parameters (LHD%, RSf and MAF), a statistically significant difference was observed between the control group and the five studied groups. To assess latent iron deficiency, we compared IDA and LID, and all three parameters showed a difference between those two groups ($p<0.05$). Concerning anaemia of chronic disease-groups (ACD-groups), only MAF and RSf showed a statistically significant difference between the three ACD-groups (ACD *vs* ACD-ID *vs* ACD-PID). Analysing the IBD groups, only MAF showed a statistically significant difference between the groups of IDA and LID. Concerning groups ACD, ACD-ID, and ACD-PID of IBD patients, no statistically significant difference was found. Comparing the control group of the first part of the study (n=267) with IBD patients without anaemia and no inflammation (n=42), LHD% demonstrated a statistical difference ($p<0.05$), contrary to MAF. Comparing the control group (n=267) with IBD patients with inflammation and without anaemia (n=15), both LHD% and MAF demonstrated a statistically significant difference.
Summary and Conclusion: This study demonstrates that LHD%, MAF and RSf could be used as screening tests of latent iron deficiency; MAF and RSf could reflect iron depletion in patients with anaemia of chronic disease. After testing the validity of these findings in the settings of IBD, we selected MAF as the most suitable haematological parameter for latent iron deficiency differentiation.

P1183

ROLE OF BMI IN PREDICTING PREVALENCE OF IRON DEFICIENCY ANEMIA

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Background: Iron deficiency anemia is the most common anemia worldwide. Interestingly, overweight and obese children and adolescents are found to have a higher incidence of iron deficiency anemia. Overweight and obese children and adolescents have been shown to have higher hepcidin levels, and inadequate responses to oral iron therapy. Hepcidin produced from hepatocytes is one of the key regulators of iron hemostasis. Hepcidin inhibits expression of iron transporter, ferroportin, in enterocytes, macrophages and hepatocytes. Inhibition of ferroportin action results in iron sequestration in the enterocytes with diminished release of iron into the circulation, thereby decreasing iron absorption. Hepcidin levels are upregulated by inflammation and iron storage but downregulated by anemia. Obesity triggers inflammatory response with increased release of cytokines and acute phase reactants in multiple organs. Obese patients are found to express higher levels of hepcidin. Therefore, we are assessing whether adult patients with high BMI (BMI≥30) are more prone to develop iron deficiency anemia.
Aims: Iron deficiency is thought to be more common in patients who have poor nutrition and low iron intake. However, there have been observations of an inverse relationship between body weight and iron saturation. Therefore, we are assessing whether Body Mass Index (BMI) can affect serum iron levels.
Methods: This is a retrospective case control study where 78 adult patients from age 18-98 years with iron deficiency anemia from Eaton Hospital in-patients as well as a community care clinic in Easton were assessed with regard to their body weight (*i.e.* BMI). Hemoglobin, serum iron, ferritin, iron binding capacity and iron saturation were measured and correlated with patients' BMI. Patients with any ongoing blood loss such as hematuria, severe menorrhagia, hematochezia or history of gastrointestinal bleeding including peptic ulcer disease, variceal bleeding, hemorrhoidal bleeding, diverticular bleeding, arteriovenous malformation were excluded. Patients who had inflammatory bowel disease, celiac disease, post-surgical malabsorption problems, gastric bypass surgery, gastrointestinal tract malignancy, chronic kidney disease, repeated blood donations, as well as pregnant patients were also excluded from the study.
Results: 67 female patients and 11 male patients aged 18-98 years with iron deficiency anemia were evaluated for body mass index. 40 patients had high BMI (BMI ≥30) and 38 patients had low BMI (BMI<30). The median age was 55. The mean hemoglobin of patients in this study was 10.8g/dL. The mean iron level in the high BMI group was 40.6, *versus* 57.92 µg/dL in the low BMI group, p value=0.08. In addition, the mean ferritin level in the high BMI group was 76.92 *versus* 198.8 ng/ml in the low BMI group (p value=0.09), using the Welch two sample t-test. The mean TIBC was slightly higher at 352.8 µg/dL in obese patients whereas patients with a low BMI had a lower mean TIBC at 312.72 µg/dL, p value 0.06. Patients with a high BMI had a mean iron saturation of 11.25%, compared to a mean iron saturation of 18.71% in the low BMI group, p value 0.007 (Fig 1).

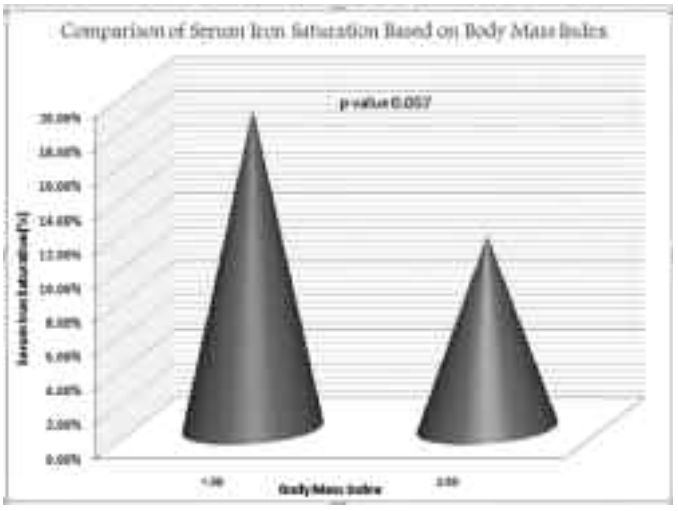

Figure 1.

Summary and Conclusion: In our study, obese patients (BMI≥30) had a higher prevalence of iron deficiency anemia in comparison to patients with BMI<30, after excluding patients with potential blood loss or iron malabsorption. Therefore, BMI is a significant predictor of iron saturation.

P1184

MICROCYTIC ANAEMIA AND IRON METABOLISM IN ERYTHROPOIETIC PROTOPORPHYRIA

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Background: Erythropoietic Protoporphyria (EPP) is a cutaneous porphyria, due to ferrochelatase (*FECH*) gene alterations. The *FECH* gene encodes the last enzyme in the heme synthesis which catalyses for insertion of iron into protoporphyrin IX (PPIX) to form heme. EPP is clinically characterized by painful long-life severe photosensitivity usually with a childhood onset and in some cases it is associated with microcytic anaemia. Accumulation of PPIX in blood red cells, plasma, skin and liver is associated with burning, pain, itching followed by erythema and oedema of the skin and elevated transaminases up to liver failure. A negative correlation between PPIX and iron and transferrin (Tf) saturation has been reported. The molecular basis for an overt phenotype are: - co-inheritance of a null mutation in the ferrochelatase (*FECH*) gene trans to a GTC haplotype, reducing expression of the remaining wild-type allele. This combination is responsible to decrease *FECH* activity to<35%; - X-linked transmission, a rarer variant of EPP called X linked-protoporphyria (XLPP), due to gain-of-function mutations in the 5-aminolaevulinate synthase 2 (*ALAS2)* gene on the X chromosome encoding the first enzyme in the heme synthesis which produces 5-aminolaevulinic acid (ALA) starting from glycin and succinyl-CoA.
Aims: The aim of this study was to re-evaluate iron status of 22 subjects (age at diagnosis: 23.5±15.7; 8-61 years) diagnosed as affected by EPP attending the Rare Disease Centre at Fondazione Ca' Granda in Milan from January 2009 to December 2013.
Methods: EPP diagnosis was first suspected by anamnesis, signs, the increase of erythrocyte protoporphyrins and the presence of a fluorescence emission peak at 632-634 nm in the plasma. The diagnosis was confirmed by direct sequencing and MLPA of *FECH* gene starting from genomic DNA extracted from peripheral blood. In all patients, we also measured iron, ferritin, Tf, Tf saturation, haemoglobin (Hb), mean corpuscular volume (MCV) and the relative percentage of free (PPIX) and zinc-protoporphyrins (ZnPP) in peripheral venous blood.
Results: Fifteen patients inherited a null mutation trans to a low expressed allele, two subjects showed the homozygous GTC haplotype and a 61 year-old man carrying only a null mutation complained cutaneous symptoms after myelodisplastic syndrome onset. In the remaining four patients *ALAS2* alteration was detected. Thirteen (59 %) of our patients presented a slight microcytic anaemia (in females Hb 12.3±1 g/dl, range 10.5-14 g/dl and MCV: 77.1±7.6 fl, range 60.4-87.1 fl; in males Hb 13.4±1.2 g/dl, range 10.9-15.1 g/dl and MCV 76.4±8.0 fl, range 63.1-95.9 fl). Ferritin was reduced in 63.6% of cases (in females 35.4±51 ng/ml, range 3-192 ng/ml; in males 41.3±31.8 ng/ml, range 10-112 ng/ml; normal values 15-150), Tf and serum iron (Fe) values were in the normal range (respectively in females Tf 309.3±39.9 mg/dl and in males 298±21.1 mg/dl; normal values 200-360; in females Fe 68.9±40.3 mcg/dl and in males 84±25.7 mcg/dl; normal values 37-145) while Tf saturation was indicative for iron deficiency only in females. A 29 year-old girl presented X-linked protoporphyria in association to Iron Refractory Iron Deficiency Anaemia (IRIDA) and in contrast to EPP patients she had no exacerbation of skin symptoms during intravenous iron treatment, as reported in literature.
Summary and Conclusion: This study confirms a relationship between EPP and iron deficiency anaemia which requires further investigation involving the iron metabolism regulation.

P1185

SIGNIFICANT IMPACT OF IRON CHELATION AFTER ALLOGENEIC HEMATOPOETIC STEM CELL TRANSPLANTATION ON DISEASE RECURRENCE: POTENTIAL ANTI-LEUKEMIC ACTIVITY

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Background: Iron overload (IO), primarily related to multiple red blood cell transfusions, is a relatively common complication in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients. Elevated pre-transplant ferritin level, a surrogate marker of iron overload, was demonstrated to be an important cause of mortality and morbidity in patients who have undergone allo-HSCT. Excessive iron accumulation results in tissue damage and organ failure, mainly as a result of the generation of free radicals that cause oxidative damage and organ dysfunction. Iron chelators have been widely used leading to normalisation for ferritine level and lower IO-related complications. As iron has a fundamental role in cell survival affecting pathways involved in DNA synthesis, cell differentiation, and apoptosis, some studies evaluated the anti-proliferative activity of iron chelators in cancer and leukemia patients on disease recurrence.
Aims: The objective of this study was to determine at a first time the impact of serum ferritin level measured at time of allogeneic HSCT in adult patients with hematological disorders on the different outcomes and to investigate at a second time the role of iron chelation on relapse incidence.
Methods: We included 158 patients, 100 males and 58 females with a median age of 45 years (18-67) who underwent allo-HSCT between 2002 and 2010. There were 83 acute myeloid leukemias, 10 chronic myeloid leukemias, 11 myelodysplastic syndromes, 7 myeloproliferative disorders, 19 myelomas, 9 non-Hodgkin lymphomas, 6 Hodgkin diseases, 5 aplastic anemias and 3 hemoglobinopathies. Sixty-seven (42%) patients were sex mismatched (F→M:37; M→F:30); for ABO compatibility, 61% were compatible, 18% had minor incompatibility and 21% had major incompatibility. Concerning the HSCT procedures, 60 patients (38%) received peripheral blood stem cell and 98 (62%) received bone marrow from 97 (61%) HLA related donors [matched, n=76; mismatched, n=21], and 61 (39%) HLA unrelated donors [matched, n=36; mismatched, n=25] after myeloablative [n=64, (41%)] or reduced intensity conditioning [n=94, (59%)]. At transplantation, 91 (58%) were in complete remission (CR) or chronic phase [CR1: n=61 (67%); ≥CR2: n=30 (33%)]. The median serum ferritin level at HSCT was 1327 microg./l (26-14136); 31(20%) patients had a level 26-500, 33 (21%) had a level 500-2500, and 94 (59%) >2500. There was no significant correlation between the different ferritin levels, disease kind and status at HSCT. After transplantation, 23 patients received iron chelating agents after a serum ferritine level of 1000 microg/l and stopped when the level decreased below 1000.
Results: The cumulative incidence of acute GVHD ≥ II at 3 months was 14% (11-16.5) with 10.5% (8-13) for grade III and 7% (5-9) for grade IV; the 1 year cumulative incidence of limited and extensive chronic GVHD were 4% (2-6) and 12.4% (9-16) respectively. After a median follow-up of 18 months (1-106), the 5 years OS probability was 65% for patients with ferritin level below 500 microg./l, 39% for level between 500 and 2500 microg./l and 28% for level >2500 micog./l, [Hazard ratio=3.5 (1.5-8.1), p=0.002]; this was explained by a significant higher TRM in patients with level >2500 [Hazard ratio=4.3 (1.02-18), p=0.04]. Interestingly, we found in multivariate analysis that patients receiving iron chelators had significantly better OS [5 years OS=59% *vs*. 34% for non-chelated patients, Hazard ratio=0.34 (0.15-0.76), p=0.008], (Figure 1a), and experienced less disease relapse [5 years relapse incidence=18% *vs*. 41% for non-chelated patients, Hazard ratio=0.22 (0.07-0.73), p=0.012], (Figure 1b).

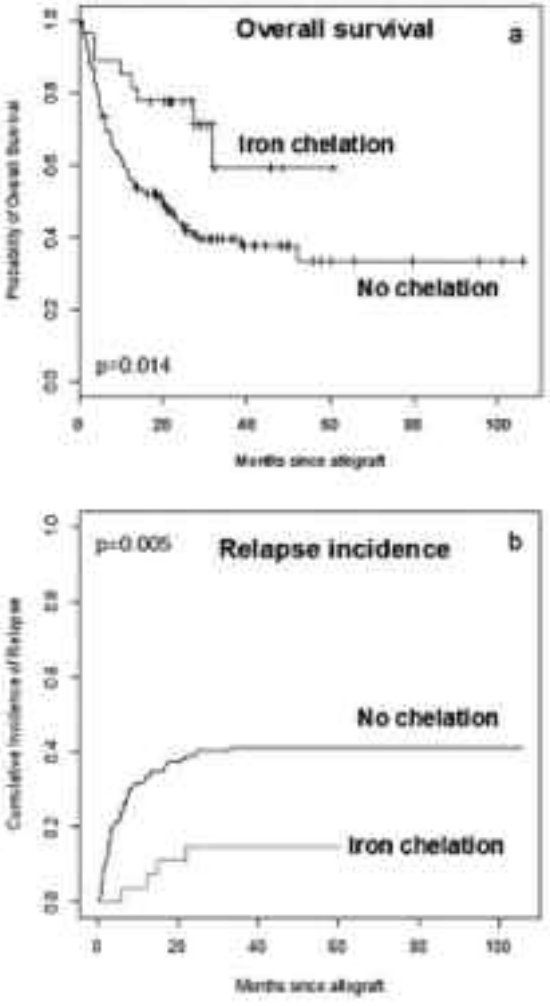

Figure 1.

Summary and Conclusion: We confirmed the negative impact of iron overload on the outcomes allo-HSCT recipients. More importantly, we demonstrated that iron chelators have a positive impact in reducing disease relapse by the possible mechanism of iron deprivation in leukemic cells. This clinical observation needs to be confirmed by prospective randomized trials.

Infectious diseases, supportive care 2

P1186

INVESTIGATION ON THE EARLIEST SIGN OF INVASIVE PULMONARY ASPERGILLOSIS (IPA) AND THE ONSET, WITH ROUTINE NON-PYREXIA PHASE CT BASED DIAGNOSTIC STRATEGY, A COHORT STUDY

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Background: IPA is an aerial infection, beginning from airwayinvasive phase (AWIP) and then developing to angioinvasive phase (AGIP). However, during neutropenia, the start of AGIP, halo sign (HS), is still considered the earliest sign of IPA. No attempt has been made to detect IPA in AWIP, much remains unknown about the onset.
Aims: Since the late 2000s, galactomannan (GM) has improved dramatically; decreased cut off (0.5) and routine measurement from admission irrespective of pyrexia are now well accepted. Conversely, chest CT (CT), considered as an earlier diagnosis method than GM, is still conducted only post pyrexia. Even on the Pyrexia Day One CT, progressed pneumonia can be detected in many cases. Therefore, we hypothesized that the earliest sign before HS can be captured by CTs in the non-pyrexia phase. This study investigates the hypothesis.
Methods: From 12/10/2010 to 9/12/2013, all acute leukemia (AL) patients without the history of IPA who received induction chemotherapy in our hospital were included. Informed consent was obtained. GM was routinely conducted twice weekly. ≥0.5 was positive. We defined the non-pyrexia phase as max temperature ≤37.3. Routine non-pyrexia phase CT (R-CT)s were conducted at admission and repeatedly conducted at 7 day intervals. Additional CTs were also conducted when GM ≥0.4, the first day of ≥38.0 pyrexia and in cases of persisting pyrexia every 3 days. All images were analyzed by senior radiologists. Abnormalities suggesting IPA were strictly determined combining all CTs and patients clinical course. EORTC/MSG criteria was used for diagnosis. Clean room entry, prophylaxis (itraconazole or voriconazole) and treatment strategy (voriconazole alone or combination) were completely unified.
Results: 220 successive AL patients were analyzed, of them, 61 patients (27.7%) were diagnosed as IPA (all probable, no proven). Patient characteristics, IPA group *vs* non-IPA group, are as follows (data is presented as median (range)). Age; 74 (46-91) *vs* 66.5 (13-93), days of absolute neutrophil counts ≤500; 27 (10-387) *vs* 24 (9-373), days of hospitalization; 41 (25-462) *vs* 35 (24-375), number of CTs; 6 (2-19) *vs* 5 (1-16) and overall mortality; 11.4% (7/61) *vs* 5.0% (8/159). IPA was successfully treated in all patients and no IPA related fatalities were observed. 4 patients were excluded for reasons such as mucor superinfection. As the earliest IPA CT sign, 98% (56/57) of patients showed airwayinvasive pattern, furthermore, only 15.7% (9/57) progressed to angioinvasive pattern. Figure shows IPA onset determined by CT for all 61 patients. Of them, 60.6% (37/61) cases were diagnosed with R-CT. CT could make diagnosis earlier than GM (5.5 (1-28) *vs* 13 (2-53)). Furthermore, figure implies IPA preexistence; the diagnosis rate rapidly falls as the days from admission increases with 81.9% (50/61) diagnosis by day 15.

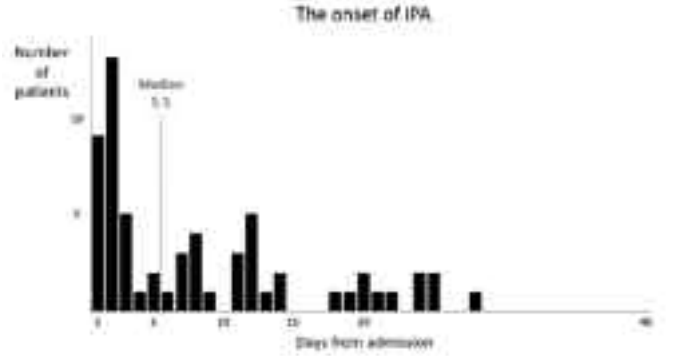

Figure 1.

Summary and Conclusion: Logically, pyrexia appears from progressed phase of infection, therefore the existence of pneumonia in the non-pyrexia phase is not unexpected. Unlike X-p, CT is not portable, which may be the main reason why R-CT has not previously been conducted. For AL patients without complete remission, immunosuppression has already occurred before admission. Therefore, IPA preexistence can be expected. We expect these new findings to be accepted in the near future. In Hematologica 2013 (98(11)), Marcio et al propose the importance of AWIP recognition; our initial study analysis was quoted as the first clinical study indicating AWIP as the earliest phase during neutropenia. In AWIP, in addition to lower fungal burden, the full advantage of excellent drug migration can be realized. Therapy from AWIP is vital; irrespective of the highest recorded risk patient background, IPA was successfully treated in all.

P1187

EFFICACY AND SAFETY OF ANIDULAFUNGIN IN THE TREATMENT OF INVASIVE CANDIDIASIS IN NEUTROPENIC PATIENTS: ANALYSIS OF POOLED DATA FROM FIVE PROSPECTIVE STUDIES

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Background: Invasive candidiasis is associated with high morbidity and mortality, especially in relation to neutropenia. Anidulafungin has been studied primarily in non-neutropenic patients.
Aims: Here we present the results of a pooled analysis of data from one randomized, double-blind, comparative study and four open-label, non-comparative studies to determine the efficacy and safety of intravenous (IV) anidulafungin in the treatment of invasive candidiasis (IC) in patients with neutropenia.
Methods: Data from neutropenic patients (baseline absolute neutrophil count [ANC] ≤500 cells/mm^3 or classified as neutropenic by the investigator) with microbiologically confirmed IC were pooled. Patients received a single IV loading dose of anidulafungin 200 mg on Day 1, followed by a once-daily maintenance dose of 100 mg for at least 14 days after the last positive culture for *Candida* species and symptom resolution. A switch to oral therapy (fluconazole or voriconazole) was permitted after at least 5–10 days of treatment with IV anidulafungin. The primary endpoint was global response (based on investigator assessment of clinical and microbiological response) at the End of IV Therapy (EOIVT) in the modified intent-to-treat population (patients who received treatment and had microbiologically confirmed *Candida* infection). Global response at the End of (all) Treatment (EOT) was a secondary endpoint. Global response of success was defined as a clinical response of cure/improvement and a microbiologic response of eradication/presumed eradication.
Results: In total, 46 anidulafungin-treated patients (mean age 56 years) were included in the analysis. The most common *Candida* species were: *C. tropicalis*, 16 (34.8%); *C. krusei*, 9 (19.6%); *C. parapsilosis*, 8 (17.4%); *C. albicans*, 7 (15.2%), *C. glabrata*, 7 (15.2%), and *C. kefyr*, 4 (8.7%). Sites of infection were: blood only, 39 (84.8%); blood and other sterile site, 2 (4.3%); and other sterile site only, 5 (10.9%). The median duration of neutropenia (in the 28 patients who had baseline and post-baseline ANC) was 16.0 days (range 1–43). The global response success rate at EOIVT was 56.5% (95% CI 42.2, 70.8). Similarly, the global response success rate at EOT was 52.2% (95% CI 37.7, 66.6). In patients with resolved or persistent neutropenia at the time of the last dose of study treatment, the global response success rate at EOIVT was 80.0% (12/15; 95% CI 59.8, 100.0) and 53.8% (7/13; 95% CI 26.7, 80.9), respectively. The safety profile was similar to previous studies. All-cause mortality by Day 14 and Day 28 post-treatment was 19.6% (9/46) and 23.9% (11/46), respectively.
Summary and Conclusion: In this group of 46 neutropenic patients with IC treated with anidulafungin, the global response success rate was comparable to published reports in which resolution of neutropenia was associated with a favourable response to treatment.

P1188

USE OF ANTIFUNGAL DRUGS IN REAL CONDITIONS OF MEDICAL PRACTICE IN HAEMATOLOGY: A FRENCH MULTICENTER PROSPECTIVE STUDY (AFHEM)

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Background: The mortality rate associated with invasive fungal infections (IFI) remains very high (≥ 40 %), justifying the necessity of gaining a strong understanding of medical practices in terms of systemic antifungal therapies. This is particularly important in haematology units that closely follow the most highly exposed populations such as long term neutropenic patients or recipients of allogeneic HSC transplantation.
Aims: Assess the use of antifungal drugs in real conditions of medical practice in haematology in France.
Methods: Multicenter, cross-sectional, prospective French observational study conducted in 24 haematology units (717 beds including 368 sterile beds with about 20% of paediatric beds) over 5 consecutive days on adult or paediatric patients suffering from haematological malignancies and hospitalized during the 5-day period.
Results: Six hundred and fifty-five patients were enrolled in the register. Among evaluable patients (N=627), 276 (44%) received at least one systemic antifungal drug during the 5 days as prophylaxis (N=215, 78%), as empirical

treatment (N=30, 11%) and as curative or pre-emptive treatment (N=40, 14%). Overall, 494 patients (paediatrics, 13%) signed a consent form to participate into the extended study, with 38% of patients transplanted (including 25% of allogeneic HSCT, 8% of autologous transplant, and 5% of both) and 63% put into a sterile room. At inclusion, 87% of patients had not developed a previous IFI, 90% did not present a persistent fever on antibiotics and 74% had no neutropenia exceeding 10 days. At inclusion 50% of the patients received both antibiotic and antiviral treatment at inclusion and 82% were administered chemotherapy and/or monoclonal antibodies and/or immunosuppressive treatment. Forty-one percent of ALL, AML or MDS patients had no antifungal treatment, 43% received antifungal prophylaxis, 6% received empirical treatment and 10% received either pre-emptive or curative treatment. Of the NHL, HL, CLL or Myeloma 68% of patients did not receive any antifungal therapy and 84% of those who received an antifungal drug received prophylaxis. As expected, transplanted patients, patients presenting neutropenia for at least 10 days and patients in partial or complete remission were more likely to receive an antifungal drug (74%, 65% and 56% of these patients, respectively). Multivariate analysis showed that persistent fever refractory to antibiotherapy, duration of hospitalization over 30 days and ALL, AML or MDS are predictive factors for documented treatment (either curative or pre-emptive). Focusing on antifungal prophylaxis, this early strategy was associated with allogeneic HSCT, entry in sterile room and antibiotherapy.

Summary and Conclusion: As IFIs are life threatening and their early diagnosis is difficult to establish, primary antifungal prophylaxis is highly recommended in current guidelines (ECIL, IDSA, ASBMT). This study shows that one third of patients hospitalised in haematological units receive antifungal prophylaxis and about half receive systemic antifungal drugs. Globally, these data provide new insights into the management of IFIs in French haematology units, which can help to improve patient management, especially identifying those who are more likely to require antifungal treatments. Empirical treatment is no longer predominating in antifungal strategy and is replaced by prophylaxis. Factors associated with antifungal prophylaxis were allograft (74% of patients), prolonged neutropenia (65%) and partial or complete response to chemotherapy (56%).

P1189

AEROSOLIZED LIPOSOMAL AMPHOTERICIN-B TO PREVENT INVASIVE PULMONARY ASPERGILLOSIS IN ACUTE MYELOID LEUKEMIA. EFFICACY AND COST-EFFECTIVENESS IN REAL-LIFE

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Background: Chemotherapy induced neutropenia can be complicated by invasive pulmonary aspergillosis (IPA). In 2008, liposomal amphotericin-B (L-AmB) inhalation was shown to prevent IPA in a placebo controlled trial. Patients with acute myeloid leukemia (AML) are the subset of hematology patients at highest risk for IPA. In 2008, L-AmB inhalation prophylaxis became standard of care for all AML patients in our hospital.

Aims: In this study, we evaluated the efficacy and cost-effectiveness of L-AmB inhalations in a prospective cohort of AML patients.

Methods: 127 consecutive AML patients received chemotherapy and prophylactically inhaled L-AmB during their first and second chemotherapy course. 108 patients treated for AML at the same sites from 2005 to 2008 served as controls. A standardized diagnostic protocol was used and probable or proven IPA served as the primary endpoint. Also, diagnostic and therapeutic costs were comprehensively analyzed and compared.

Results: A significant decrease in probable/proven IPA in the L-AmB inhalation group was observed(L-AmB 9%, control 23%, p=0.0064). Systemic antifungal therapy given at any time during the entire AML therapy decreased from 49· 5% to 27· 0%. Per patient equipment and drug costs for the L-AmB inhalation (1292 euro/patient) were more than compensated by a decrease in costs for diagnostics and therapeutic voriconazole use (minus 1816 euro). No L-AmB inhalation related serious adverse events were seen.

Summary and Conclusion: In an unselected AML patient group, L-AmB inhalation resulted in a significant and substantial decrease in IPA and was cost saving. Now that azole resistant becomes more frequent, non-azole based prophylaxis may become an attractive strategy.

P1190

HIGH RISK OF INVASIVE FUNGAL INFECTIONS (IFI) IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) PATIENTS RECEIVING INDUCTION AND SALVAGE CHEMOTHERAPY WITH ITRACONAZOLE PROPHYLAXIS – TIME TO CHANGE GAME PLAN

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Background: Background: IFI is a major cause of morbidity and mortality in patients receiving chemotherapy for hematologic malignancies. It is well reported in acute myeloid leukemia (AML) patients and patients undergoing bone marrow transplant. IFI in acute lymphoblastic leukemia (ALL) patients is less well studied, despite more intensive use of steroids in chemotherapy regimens for these patients.

Aims: Aims: We aim to determine IFI incidence in adolescent and adult ALL patients receiving chemotherapy, examine the clinical features and outcomes of patients with IFI and efficacy of itraconazole as antifungal prophylaxis.

Methods: Methods: Patients treated with chemotherapy for ALL diagnosed between January 1999 and May 2013 were included in the study. A retrospective review of casenotes and data in Leukemia Registry (institutional board approved) was made for clinical profile, disease characteristics and IFI management. IFI was defined using EORTC criteria (De Pauw, CID 2008).

Results: Results: Twenty nine cases of IFI were diagnosed, giving an incidence of 13.9%, using patients undergoing chemotherapy as a denominator. The median age for patients developing IFI was 36 years (19-61). The main chemotherapy regimens used included HCVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate, cytarabine), FLAG (fludarabine, cytarabine, GCSF) and HK-SG ALL protocol. Itraconazole was the antifungal prophylaxis used in all cases. Nineteen (65.5%) cases occurred during induction and salvage chemotherapy. Eighteen cases (62%) had a duration of neutropenia (ANC<500/mm3) lasting longer than 14 days in the admission with IFI. There were 25 proven (86.2%) and 4 probable cases. There was no significant difference in incidence of IFI during the period of heavy construction around haematological wards in May 2007-2013 compared to the period 1999 – April 2007. The IFI incidence was 13.6% and 14.2% respectively. CT thorax was done in 26/29 (89.7%) of cases, with 12/26 (46.2%) demonstrating classical criteria of IFI (halo, air crescent sign, cavity in consolidation). Amongst the cases, aspergillosis accounted for 10, Candida bacteremia in 4, fusariosis in 2, mucormycosis in 1, trichosporon infection in 1, cryptoccoccal infection in 1 and other moulds in 4. IFI did not result in a delay in chemotherapy in only 17.2% of cases. Mortality attributable to IFI was 31.3%, with patients surviving less than 6 months from IFI diagnosis.

Summary and Conclusion: Conclusions/Summary: The incidence of IFI in ALL patients on induction and salvage chemotherapy is unacceptably high (13.9%). This incidence in AML patients receiving chemotherapy for the same period was 8.9%. The majority of infections occurred during induction and salvage chemotherapy, with patients having prolonged neutropenia. These invasive fungal infections resulted in significant delay in chemotherapy. Mortality directly attributable to IFI was high at 31.3%. Alternative antifungal prophylaxis, rather than itraconazole use, is urgently indicated.

P1191

A SINGLE-INSTITUTION RETROSPECTIVE ANALYSIS OF ANTIFUNGAL TREATMENTS IN ONCO-HEMATOLOGIC PATIENTS

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Background: Invasive fungal infections (IFIs) are a leading cause of morbidity and mortality in hematologic patients (pts). The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG), have classified IFIs as proven, probable or possible based on diagnostic certainty.

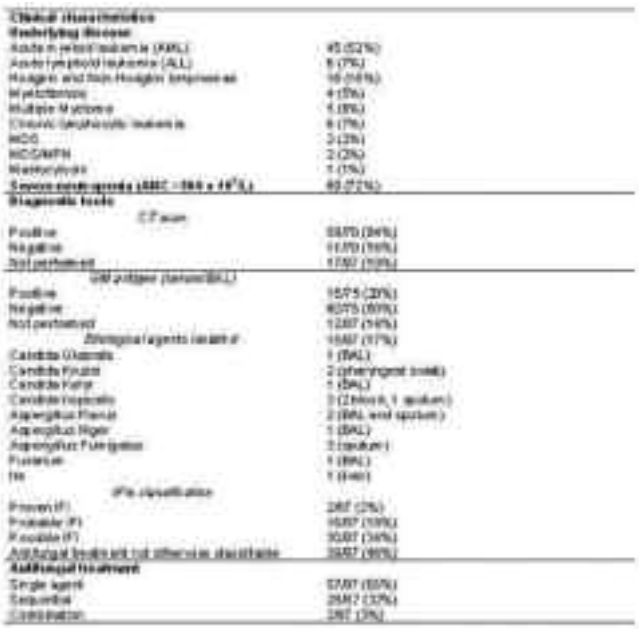
Aims: To describe antifungal treatments (AFtx) in a series of consecutive hematologic pts; to test the applicability of the EORTC/MSG criteria in a real life clinical setting.

Methods: Among 700 consecutive pts admitted to our Unit from January 2010 until July 2013, we selected those who underwent AFtx, for a total of 87 AFtx: 83 pts received AFtx, 4 was treated twice during 2 different hospitalizations. The clinical variables analyzed were: hematologic disease, neutropenia, CT scan (CTs), galactomannan antigen (GMa), mycological cultures, EORTC/MSG criteria, AFtx type (single agent, sequential, combination) and outcome.

Results: Table 1 summarizes prognostic factors, diagnostic results, IFIs classification and AFtx performed in our pts. Chest CTs (cCTs) results were distributed as follows: 25/45 AMLs were positive, 8 negative and 11 not done; 3/6 ALLs were positive and 3 negative; 13/16 lymphomas were positive, 1 negative, and 2 not done; finally, 16/20 of the group of "other hematologic malignancies" were positive and 4 not done. Two pts (1 AML, 1 ALL) underwent abdomen CTs and laparoscopic biopsies for hepatosplenic mycoses. Serum or BAL GMa was positive in 6/38 (16%) AMLs, 1/6 (17%) ALLs, 4/15 (27%) lymphomas and 4/16 (25%) "other hematologic malignancies". GMa was positive in 13 (24%) and negative in 41 (76%) of the 54 positive CTs. Conversely, GMa was positive only in one of the 11 negative CTs (9%). Yeasts were isolated in 7, moulds in 8 cases; GMa was positive in 3 (20%) of these (A niger, BAL; A fumigatus, sputum; Fusarium, BAL). The 87 cases could be classified according to EORTC/MSG criteria as follows: 2 proven (2%), 16 probable (18%) and 30 possible (35%); nevertheless, 39 pts (45%) could not

be classified because they lacked alternatively clinical or host criteria. Multiple AFtx were administered to 16/45 (36%) AMLs; 6/16 (37%) lymphomas; 9/20 (45%) "other hematologic malignancies". During hospitalization, 13 pts died (15%) and presented the following characteristics: CTs: 7 positive (54%), 1 negative (8%), 5 not performed (38%); GMa: 9 negative (69%), 4 not searched (31%); mycological cultures: 1 positive (A flavus, sputum); IFIs classification according to EORTC/MSG criteria: 4 cases (31%) classified as possible, while 9 (69%) were not classifiable; 9 pts (69%) were treated with mono AFtx, 4 (31%) with sequential or combination AFtx.

Table 1.



Summary and Conclusion: In our series the prevalence of AFtx was 12% (87/700) in 43 months. The worst outcome was observed in complex cases with a poor diagnostic definition characterized by requirement of combination AFtx, CTs and GMa search not performed, no mycological isolates, not applicability of EORTC/MSG criteria. The fact that 39 cases could not be classified according to EORTC/MSG criteria should be further investigated: it may reflect a low discriminating power of these criteria in onco-hematologic setting or may highlight the need for clinical studies and/or new diagnostic tools (such as 1-3-β-D-glucan determination) in order to diminish the number of cases in whom an AFtx is inappropiately delivered or, on the contrary, dangerously postponed.

P1192

COMPARISON OF GALACTOMANNAN DETECTED IN SERUM/ BRONCHOALVEOLAR LAVAGE FLUID AND TYPE OF ABNORMALITY ON PULMONARY HIGH RESOLUTION COMPUTER TOMOGRAPHY IN PATIENTS WITH PULMONARY INVASIVE ASPERGILLOSIS

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Background: Up to now there have been missing data in the literature concerning relationship of galactomannan (GM) detected in bronchoalveolar lavage (BAL) fluid and type of abnormality on pulmonary high resolution computer tomography (HRCT) in patients (pts.) with pulmonary invasive aspergillosis (IPA).
Aims: To evaluate relationship between sensitivity of GM detected in BAL fluid and serum (S-GM) and type of abnormality on pulmonary HRCT in pts. with IPA.
Methods: We retrospectively analyzed mentioned relationship in pts. with probable and proven IPA entered in Fungal InfectioN Database (FIND)" *aspergillus* between 2001 and 2012.
Results: In total there were 143 IPA cases with GM detected in BAL fluid analyzed at the time of diagnosis. Overall positivity of GM in BAL fluid (1 sample ≥ 0.5 IP) was 86% in these pts. Sensitivity of GM in BAL fluid was significantly higher in pts. with pulmonary nodules than infiltrates on HRCT (89.7% *vs.* 76.2%; p=0.043). There was no statistically significant relationship between value of GM in BAL fluid and type of HRCT finding (p=0.690). S-GM was detected in 312 pts. with IPA. Overall positivity of S-GM (2 consecutive samples ≥ 0.5 IP) was 68.6% in these pts. Similarly to GM in BAL fluid there were proved statistically significant difference in sensitivity of S-GM in pts. with nodules and infiltrates on HRCT (78.0% *vs.* 62.0%; p=0.004). There was also found no statistically significant relationship between value of S-GM and type of HRCT finding (p=0.321).
Summary and Conclusion: Our study proved higher sensitivity of both GM in BAL fluid and S-GM in IPA pts. with more specific findings – nodules compare to non specific findings – infiltrates on HRCT.

P1193

CLINICAL EFFECTIVENESS OF PROPHYLACTIC VORICONAZOLE IN CHILDREN TREATED FOR HAEMATOLOGICAL MALIGNANCIES

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Background: Invasive fungal infections (IFI) have become high prevalence in patients with haematologic malignancies receiving chemotherapy. The risk of IFI is associated with the degree and duration of neutropenia, the disruption of mucosal barriers and the prolonged use of antibiotics and corticosteroids. Prophylaxis includes the use of antifungal drugs in all patients at risk. Voriconazole is a triazol antifungal agent that exhibits broad antifungal activity against both Aspergillus and Candida (including non – albicans) species, which has been used for the treatment of invasive candidiasis and aspergillosis. Its use as a prophylactic agent is under study, especially in children
Aims: The aim of the study was to evaluate the efficacy and safety of voriconazole as antifungal prophylaxis in children suffering from haematological malignancies with chemotherapy induced neutropenia.
Methods: The files of 136 children were retrospectively reviewed. They divided into two groups: group A of 69 children [42 Acute Lymphoblastic Leukemia (ALL), 1 mature B ALL, 8 Acute Myelogenous Leukemia (AML), 16 Non – Hodgkin Lymphoma (NHL), 2 Hodgkin Disease (HD) and group B of 67 [40 ALL, 1 mature B ALL, 5 AML, 14 NHL, 7 HD]. All patients were treated at a single institution, between January 2006 and December 2013 and received at a fungal prophylaxis with voriconazole during chemotherapy induced neutropenia. Age, gender, diagnosis, treatment regimen and laboratory investigations (WBC counts and liver function tests) were recorded. Voriconazole was at a dose of 4mg/Kg bid in group A and 7mg/Kg bid in group B, intravenously or orally.
Results: In total, 467 patient – cycles (236 in group A and 231 in group B) were studied. Median duration of neutropenia after chemotherapy during which time voriconazole prophylaxis was administered, was 17 days. Treatment success rate defined as absence of proven, probable, possible or suspected IFI was 97.8% [95.64% (229/236) for group A, 98.2 (231/227) for group B]. Proven IFI was documented in two patients with ALL (1 pulmonary aspergillosis, 1 Candida albicans fungemia, group A), 2 had probable IFI, (splenic candidiasis, 1 group A and 1 group B) and in 7 cases were considered suspected IFIs (4 group A and 3 group B). No severe adverse reactions or interactions with concurrent medications were observed that could be attributed to voriconazole administration.
Summary and Conclusion: These results suggest that voriconazole is an effective and well – tolerated alternative for antifungal prophylaxis in children with hematological malignancies during chemotherapy induced neutropenia. Randomized, double – blind, case control studies are needed to confirm the efficacy of this approach in comparison to standard treatments.

P1194

IMPACT OF TOLL-LIKE RECEPTOR 4 POLYMORPHISMS ON INFECTIOUS COMPLICATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Infectious complications continue to be one of the major causes of morbidity and mortality in patients with acute myeloid leukemia (AML). Several single nucleotide polymorphisms (SNPs) may contribute to genetic susceptibility to infections or even sepsis. Recent studies have identified a potential association of two SNPs of the Toll-like receptor 4 (TLR-4) gene (Asp299Gly and Thr399Ile) and infectious events.
Aims: We sought to investigate the impact of these TLR-4 SNPs on developing infections in 172 patients with newly diagnosed AML during anthracycline-based induction chemotherapy (cytarabine combined with idarubicin or mitoxantrone). The incidences of neutropenic fever, blood stream infections, pneumonia, central venous catheter infections and the occurrence of sepsis were analysed.
Methods: Analysis of TLR-4 SNPs was performed by pyrosequencing and revealed 151 of 172 patients to be homozygous for the wild-type genotype. In contrast, 20 patients (11.6 %) were heterozygous for both SNPs while one patient (0.6 %) was heterozygous for the Thr399Ile SNP.
Results: We can demonstrate that patients carrying the TLR-4 SNPs developed significantly more episodes of neutropenic fever (p=0.031) and the duration of fever was significantly longer (p=0.032). The probability of pneumonia was also significantly higher among patients carrying the polymorphisms (28% *vs*. 65%, p=0.004). In contrast, no difference was found between patients with TLR-4 wild-type genotype and those heterozygous for both SNPs with regard to blood stream infections and the occurrence of central venous catheter infections. Importantly, the detection of TLR-4 SNPs shows a strong association with the development of sepsis (40% *vs*. 85%, p=0.00019).
Summary and Conclusion: To our best knowledge, this study represents the first analysis demonstrating that a haplotype containing two commonly segregated polymorphisms (Asp299Gly and Thr399Ile) of the human TLR-4 gene influences the risk of serious infections in patients with acute myeloid leukemia undergoing induction chemotherapy.

P1195

THE EXPRESSION OF TOLL-LIKE RECEPTORS AND DEVELOPMENT OF SEVERE SEPSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIAS

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Background: Toll-like receptors (TLRs) play an important role in the host defense against microorganisms. TLRs are mainly expressed in human immune related cells such as monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells. They effect is connected with secretion of cytokines and chemokines that recruit immune cells death that limits microbe expanding. Sepsis remains a common cause of mortality in patients with acute myeloid leukemia (AML) treated with intensive induction chemotherapy. Toll-like receptors play a key role in the mediation of systemic responses to pathogens during sepsis. The expression of TLRs and their association with the development of sepsis in patients with acute myeloid leukemia remains unclear.
Aims: The aim of this study was to investigate the possible associations between expression of TLR2, TLR4 and TLR9 and occurrence of sepsis in patients treated with intensive induction chemotherapy for AML.
Methods: 103 patients with newly diagnosed acute myeloid leukemia (AML) were evaluated (47 females and 56 males). The median age of patients was 51 years. The diagnosis was performed according to the WHO criteria for AML. The healthy control group included 20 age-matched individuals (9 females and 11 males). Bone marrow samples were taken before induction therapy. Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured. The relative quantitation was indicated by cycle threshold (Ct) values. The Ct value of the target genes was normalized (*Δ*Ct) to the Ct value of the GUS gene of the samples. The results were statistically analysed using 'STATISTICA 8,0'. Statistical analysis was performed by means of Mann-Whitney's U-test and p<0,05 indicated a significant difference.
Results: All patients with AML were treated with intensive induction chemotherapy. Prophylactic oral chinolones were used in all patients. Neutropenic fever occured in 98 patients (95%). In 62 patients (60%) infectious agent was found. We identified 20 episodes of severe sepsis (20%). 10 patients with symptoms of severe sepsis died from infection. Gram-negative bacteria were more commonly found in patients with sepsis. TLR2 and TLR4 mRNA expression was higher in patients with neutropenic fever than in group with neutropenia without sign and symptoms of infection after chemotherapy although the difference was not statistically significant. Among the patients with neutropenic fever the mRNA expression of TLR2 and TLR4 was significant higher in septic patients than in patients without sepsis symptoms (ΔCt TLR2 0,93±0,82 *vs* 0,78±0,85 and *Δ*Ct TLR4 0,35±0,29 *vs* 0,38±0,25). Moreover we observed that expression of TLR2 and TLR4 was significant higher in patients with AML and bacterial infection in comparison to group with separate fungal infection (ΔCt TLR2 1,15±1,06 *vs* 0,66±0,51 and *Δ*Ct TLR4 0,45±0,38 *vs* 0,21±0,19). The results are shown in table 1.

Table 1. Correlation between mRNA expression of TLRs and sepsis in AML patients.

	Sepsis n=20	No sepsis n=83	p
ΔCt TLR2	0,93±0,82	0,78±0,85	<0,01
ΔCt TLR4	0,35±0,29	0,38±0,25	<0,01
ΔCt TLR9	0,002±0,001	0,004±0,003	ns
	Bacterial infection n=43	**Fungal infection n=20**	**p**
ΔCt TLR2	1,15±1,06	0,66±0,51	<0,01
ΔCt TLR4	0,45±0,38	0,21±0,19	<0,01
ΔCt TLR9	0,002±0,001	0,001±0,002	ns

n=number of patients
ns=not significant

Summary and Conclusion: Our results suggest that TLRs could be an independent factor for development of sepsis in patients with acute myeloid leukemias after intensive induction chemotherapy. This observation should be validated by larger study.

P1196

ANTIBACTERIAL PROPHYLAXIS AND EMERGING RESISTANT STRAINS IMPACT ON EPIDEMIOLOGY AND OUTCOME OF BLOODSTREAM INFECTIONS IN ACUTE LEUKAEMIA: A PROSPECTIVE STUDY BY THE RETE EMATOLOGICA LOMBARDA (REL)

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Background: Bloodstream infections (BSI) are a considerable problem in acute leukaemia (AL) patients (pts), with mortality ranging from 5 to 10%. Moreover, Gram-negative rods (GNR) and multiresistant (multiR) bacteria are emerging pathogens and may negatively impact on the epidemiologic scenario.
Aims: In order to better define the very recent epidemiology and outcome of BSI in a real-life setting, we planned a prospective study collecting all consecutive febrile/infectious episodes occurring in AL pts admitted to 9 haematological institutions participating to REL, representing about 75% of the entire AL population treated in Lombardy.
Methods: From Dec-2012 to Jan-2014, all febrile/infectious episodes were recorded. The following data concerning BSI were analysed: age, gender, type and phase of leukaemia, neutropenia, fluoroquinolone (Fq) prophylaxis, presence of central venous catheter (CVC), concomitant pulmonary infiltrates, antibiotic resistance.
Results: In 158 AL pts (M/F 92/66; median age 54y, range 19-78; AML/ALL 132/24), 216BSI were diagnosed. In 120 (55%) BSI occurred in pts on Fq prophylaxis. In 48 (22.2%) pneumonia was also present. CVC-related BSI were observed in 29.2% of cases. Gram positive cocci (GPC) were isolated in 93/216 BSI (43.1%); GNR in 88 (40.7%), polymicrobial (PMB) aetiology in 33 (15.3%) and fungi (F) in 2 (0.9%). Coagulase-negative staphylococci (CoNS) were the most frequent GPC (69/216, 31.9%); *S. aureus*, *S. viridans* and enterococci were observed in 6 (2.8%), 11 (5.1%) and 20 (9.3%) cases, respectively. Methicillin-resistant strains accounted for 90.6% of all staphylococci and vancomycin-resistant strains for 10% of enterococci. CVC-rel BSI was independently correlated with GPC aetiology (GPC 60% *vs* GNR 36.5%) (p=0.001). Considering GNR, enterobacteriaceae were isolated in 88/216 BSI (40.7%) and *P. aeruginosa* in 20 (9.3%). GNR occurred more frequently during consolidation cycles (49% *vs* 34.2%) and in pts not on Fq prophylaxis (47.9% *vs* 35%). Both conditions were independent risk factors at multivariate analysis (p=0.009 and p=0.004, respectively). Fq resistant strains were recorded in 91% of isolates. ESBL+ strains, which accounted for 25% of enterobacteriaceae, were also more frequent in consolidation cycles (16.7% *vs* 4.7%, p=0.005) and Fq prophylaxis (14.2% *vs* 5.2%, p=0.03). Carbapenemase producing (CP)

strains occurred in 8% of enterobacteriaceae. Among *P. aeruginosa* strains, 15% were multiR. Thirty-day mortality was 6.5% (14/216); it was lower for GPC (3.2%) and similar for GNR and PMB BSI (9.1%). CP enterobacteriaceae or multiR *P. aeruginosa* BSI (30d mortality: 41.7%; OR 71.2, CI 8.7-580.8, p<0.0001) but not ESBL+ strains were independent predictors of death. Furthermore, having relapsed/resistant AL (15.6%; OR 10.5, CI 2.0-55.3, p=0.006), and the presence of concomitant pulmonary infiltrates (20.8%; OR 15.3, CI 3.0-77.9, p=0.001) significantly correlated with the risk of death at multivariate analysis.
Summary and Conclusion: In our real-life prospective study, the very recent bacterial epidemiology of AL pts was sustained by similar percentages of GPC and GNR. Fq prophylaxis was nearly ubiquitous and significantly predicted GNR and ESBL+ aetiology, which occurred even during consolidation cycles. While ESBL+ did not impact on survival, both multiR- and CP-bacteria correlated with a higher risk of death. Interestingly, also a concomitant diagnosis of pneumonia during BSI was a strong predictor of a poorer outcome. The usefulness of Fq prophylaxis has to be reanalysed in this new epidemiologic scenario.

P1197

NOSOCOMIAL MULTIDRUG-RESISTANT KLEBSIELLA PNEUMONIAE SPREAD AS THE MAJOR CAUSE OF SEVERE INFECTIONS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES
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Background: Bacterial infections are more frequent in persons with impaired function of the immune system and/or with neutropenia. In neutropenic patients with haematological malignancies the use of chemotherapeutic drugs contributes to a further immunosuppression with reduction of body's defence mechanisms. In the last few years multidrug resitant *K.pnemoniae* is an emerging threat as nosocomial pathogen.
Aims: The aim of this study was to determine the prevalence of *Klebsiella pneumoniae* strains in patients with haematological malignancies in our ward, its spread, the origin of the infectious agent and in future to assess the clinical significance.
Methods: More than 1600 inpatients have been investigated in our haematological ward during three years; from 2010 up to October 2013. Hemocultures from 460 hospitalized patients with suspected blood stream infection were performed and isolated strains were identified by standard microbiological procedures.
Results: In 170 inpatients (37%) the presence of isolated strains was revealed. *Klebsiella pneumoniae* was present in 20% of the patients (n=35); 22 out of 35 cases occurred in acute myeloid leukaemia patients (AML) all presenting with severe neutropenia. The 90% of the isolates belonged to a multidrug-resistant strain showing *in vitro* resistance mainly to carbapenems and fluoroquinolones. Around 70% of all the resistant *Klebsiella pneumoniae* isolates were sensitive only to colistin. The mortality among hospitalized patients with K *pneumoniae* was of 40% (n=14) and 93% of them were affected by multidrug resistant K *pneumoniae* while the 89% of multidrug resistant K *pneumoniae* was detected in 60% of patients (n=21) with a good outcome. During the four year study period there has been a dramatic increase in the prevalence of K. *pneumonia spp* of about five-fold.
Summary and Conclusion: The first strain of multidrug resistant K *pneumoniae* was identified in our ward in 2010, since then there has been a dramatic spread in the occurrence of this pathogen during the study period. The infections due to K. *pneumonia* occurred almost in patients treated with aggressive therapy or in pluritreated patients presenting with profound and prolonged cytopenia. Further, this study will allow us to evaluate emergence of multidrug resistant strains among K *pneumoniae* species in our ward in order to implement efficient infection control to limit the spread of this pathogen.
Keywords: *Klebsiella pneumoniae,* multidrug-resistant strain, haematological malignancy

P1198

THE INCREASING PROBLEM OF BLOODSTREAM INFECTIONS CAUSED BY MULTI-DRUG RESISTANT BACTERIA IN HEMATOLOGIC AND HSCT PATIENTS
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Background: Multi-drug resistant (MDR) bacteria infections are an increasing problem in recent years.
Aims: We evaluate the incidence and outcome of bloodstream infections (BSI) caused by multi-drug resistant (MDR) bacteria in patients (pts) with hematologic diseases and in hematopoietic stem cell transplantation (HSCT) recipients.
Methods: 256 BSI have been recorded between october 2008 and october 2013 in 187 pts. The characteristics and outcome of MDR-BSIs have been evaluated. MDR-BSIs and multi-drug-sensible bacteria-BSI (MDS-BSI) have been compared. Moreover the results were evaluated according to the status of underling hematologic disease and the HSCT.
Results: 316 isolates have been documented in 256 BSIs. Among these, 55 (17%) bacteria were MDR and 261 (83%) were MDS. MDR isolates were: MDR *Pseudomonas aeruginosa*, 28/55 (51%); *Escherichia coli* ESBL, 16/55 (29%); *Stenotrophomonas maltophilia*, 6/55 (11%); *Klebsiella pneumoniae*-KPC, 4/55 (7%); *Enterococcus sp*-VRE, 1/55 (2%). A progressive increase of MDR-BSI was documented in the last two years (with 2 episodes/month in 2013). Septic shock and infection related mortality were significantly higher in the MDR-BSI compared to MDS-BSI group (36% *vs* 12% and 35% *vs* 11%, respectively – P less than 0,001). Occurrence of septic shock was particularly elevated in KPC-BSI (75%) and in MDR- *Pseudomonas aeruginosa*-BSI (50%). Pts with refractory/relapsed hematologic disease had higher infection mortality rate (70%). In the MDR-BSI group, a higher mortality rate was observed in HSCT recipients, (52% in HSCT *vs* 20% in not-HSCT, P less than 0,05).
Summary and Conclusion: MDR-BSI (particularly MDR-*Pseudomonas aeruginosa*-BSI and KPC-BSI) are a serious and growing problem in hematologic pts. The onset of MDR-BSI is often associated with septic shock and with high mortality rate, despite a target and combined antibiotic therapy. Mortality MDR-BSI related is particularly high in HSCT recipients (52%) and in pts with refractory/relapsed hematologic disease (70%). These results underline the urgent need of guidelines for surveillance and treatment of hematologic and HSCT pts with MDR bacteria colonization or/and with MDR active infection.

P1199

INTERVENTIONAL STRATEGIES TO CONTAIN COLONIZATION AND INFECTIONS CAUSED BY CARBAPENEMASE PRODUCING KLEBSIELLA PNEUMONIA IN HAEMATOLOGICAL MALIGNANCIES:A SINGLE CENTRE EXPERIENCE
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Background: The emergence and dissemination of Carbapenemase producing *Klebsiella pneumoniae* (KPC) represent a serious threat to public health and is associated with high mortality rates in patients with hematologic malignancies. Several risk factors (prior exposure to or current use of antibiotics, extended stay in hospital, neutropenia, sharing a room with a know carrier etc) have been identified for colonization in hematological patients. However, data on how to contain their spread in haematological setting are still surprisingly limited.
Aims: We carried out a prospective investigation to assess the prevalence of KPC colonization among hematologic patients, the impact of a strategy control to limit the spread and to evaluate the efficacy of an implementation of more extensive active surveillance.
Methods: Infections caused by multiresistant pathogens have been recorded in our Unit for a two year period (January 2012-December 2013). From January 2012 to July 2013, we developed an intensive control infection program, (Plan A): 1. Weekly colonization screening for KPC. 2. Educate healthcare personnel about KPC. 3. Promotion of hand hygiene. 4. Physical separation of carriers from non-carriers. 5. A double-carbapenem plus colistin therapeutic empiric regimen was given for blood stream infections. Since July 2013, we decided to implement additional measures, including (Plan B): 1. Drastic reduction in the number of beds. 2. 2% chlorhexidine body washing for colonized patients. 3. Ensure access to adequate hand hygiene stations (*i.e.*, clean sinks and/or alcohol) and ensure they are well stocked with supplies (*e.g.* towels, soap, etc.). 4. Donning gown and gloves before entering the affected patient's room and removing the gown and gloves and performing hand hygiene prior to exiting the affected patient's room.
Results: Since January 2012 perianal swabs were obtained weekly from 466 consecutive patients, for a total of 831 admissions and 10.125 days of hospital stay. KPC colonization was present in 40 out of 466 screened patients at some time during their (often multiple) hospitalizations (8.58%); 10 bloodstream infections were reported in 9 patients (25%). Three deaths (3/10, 33%) due to KPC were reported before implementation screening (overall mortality rate was 8% of colonized patients). KPC spontaneous decolonisation was achieved in 8/40 pts (20%) after a median duration of 85 days (range 21-115 days). Most patients who recovered from KPC infection or were just colonized by KPC went on to receive additional chemotherapy without any life threatening KPC infection occurring (only one patient reported two septic episodes). Since screening cultures or further clinical cultures identified a progressive increase KPC-colonized or -infected patients (see in table 1) we decided to implement additional measures (Plan B). Therefore, from fourth trimester of study, the rate of new colonizations was very low: 2 patients (4.17%) have been reported, *vs* 15 patients (30%) in the third trimester.

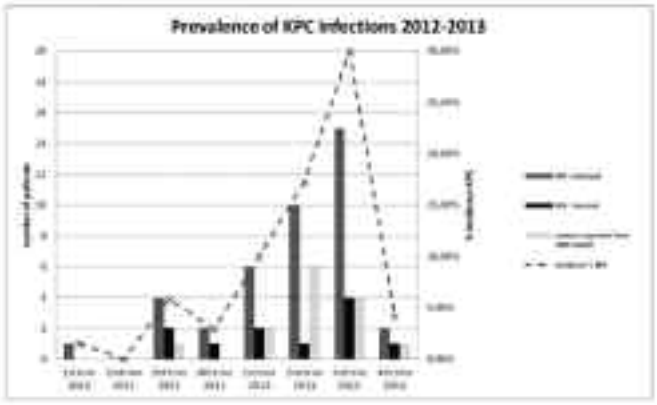

Figure 1.

Summary and Conclusion: The prevalence of KPC colonization in our hospital is high among patients with hematologic diseases. An implementation of additional measures, sharing the patients in single bed-rooms and consequently limiting transfer of cases from other wards, was able to contain KPC colonization and infection. However, the success of our preliminary interventions should be monitored constantly; besides, to prevent the emergence and further spread of KPC, a coordinated regional control effort among healthcare facilities should be recommended.

P1200

CLOSTRIDIUM DIFFICILE INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY IN CZECH AND SLOVAK REPUBLIC – A RETROSPECTIVE ANALYSIS

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Background: *Clostridium difficile* infections (CDI) with associated diarrhea represent an important cause of morbidity and mortality. Despite its importance, there is still lack of data about this infection in the group of hematological patients.
Aims: To analyze the risk factors, clinical manifestation, treatment and its outcome in this high risk patients group.
Methods: We performed a retrospective analysis of CDI episodes in 4 hematological centers in Czech and Slovak Republic occurred between 1/2007 and 12/2012. CDI was defined as a diarrhea and/or ileus and/or toxic megacolon and microbiological evidence of toxin-producing *Clostridium difficile* in stool without evidence of another cause of these symptoms. Pts. with targeted antibiotic therapy shorter than 3 days were excluded from analysis of treatment outcome. Recurrence was defined as reappearance of diarrhea and other symptoms within 1 month after therapy.
Results: 208 episodes that occurred in 193 pts. were analyzed (male 38.5%, female 61.5%, mean age 57.3 years [18 - 88] years,). 76 pts. (36.5%) received induction/consolidation therapy for acute leukemia, 26 pts. (12.5%) underwent allogeneic stem cell transplantation (SCT) and 21 pts. (10%) autologous transplantation before CDI onset. Neutropenia was present in 102 pts. (49%) and 82 pts. (39.5%) had neutropenia grade 3 and 4 CTCAE 4.0. Before CDI onset, 132 pts. (63.5%) had antibiotic therapy (prophylactic or curative for another infection) - 23% quinolones, 11% penicillins, 19% cephalosporins, 46% combination, 1% others. 24 pts. (11.5%) were on parenteral nutrition. 49% of all episodes fulfilled criteria for severe CDI and 51% were mild/moderate CDI episodes. In pts. without neutropenia were 42.8% severe CDI, with neutropenia gr. 3 and 4 were 58.5% severe CDI and in pts. after SCT were 34.5% severe CDI. Initial therapy for CDI was: oral metronidazole 77%; intravenous metronidazole 4.8%; oral and intravenous metronidazole 1.4%; oral vancomycin 0.5%; intravenous vancomycin 2.4%; oral metronidazole and oral vancomycin 4.3%; oral metronidazole and intravenous vancomycin 4.3%; intravenous metronidazole and oral vancomycin 3.3%; intravenous metronidazole and intravenous vancomycin 2%. Colectomy was necessary in one case. Time to initial response was 6 days (mean). Overall cure rate was 92% and recurrence rate 8.3%. For oral metronidazole cure rate was 95% and recurrence rate 7.9% and for intravenous metronidazole 80% and 0%, respectively. Overall cure rate in pts. with neutropenia was 87.8% and recurrence rate 8.3%. In non-neutropenic pts. cure rate was 95.2% and recurrence rate 8.3%. In the group of pts. after allogeneic SCT was cure rate 96.1% and recurrence rate 4%.
Summary and Conclusion: Our study proved severe morbidity of CDI in pts. with hematological malignancy, where half of episodes were severe infections. Surprisingly, the overall cure rate using standard therapy (metronidazole +/- vancomycin) and frequency of recurrence was similar to non-hematological populations. Supported by Masaryk University MUNI/A/0830/2013, supported by *CELL*, supported by unrestricted grant of Astellas ltd.

P1201

INCIDENCE AND OUTCOME OF INFECTIOUS COMPLICATIONS AMONG PEDIATRIC CANCER PATIENTS RELATED TO PERMANENT CENTRAL VENOUS CATHETERS

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Background: Background: Central venous access is an important treatment modality in the current management of critically ill patients. Recent studies suggest that by strict adherence to current guidelines for insertion an maintenance, the incidence of CLA-BSI can be dramatically reduced. Proper diagnosis and treatment of CLA-BSI can reduce the morbidity and mortality associated with infection.
Aims: To asses the incidence of permanent catheter –related morbidities including blood stream infections and catheter related septicemia, systemic infection as toxic endocarditis, toxic myocarditis among immunocompromised pediatric patients, finally outcome of portcath infection(regarding salvalgibilty of the line and mortality related to central line infection.
Methods: A study done on 78 out of 151 pediatric cancer patient below age of 18 years who inserted portcath receiving chemotherapy with microbiological documented permentant central line infection in the period between 1st of October 2010 till the end of March 2012.Each episode was analyzed regarding causative organism, morbidities related as septicemia, toxic myocarditis and endocarditis, cause of removal of central line and mortality related to central line infection.
Results: There is total number of 107 episodes of portcath infection, gram + ve organisms 60/107(56%), gram – ve organisms 45/107(42%) and finally candida 2/107(2%). The most common organisms was coagulase – ve staph (28.03%).Among patients with gram + ve organisms(40 patients) 34patients(34/40)(85%) had normal echo, four patients 4/40(10 %) had septic myocarditis in form of impaired contractility,two patients 2/40 (5%) with impaired contractility &valvular affection with vegetations& valvular regurge.Regarding patients with gram – ve organisms episodes (38 patients (33/38)(86.8%) had normal echo, three patients (7.89%) had septic myocarditis and finally two patients(5.2%) with impaired contractility &valvular affection with vegetations. Porta- cath were removed in 53 patients (67.9%)due to failure of sterilization(45.3%) and sepsis(54.7%) while in 25 patients (32.1%) portcath were salvaged. 7 patient died from central line infection (9%), 5 patient died from septic shock with multiorgan failure, while 2 patient died from heart failure with impaired cardiac contractility & severe valvular affection, gram + ve were isolated in 5 patients who died while gram – ve were isolated in 2 patients.
Summary and Conclusion: gram + ve pathogens are most common cause of permenant central line(portacath) infection which required better infection control, high mortality rate related to portcath systemic infection enforce us to select patients who are in urgent need for portcath insetion.

Non-malignant hematologic disorders

P1202

A SINGLE-INSTITUTION EXPERIENCE OF PERIPHERAL INSERTED CENTRAL VENOUS CATHETERS IN ONCO-HEMATOLOGIC PATIENTS

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Background: Peripherally inserted central catheters (PICCs) represent intravenous accesses with a duration intermediate between short term (jugular or subclavian central venous catheters, CVCs), and long term devices (port-a-caths). PICCs are inserted in a peripheral vein of the arm, preferentially the basilic vein in the upper third of it, without general anesthesia or sedation. PICCs are easier to insert and safer than conventional percutaneously inserted CVCs or port-a-cath. PICC lines provide a reliable central venous access in different categories of patients, but only few prospective studies have been performed in onco-hematologic patients.
Aims: To evaluate the feasibility and the safety of the positioning and use of PICCs in onco-hematologic patients by a "Hematology Unit" team.
Methods: We evaluated 53 patients affected by hematologic malignancies (23 non-Hodgkin's lymphomas, 20 acute myeloid leukaemia, 2 acute lymphoblastic leukaemia, 4 mycosis fungoides and 2 Hodgkin's lymphomas) who required a central line insertion: 51 cases were eligible for PICC positioning. Informed consent was obtained from all patients. Thirteen PICCs were single-lumen, 38 were bi-lumen; all were of power-injectable polyurethane, except one, represented by a valved silicone PICC. All PICCs were positioned by a specifically trained team composed of one hematologist physician and 3 nurses. They were inserted by ultrasound-guided venipuncture and the microintroducer technique, under strict asepsis and maximal barrier precautions. Peripheral veins of the arm were cannulated under local anesthesia, without sedation. Chest X-ray was routinely performed to verify a correct location of the tip (ideally in the proximity of cavo-atrial junction).
Results: Of the 53 patients evaluated, 51 received PICC insertion (24 females and 27 males; median age 53, range 22-83); two patients were judged not suitable for the procedure because of inadequate vein diameter. PICC remained in place for a median period of 105 days. No major insertion-related complications were observed. Late complications were infections (5/51 patients, 10%) and thrombosis (12/51 patients, 23%). Infections represented a cause of PICC removal in 5 (10%) cases, thrombosis in 7 (14%) cases. After observing in the first 28 patients a thrombosis incidence of 32%, we started performing a 7 days prophylaxis with LMWH following PICC insertion. In the next consecutive 23 patients we reported a great reduction in thrombosis rate (from 32% to 13%) and in no cases thrombosis represented a removal cause. We compared the PICC results with those of a series of 390 jugular/subclavian CVCs inserted at our Unit from 2009 to 2012 and, regarding removal causes, we observed a lower incidence of both infections (9.8% PICC *vs* 22.5% CVC) and thrombosis, after the introduction of LMWH prophylaxis (0 *vs* 2.4%).
Summary and Conclusion: Our data suggest that PICCs are a safe and effective alternative to conventional CVC in patients receiving chemotherapy for hematologic malignancies, characterized by an higher risk of septic and coagulative (hemorrhagic/thrombotic) complications. In particular, regarding removal reasons, we observed that infections and thrombosis, when LMWH prophylaxis is performed, are significantly lower in PICCs compared to jugular/subclavian vein positioned CVCs. Furthermore, we demonstrated that this procedure may be successfully pursued by a team composed of Hematologic Unit personnel adequately trained, with positive results in terms of team motivation and improvement of the clinical processes.

P1203

USEFULNESS OF PRESEPSIN (SOLUBLE CD14-ST) AS A AN EARLY DIAGNOSTIC MARKER OF SEPSIS IN LEUKEMIA

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Background: Chemotherapy-related febrile neutropenia (FN) is a frequent complication in patients with acute leukemia. Neutropenia is a major risk factor for infection in these patients. Among patients with FN, the main symptom of infection upon initial presentation may be fever only. Therefore, a standard approach for these patients includes immediate hospitalization, empirical treatment with intravenous broad-spectrum antibiotics until they become afebrile and neutrophil count has recovered. However, in only 20–30% of children with FN a bacterial infection is determined, which results in overtreatment of these patients with consequent increasing risk of bacterial resistance development. Therefore, biomarkers having the potential to predict infectious process in FN leukemic patients are of great interest. To date, there have been many studies performed in FN patients, which assessed various inflammation-related biomarkers in terms of prediction of severe infection, however, due to a heterogeneity between the studies, limited data describing the predictive value of different biomarkers is available. Currently, Procalcitonin (PCT) is used as a marker to diagnose sepsis or severe sepsis. In comparison to other markers that have traditionally been reported, PCT gives a high rate of specificity for sepsis diagnosis. However, the concentration of PCT in the human blood is elevated in various conditions, such as in severe trauma, surgical invasive procedures, and critical burn injury, which leads to SIRS. It is also necessary to be aware of false-positive results. Therefore, more reliable biomarkers for the diagnosis of sepsis are needed. C-reactive protein (CRP) has been used for many years but its specificity has been challenged. CD14 is a cell surface glycoprotein, which is constitutively expressed in a majority of innate immune response cells and exists either in an anchored membrane form, or in a soluble form (sCD14-ST, presepsin). Presepsin, which is approximately 13 kDa, has been identified as a protein whose levels increase during sepsis and systemic inflammatory response syndrome. Presepsin is a more specific and sensitive marker for the diagnosis of sepsis compared with interleukin-6 (IL-6) and PCT. Presepsin concentrations in blood were increased faster than PCT and CRP in sepsis patients. Additionally, the measurement of Presepsin concentrations is useful for evaluating the severity of sepsis. In this study, we evaluated the diagnostic efficacy of Presepsin POCT assay system based on the chemiluminescent enzyme immunoassay (CLEIA) principle and its usefulness in the early diagnosis of infection in acute leukemic patients.
Aims: Was to study the circulating level of Presepsin and to correlate this level with patients' body temperature, complete blood count(CBC) items and CRP level as an inflammatory marker, in AML patients presenting with manifestations of sepsis.
Methods: 160 AML subjects with manifestations of sepsis (group I) and 40 healthy controls (groupII) were subjected to full history taking, complete physical examination and laboratory investigations including CBC, CRP and Presepsin.
Results: It was found that the mean value of Presepsin is higher in patients with sepsis than in healthy controls with a statistical significance. Using a cutoff level of 198 pg/ml yielded a sensitivity of 82.5% and specificity 90% with AUC 0.895. The mean cutoff level in controls was 132.5 pg/ml. Presepsin performance was better than CRP, where a 96 mg/L cutoff had to be set to obtain similar specificity and sensitivity while mean cutoff in healthy subjects was 2.3 mg/L. Also, there was a significant positive correlation between Presepsin level and patients' body temperature, total WBCs count, absolute neutrophil count.
Summary and Conclusion: it was found that Presepsin level is a mirror to the degree of severity of sepsis. To our knowledge this is the first study performed in hematological malignancies related sepsis evaluating Presepsin diagnostic power as a new promising biomarker

P1204

SEPTIC MARKERS IN HEMATO-ONCOLOGY: PROCALCITONIN IS MORE SPECIFIC THAN C-REACTIVE PROTEIN IN THE STUDY OF FEVER IN PATIENTS WITH HEMATOLOGIC DISORDERS

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Background: Fever is one of the most frequent, relevant and non-specific clinical findings in hemato-oncology patients, in whom it can be secondary to the neoplastic disease, or result from a concurrent infectious complication. Biomarkers used to clarify the etiology of a fever include C-reactive protein (CRP), an acute-phase protein that is non-specifically elevated in neoplastic disease, and procalcitonin (ProCT), whose high specificity for bacterial infection can help reduce the inappropriate use or duration of antibiotherapy. Although its validity has been demonstrated, the use of ProCT in clinical practice in hemato-oncology is non-standardized and irregular.
Aims: We aim to analyze the value added by ProCT to the management of hematology patients with a fever.
Methods: We reviewed all simultaneous determinations of CRP and ProCT in our center in the 5 years since its introduction into our Lab. Patients were stratified as "Hematologic" and "Non-Heme", "ProCT+" (≥0.5ng/mL), "CRP+" (≥0.5mg/dL) and "RF" (renal failure, creatinine≥1.2 mg/dL); microbiological culture was the gold-standard comparator.
Results: We analyzed 8512 patient-events (pts), 165 of whom were Hematologic, with a rate of ProCT+ higher than Non-Heme pts (53.3% *vs* 34.8%, p<0.001), a similar mean ProCT (4.6 *vs* 3.1ng/mL, p=NS), but a higher mean CRP (15.1 *vs* 10.9 mg/dL, p<0.001). In 61.7% of Non-Heme pts, CRP and ProCT results were discordant: 64.1% of CRP+ pts were ProCT- and 9.6% of CRP- were ProCT+. In Hematologic pts, discordance was only 45.4% (p<0.001) and no CRP- Hematologic pts were ProCT+; however, 46.0% of CRP+ pts were ProCT- (p<0.001) – this CRP+/ProCT- discordance was found in 25.0% of multiple myelomas, 26.9% of lymphomas, 53.5% of acute leukemias, 68.6% of auto-stem-cell transplants (SCT) and 72.7% of benign cytopenias (p<0.001). ProCT+ Hematologic pts were twice as likely as ProCT- pts to have positive bacterial cultures (37.5% *vs* 15.6%, p<0.001), with a further 11.4% of ProCT+ patients (but 0% of ProCT-Negative) positive for fungi, for a

total of 48.9% *vs* 16.7% positive cultures (p<0.001). In comparison, only 33.7% (27.6%+6.1%) of PCR+ pts had positive cultures (p<0.001). ProCT-/CRP+ positive-cultures were found in *Pseudomonas aeruginosa* colonization of sputum, coagulase-negative *Staphylococci* contamination and patients under antibiotherapy for 4-7 days. The percentage of CRP+ and CRP- pts with RF was comparable (30.8% *vs* 27.3%, p=NS); however, ProCT+ pts were twice as likely to have RF (47.6% *vs* 21.5%, p<0.001), leading to a false-positive rate of 41% in patients with RF, findings which were replicated in the Non-Heme population and in multivariate analysis.
Summary and Conclusion: The hematologic population had a higher mean CPR than the general population, with a higher rate of false-positives with CRP than with ProCT; only ProCT was able to identify respiratory tract colonization, culture contamination by commensals and good-response to antibiotherapy. Only a third of CRP+ auto-SCT recipients and half of CRP+ acute leukemias were ProCT+, suggesting that the value of ProCT as an infectious marker may be particularly relevant in these subpopulations. Renal failure increased the rate of false positives with ProCT, while not influencing CRP, suggesting that ProCT's utility may be lessened in renal patients. In conclusion, we suggest that ProCT is a valid septic marker in Hematologic patients, with higher specificity than CRP in the identification of infectious fever, and appears to be particularly useful in acute leukemia or auto-transplant patients without renal failure.

P1205

NOVEL HOMOZYGOUS MUTATION AFFECTING THE INTERNAL TRANSLATIONAL START SITE OF THE VON HIPPEL-LINDAU TUMOR SUPPRESSOR GENE IS ASSOCIATED WITH CONGENITAL ERYTHROCYTOSIS

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Background: Erythrocytosis is characterized by increased red blood cell mass. It is a heterogeneous disorder, which can either be acquired or congenital. An important cause of congenital erythrocytosis involves genetic defects in the oxygen sensing pathway, in particular, the PHD2 (HIF-prolyhydroxylase-2) – von Hippel-Lindau tumorsuppressor (VHL) – Hypoxia-Inducible Factor (HIF)-2α axis. Defective oxygen sensing ultimately results in inappropriately increased production of erythropoietin (EPO), a key regulator of erythropoiesis. Mutations in PHD2 and HIF-2α show a dominant pattern of inheritance, whereas mutations in VHL that are associated with erythrocytosis are generally inherited in an autosomal recessive manner. The most frequenct cause of congenital erythrocytosis is Chuvash erythrocytosis, caused by a p.Arg200Trp change in VHL. Intriguingly, mutations in VHL can also cause von Hippel-Lindau syndrome. This dominantly inherited disease predisposes affected individuals to the development of specific benign or malignant tumors.
Aims: We set out to investigate the molecular basis of apparantly congenital secondary erythrocytosis in three patients from two unrelated families. Both families originated from adjacent regions in Northern Morocco. All three patients displayed extremely high EPO levels, ranging from 9,000-13,500 U/L. None of the patients or family members involved in this study was diagnosed with VHL-associated tumors.
Methods: Coding regions of the genes encoding hemoglobin (α-globin, genes: *HBA1, HBA2,* and β-globin, gene: *HBB*), bisphosphoglycerate mutase (BPGM, gene: *BPGM*), HIF-prolyl hydroxylase 2 (PHD2, gene: *EGLN1*), Von Hippel-Lindau tumor suppressor (VHL, gene: *VHL*), and HIF-2a (HIF2A, gene: *EPAS1*) were investigated for mutations by PCR and subsequent DNA sequence analysis.
Results: A novel mutation was detected in exon 1 of the *VHL* gene: c.162G>C. All three affected individuals were homozygous for this mutation, which predicts the substition of methionine by isoleucine at position 54 of the long isoform of VHL, pVHL30. Apart from encoding this rather conservative amino acid change, c.162G>C also abolishes the internal translational start codon that directs transcription of the second and shorter isoform of pVHL, pVHL19. The c.162G>C mutation is therefore expected to result in the exclusive production of only the long pVHL30 isoform, rendering our patients *de facto* natural "knock-outs" for the pVHL19 isoform.
Summary and Conclusion: We report on a novel c.162G>C (p.Met54Ile) mutation in *VHL*. This mutation co-segregated with the phenotype of erythrocytosis in three patients from 2 unrelated families. c.162G>C is the most upstream *VHL* mutation so far described in patients with congenital erythrocytosis. Apart from encoding a p.Met54Ile change the mutation is predicted to result in the complete loss of the pVHL19 isoform. Because of our patients' extremely high EPO levels which were, on average, 800 times higher than reported on other patients with erythrocytosis, in particular Chuvash erythrocytosis, we postulate that pVHL19 is an important regulator of EPO production, whereas pVHL30 may be the more potent tumor suppressor.

P1206

NICOTINAMIDE (VITAMIN B3) TREATMENT INCREASES RESPONSE TO G-CSF IN SEVERE CONGENITAL NEUTROPENIA PATIENTS

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Background: Severe congenital neutropenia (SCN) often manifests with life-threatening bacterial infections. G-CSF treatment is aimed at keeping neutrophil levels of at 1000/ul to prevent infectious complications. Some SCN patients require high doses of G-CSF to reach this goal, which makes the treatment more painful, especially in small children and increases the costs. A small group of SCN patients is not responsive to G-CSF therapy at all. There is also some evidence that higher doses of G-CSF increase the risk of leukemic transformation in SCN. Other therapeutic options in addition to or in combination with G-CSF are required. Previously we demonstrated that nicotinamide (vitamin B3) increases neutrophil counts in healthy volunteers, acting through NAD^+/SIRT1 protein deacetylation pathway (Skokowa J. et al, Nature Medicine 2009). We aimed to evaluate whether nicotinamide treatment of SCN patients will lead to neutrophil production.
Aims: To asess the effect of nicotinamide treatment in SCN patients.
Results: We treated 2 newly diagnosed patients with SCN with nicotinamide alone, and even though we observed significant increase in mean neutrophil counts (from average 100 to 350 cells/µl), its effect was not enough to ameliorate the risk of infectious complications and the standard G-CSF treatment was applied. Five patients with SCN, receiving G-CSF, were additionally treated with nicotinamide (20 mg/kg/d). One of them had a poor response to G-CSF in a dose of 40 µg/kg/d. Upon nicotinamide treatment the desired neutrophil counts were achieved without further increase of the G-CSF dose. Upon continuous treatment of this patient we managed to decrease the G-CSF dose to 15 µg/kg/d, with good clinical and laboratory results. Overall in our group of patients, nicotinamide treatment allowed to decrease G-CSF dose 2.1 fold. We also used nicotinamide treatment in two cyclic neutropenia (CyN) patients. In one patient complete replacement of G-CSF by nicotinamide could be achieved. This patient is being treated with 5 mg/kg/day of nicotinamide for more than three years now. In another CyN patient treatment with nicotinamide increased the neutrophil counts during the nadir of the cycles from average 100 to average 400 cells/µl and improved the her quality of life.
Summary and Conclusion: Taken together, in our group of SCN and CyN patients nicotinamide treatment allowed to significantly decrease the G-CSF dose with continuous clinical and laboratory responses. We propose that use of nicotinamide in combination with a reduced dose of G-CSF for treatment of neutropenia patients is promising and should further be investigated.

P1207

LONG-TERM OUTCOME OF 100 CHILDREN WITH MODERATE APLASTIC ANEMIA TREATED WITH HORSE ANTITHYMOCYTE GLOBULIN AND CYCLOSPORINE

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Background: The proportion of patients with moderate aplastic anemia (MAA) is relatively small, ranging from 8% to 25% in pediatric series of AA. British guidelines recommend only observation without specific therapies until patients with MAA become transfusion dependent. Because the duration of aplasia before immunosuppressive therapy (IST) correlates inversely with the response, the response to IST is generally poor in patients who progress slowly from MAA to severe AA. The prospective randomized EBMT study demonstrated the superiority of a combination of antithymocyte globulin (ATG) and cyclosporine (CsA) over CsA alone in terms of the hematopoietic response and failure-free survival (FFS). We adopted this strategy and treated 100 MAA children with ATG and CsA.
Aims: To clarify the long-term outcome of children with moderate aplastic anemia treated with horse antithymocyte globulin and cyclosporine.
Methods: From October 1992 to December 2010, 100 children with newly diagnosed MAA were enrolled in successive studies. The eligibility criteria were<18 years old, recently diagnosed with MAA (within 180 days), and no prior treatment for AA. MAA was defined if at least two of the following criteria were fulfilled: absolute neutrophil count<1.0×10^9/L, reticulocyte count<60×10^9/L, or platelet count<50×10^9/L, with hypocellular bone marrow.

Patients who filled the criteria for severe AA and those with congenital bone marrow syndrome were excluded. All patients received first-line therapy with horse ATG (lymphoglobulin) and CsA. The hematologic response rate at 3 and 6 months after IST, 10-year overall survival (OS), and FFS were analyzed. Treatment failure was defined as the need for salvage therapy (second IST and stem cell transplantation [SCT]) for non-response, relapse, development of a clonal disease (myelodysplastic syndrome [MDS], leukemia, or paroximal nocturnal hemoglobinuria) or death, whichever came first.
Results: At 3 months, 8 patients achieved complete response (CR) and 36 achieved partial response (PR), with an overall response rate of 44%. At 6 months, 13 patients achieved CR and 41 achieved PR, with an overall response rate of 54%. Treatment failure was observed in 50 patients; 37 received salvage therapy, 11 relapsed and 2 developed to MDS. As a result, the probability of 10-year FFS was 48.4% (95% confidence interval [CI]; 38.9-60.1%). At the time of analysis, 17 patients received a second IST, of whom 7 patients responded. A total of 40 patients received SCT as second- or third-line therapy (unrelated donor, n=15; HLA-matched sibling donor, n=20; HLA-mismatched related donor, n=3; and cord blood, n=2). Although 37 patients were rescued by SCT, 3 died after unrelated donor transplantation due to cardiotoxicity, graft failure and graft-*versus*-host disease. Consequently, the probability of 10-year OS was 96.9% (95% CI, 93.5–100%) with a median follow up of 93 (range, 29–221) months. Median age in the treatment failure group was significantly lower than in the failure-free group (7.5 and 10 years, respectively; p=0.041). Median disease duration in the treatment failure group was significantly longer than in the failure-free group (27.5 and 17 days, respectively; p=0.004). The response rate at 3 and 6 months in the failure-free group was significantly higher than in the treatment failure group (62% and 76% *versus* 26% and 32%; p<0.001).
Summary and Conclusion: Although the long-term OS of MAA children treated with horse ATG and CsA was excellent, 40% of the patients required salvage therapy with SCT. Development of more effective non-SCT therapies is necessary for children with MAA.

P1208

CLINICAL FEATURES, GENETICS AND OUTCOME OF PEDIATRIC PATIENTS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN KOREA: REPORT OF A NATIONWIDE SURVEY FROM KOREA HISTIOCYTOSIS WORKING PARTY

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disease caused by dysregulated immune responses and overwhelming inflammation. Although our understanding of HLH has increased significantly, a large-scale, nationwide study of this heterogeneous disease is still lacking.
Aims: We analyzed a nationwide registry of pediatric hemophagocytic lymphohistiocytosis (HLH) patients in Korea to assess the clinical and genetic features and treatment outcomes in pediatric HLH.
Methods: The Korea Histiocytosis Working Party retrospectively analyzed data on 251 pediatric patients diagnosed with HLH between 1996 and 2011.
Results: In the study cohort, 31 cases were categorized with familial HLH, 61 with presumed secondary HLH, and 159 with unspecified HLH. Of 217 evaluable patients, 91 (42%) had concomitant Epstein-Barr virus infection. Of 238 evaluable patients, central nervous system (CNS) involvement, which was more frequent in the familial group, was evident in 81 cases (34%). Genetic tests revealed a predominant UNC13D mutation with a high incidence of two recurrent splicing mutations (c.118-308C>T and c.754-1G>C). The 5-year overall survival rate was 68% (43% in the familial group, 80% in the presumed secondary group). The 5-year overall survival rate among 32 patients who underwent allogeneic hematopoietic stem cell transplantation was 64%. The type of donor and conditioning regimen did not influence the outcome, while cord blood graft tended to be associated with a poorer outcome, but without statistical significance (P=0.115). In multivariate analysis, a younger age at diagnosis, severe cholestasis, and a coagulation abnormality were independent prognostic factors for survival. Responses during initial treatments were also significant indicators of outcome.
Summary and Conclusion: Our study showed the unique predominance of a UNC13D mutation and vulnerability to Epstein-Barr virus infection in Korean children with HLH, and emphasizes the prognostic significance of age, liver dysfunction, and treatment responses in this disease. A multicenter prospective trial that builds on the present results is warranted to identify subgroups of patients with a poor prognosis and identify optimal treatments.

P1209

IMPROVEMENTS IN HEMOGLOBIN LEVELS AND FATIGUE IN A RANDOMIZED, DOUBLE BLINDED, PLACEBO CONTROLLED STUDY (MCD3282001) OF SILTUXIMAB IN PATIENTS WITH MULTICENTRIC CASTLEMAN'S DISEASE (MCD)

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Background: MCD is a rare lymphoproliferative disorder characterized by excess interleukin (IL)-6 production. Symptoms include anemia, fatigue, weakness, and loss of appetite. Siltuximab, a chimeric mAb against human IL-6, is undergoing clinical trials in MCD.
Aims: To evaluate the relationship of fatigue by patient reported outcomes (PROs) and changes from baseline in hemoglobin (Hb) levels in study CNTO328MCD2001 in MCD patients.
Methods: Patients received best supportive care and were randomly assigned 2:1 to siltuximab 11 mg/kg (n=53) or placebo (n=26) given by 1-hour intravenous infusions every 3 weeks. Primary endpoint was durable tumor and symptomatic response by independent radiological review and improvement/stabilization in clinician reported MCD-related symptoms for ≥18 weeks. PRO measures included an MCD-symptom scale (SS) developed for this study, FACIT-F (a measure of patient reported fatigue where higher scores indicate less fatigue) and SF-36. The MCD-SS fatigue domain included items related to tiredness, fatigue, lack of energy and feeling weak. Descriptive statistics were obtained at each cycle. Association between fatigue and Hb levels were explored by area under the curve analysis (AUC).
Results: Median age was 48 years, 48% were Asian, 39% were white, 66% were male, 58% had prior systemic therapy, 30% were on corticosteroids. MCD symptoms at baseline (siltuximab/placebo) included fatigue (89%/81%), malaise (62%/58%), and night sweats (47%/62%). For the primary endpoint a higher percentage of durable tumor and clinician-reported symptomatic response was observed with siltuximab compared with placebo (34% *vs*. 0%; *P*=0.0012). Of patients with anemia at baseline 19/31 (siltuximab; 61%) and 0/11 (placebo; 0%) showed a ≥15 g/L Hb increase at Week 13 (95% CI: 28.3, 85.1; *P*=0.0002). Of patients with baseline Hb levels below the lower limit of normal, 13 (42%; 95% CI: 24.5, 60.9) in the siltuximab group and none in the placebo group had Hb values return to normal or higher at Week 13. For the MCD-SS fatigue component, patients treated with siltuximab improved more than placebo (AUC, *P*=0.02, Figure). Improvements in FACIT-F scores at Cycle 4 Day 1 were positively associated with the Hb response at Week 13. Patients with an increase in Hb of ≥15 g/L (n=19) reported more improvement in fatigue (FACIT-F mean [± SD] change from baseline score of 9.6 [±10.04]) compared with patients with Hb increases of<15 g/L (n=23; mean change of 1.0 [±8.92]). For SF-36, 5 of 8 domain scores statistically favored siltuximab (role physical and role emotional, vitality, bodily pain, and mental health). Improvements in fatigue were supported by enhancements in other aspects of functioning and well-being, providing broad support for the significance of fatigue reduction on functional status and well-being. As reported previously, the overall safety profile was similar between treatment arms despite longer treatment with siltuximab.

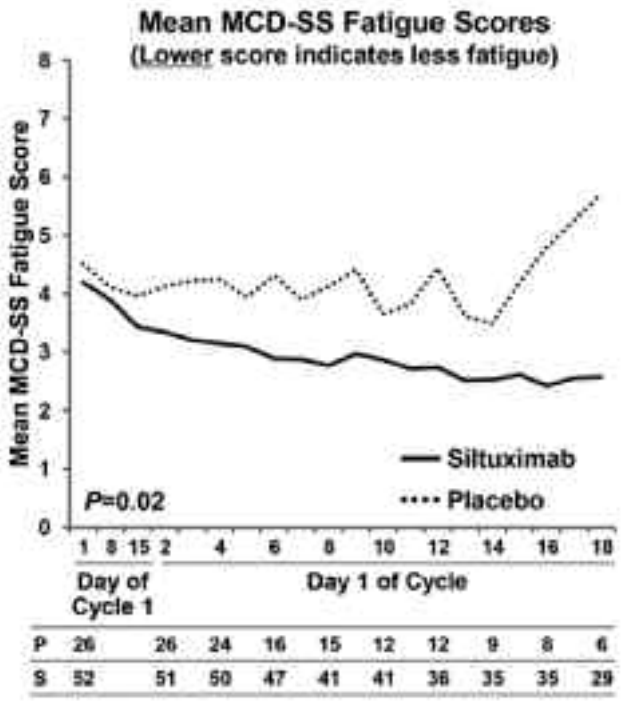

Figure 1.

Summary and Conclusion: MCD patients treated with siltuximab reported significantly greater improvements in fatigue than placebo patients measured by multiple PRO instruments. Improvements with fatigue were positively associated with increases in Hb from baseline.

P1210

RITUXIMAB IS THE FIRST-LINE THERAPY FOR IDIOPATHIC MULTICENTRIC CASTLEMAN'S DISEASE: TWELVE YEARS EXPERIENCE IN A SINGLE INSTITUTE

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Background: Multicentric Castleman's disease (MCD) is an indolent lymphoproliferative disorder of unknown etiology. Most cases of MCD are HIV-associated, and patients with HIV-negative idiopathic Castleman's disease (iMCD) are few and far between. Therefore, no established optimal treatment is available for iMCD. In Japan, almost all patients with Castleman's disease are HIV-negative, and they are mainly treated by corticosteroids and tocilizumab (anti-IL6 receptor antibody).
Aims: We have reported on rituximab therapy for iMCD (Br J Haematology 2003, Eur J Haematology 2006). Therefore, we reviewed the records of all iMCD patients at our hospital treated with rituximab therapy, and compared them with those of patients who received tocilizumab therapy (median follow-up period 64 months, range 5 to 133 months).
Methods: Eight HIV-negative patients with iMCD (4 men and 4 women, median age 49 years, range 26-61 years) were referred to Takamatsu Red Cross Hospital over a twelve-year period. Six patients were initially treated by rituximab, and two were treated by tocilizumab. After clinical evaluation, rituximab was administered intravenously at the standard dose of 375 mg/m^2 weekly for 4 or 8 times without chemotherapy, and two patients were administrated tocilizumab at 8mg/kg biweekly. We analyzed the clinical course of iMCD after therapy in the follow-up period.
Results: In the follow-up at 43 to 133 months, three of the six patients (50%) with iMCD achieved almost complete remission with 4 rituximab infusions. Two of three rituximab-resistant patients received second-line therapy at 2 and 56 months (melphalan and tocilizumab).Under repeated tocilizumab infusion, three patients treated by tocilizumab (two patients with first-line therapy and one patient with second-line therapy after rituximab) did not require other therapy. However, one patient needed a dose escalation of tocilizumab (8mg/kg biweekly to 8mg/kg weekly). All eight patients survived and were living well during the observation period.
Summary and Conclusion: Four rituximab infusions achieved durable response in 50% of rituximab-treated iMCD patients without additional rituximab therapy. Tocilizumab is as effective as rituximab, but durable repeated infusion is necessary to maintain IL6-antagonist induced remission. Therefore, rituximab is the most appropriate first-line therapy in iMCD.

P1211

INFLAMMATORY AND ANEMIA-RELATED MARKERS IN A PHASE 2, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF SILTUXIMAB (ANTI-IL-6 MONOCLONAL ANTIBODY) IN MULTICENTRIC CASTLEMAN'S DISEASE PATIENTS

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Background: Multicentric Castleman's disease (MCD) is a rare, lymphoproliferative disease driven by dysregulated interleukin (IL)-6 production. A double-blind placebo controlled study in MCD showed significant improvement in durable tumor and symptomatic response (34.0% *vs* 0.0% with placebo; *P*=0.001), and consistent improvement in secondary endpoints with similar frequencies of adverse events, despite longer drug exposure in the siltuximab group (Wong *et al.* 2013 Blood 122:21; 505). Herein, we examine siltuximab-induced changes in inflammatory and anemia-associated markers.
Aims: The clinical study's primary objective was to demonstrate that siltuximab plus best supportive care (BSC) is superior to BSC in terms of durable tumor and symptomatic response in MCD patients. The secondary objectives included biomarker evaluation apart from other measures of efficacy. The effects of siltuximab neutralization of IL-6 on C-reactive protein (CRP), hemoglobin (Hb), hepcidin, and iron parameters were studied.
Methods: Subjects (N=79) were randomly assigned 2:1 to siltuximab 11 mg/kg every 3 weeks (n=53) or placebo (n=26). IL-6, CRP, Hb, iron, transferrin saturation, ferritin, and hepcidin were measured in a central laboratory. Association of these markers with clinical response was determined by appropriate statistical methods.
Results: Systemic levels of IL-6 at baseline (median IL-6 level was 6.78 pg/mL, range 0.38-50.64 pg/mL) and CRP (median 11.20 mg/L, range 0.10-181.00 mg/L) were significantly correlated (r=0.708; *P*<0.0001). Posttreatment IL-6 levels were not evaluated due to assay interference by siltuximab-IL-6 complexes. Thus, CRP was measured as an indicator of bioactive IL-6. CRP levels rapidly decreased (median decrease of 92%) post treatment by Cycle (C) 1 Day (D) 8 with sustained suppression until end-of-treatment in the siltuximab group compared with 3% increase at C1D8 in the placebo group. Baseline CRP levels differed between responders and non-responders by durable tumor and symptomatic response (median values 34.50 *vs*. 6.32 mg/L, *P*=0.0522), and by best tumor response (median values 38.00 *vs*. 5.09 mg/L, *P*=0.0118) in the siltuximab group, wherein *P* values refer to the log transformed values. A prediction of durable tumor and symptomatic response based on log transformed baseline CRP value using a logistic model applied to siltuximab treated subjects showed a sensitivity of 28% and specificity of 83%. Hb response (change from baseline of ≥15 g/L at Week 13) was observed in 61% of subjects in the siltuximab group and 0% in the placebo group (*P*=0.0002). Levels of the iron regulating peptide hormone, hepcidin showed a median decrease of 47% (from 127.70 to 59.65 ng/mL) from baseline as early as C1D8 in the siltuximab group and 11% increase from baseline in the placebo group (from 110.00 to 112.10 ng/mL). A trend in Hb improvement with decrease in hepcidin was observed (r=-0.298; *P*=0.0336). Analysis of posttreatment changes in hepcidin levels at C1D8 (maximum change) with changes in iron parameters at C2D1 (maximum change) indicated statistically significant correlations for Hb (r=-0.395; *P*=0.0061), total iron binding capacity (TIBC) (r=-0.354, *P*=0.0169, and ferritin (r=0.599, *P*<0.0001) only in the siltuximab group. Further, higher median changes from baseline in ferritin (>2 fold decrease), Hb (4 fold increase), and TIBC (2 fold increase) were observed in anemic subjects compared to non-anemic subjects in the siltuximab group.
Summary and Conclusion: Siltuximab is an active agent in MCD. Sustained suppression of CRP, decreased hepcidin levels, significant Hb increases and improved iron parameters such as, TIBC and ferritin were observed. These results support normalization of inflammatory markers by IL-6 neutralization as reflected by the suppression of CRP levels and indicate inhibition of the IL-6-hepcidin pathway as one of the mechanisms by which anemia is resolved following siltuximab treatment.

Platelet disorders

P1212

OXIDANT STATUS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) AND THE ROLE OF AN ADJUVANT ANTIOXIDANT THERAPY

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Background: Free oxygen radicals may have an effect on the structural and functional behavior of platelets and might play a role in pathogenesis of thrombocytopenia in both, newly diagnosed (ND) and chronic ITP.
Aims: To assess oxidant -antioxidant systems initially in patients with (ND) and chronic (ITP) and evaluate effect of antioxidant therapy on, platelet count, bleeding score (BS) and oxidant status during 6 months follow-up.
Methods: An interventional 6 months prospective randomized open-labeled study registered as (NCT 01763658); in patients aged 1-18 years with ITP; ND and chronic. Patients were randomly allocated to one of two groups. Group 1(interventional arm) included 52 ITP patients (26 ND ITP patients and 26chronic ITP patients), all received antioxidant therapy; one tablet/ 20 kg (1 tablet contains: Vit. A 2000 IU, Vit C 90 mg, Vit E 15 mg and selenium 55 ug),while Group 2 (non-intervention arm), included 26 ITP patients;13(ND) and 13 chronic ITP, did not receive the antioxidant therapy. Both groups were comparable and matched with healthy controls (n=30).The primary efficacy endpoints were the difference between both groups in the change from baseline to 6 months in BS using ITP specific bleeding assessment tool (ITP-BAT), mean platelet count, total antioxidant capacity (TAC), catalase (CAT), reduced glutathione (GSH)(markers of antioxidant status) and serum malondialdehyde (MDA) levels. The safety endpoint was the occurrence of serious or life threatening bleeding, complete response to therapy, and adverse events related to therapy.
Results: Baseline platelet count and markers of antioxidant status were significantly lower in (ND) ITP patients than both healthy controls (P<0.001) and chronic ITP patients (p<0.05). In addition, in this group, baseline MDA, was significantly higher than both healthy controls and chronic ITP patients (P<0.001). In chronic ITP group, although baseline platelet count and markers of antioxidant status were significantly lower than those in the control groups (P<0.001), MDA was significantly higher (P<0.001). In (ND) ITP patients, end of study levels of TAC, CAT, GSH and BS were significantly higher in patients receiving antioxidant (p<0.05) whereas MDA was statistically lower (P<0.001). No difference in both groups regarding mean platelet count (p=0.227).In chronic ITP patients, end of study levels of TAC, CAT, GSH (p<0.001), mean platelet count(p=0.03) and BS(p=0.04) were statistically higher in patients receiving antioxidant whereas MDA was statistically lower (P<0.001). A positive correlation was found between platelet count and TAC in ITP patients (r: 0.408, P<0.05) and a negative correlation between platelet count and MDA levels in ITP patients(r: 0.491, P<0.004).
Summary and Conclusion: High oxidative stress was reported in newly diagnosed ITP and may be an association and plays role in its pathophysiology. Scavenging of oxygen free radicals ameliorated the oxidative stress, thrombocytopenia and bleeding in ITP patients.

P1213

EPIDEMIOLOGY OF INCIDENT IMMUNE THROMBOCYTOPENIA IN FRANCE: A NATIONWIDE POPULATION-BASED STUDY

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Background: Epidemiology of immune thrombocytopenia (ITP) is not well known.
Aims: The purpose of this study is to assess ITP incidence at a nationwide level (France) with recent data (mid-2009 to mid-2011, corresponding to 129 248 543 person-years).
Methods: Data source is French health insurance database, which collects prospectively data regarding demographic characteristics, out-hospital expenditures (including drugs) and hospital stays for every patient. We selected ITP patients through hospital stays and long-term disease diagnosis codes (D69.3 code of the ICD-10). Date of diagnosis was refined thanks to out-hospital ITP drug dispensing. We studied incidence by age, gender, calendar month and administrative regions. To detect the regions of low and high incidence, we used Local Indicators of Spatial Associations method after direct standardization on age and gender repartition in France. We also assessed 1) the proportion of secondary ITPs through long-term disease and in-hospital diagnosis codes, 2) the percentage of ITPs becoming persistent or chronic (defined as a long-term disease attribution, two hospital stays more than three months apart, splenectomy or rituximab exposure, or a more than 3-months out-hospital drug dispensing), and 3) the percentage of severe bleeding at disease onset, assuming that all patients with severe bleeding were hospitalized.
Results: We identified 3771 incident ITP patients. ITP incidence was 2.9/100 000 person-years (95% confidence interval: 2.83-3.01) with peaks among children and after 60 years. ITP was more frequent among males in these subgroups especially after 75 years (9/100 000 person-years). It was significantly lower in overseas Caribbean French departments, suggesting a lower incidence among Afro-American people. Seasonal variations were observed (peak in winter, nadir in summer). Incidence was statistically lower in north-west France and higher in south France. Persistence or chronicity occurred in 36% of pediatric ITP cases, compared with 67% of adults. Among adults, 18% of ITP cases were secondary. Malignant lymphoid disorders (mainly lymphoma and B-cell chronic leukemia) were observed in 5.9% of incident adult ITPs. Other main causes of secondary ITP were connective tissue diseases (2.5%, mainly systemic lupus erythematosus), myelodysplastic syndromes (2.3%), immune deficiencies (excluding HIV infection, 1.7%), and HIV infection (0.9%). Forty-seven patients (1.63%) had Evans' syndrome. ITP was secondary in 2,4% of children cases. Severe gastrointestinal or central nervous system bleeding at ITP onset were rare (<1%), increasing with age.
Summary and Conclusion: This study adds new information on ITP epidemiology, *i.e.* seasonal variations, a north-south gradient in France and a lower incidence in a dominant Afro-American population (Caribbean area). It confirms age and gender repartition, the frequency of classical causes of secondary ITP and rarity of severe bleeding at disease onset.

P1214

RITUXIMAB REVERSED THE INCREASE IN SPLENIC T FOLLICULAR HELPER CELLS DURING IMMUNE THROMBOCYTOPENIA

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Background: Mainly localized into secondary lymphoid organs, T follicular helper cells (TFH) have been recognized as the major T cell subset that promotes B cell proliferation and differentiation into plasma cells, and thus, the production of immunoglobulins. Animal models and the expansion of blood TFH during systemic lupus erythematosus and Sjögren syndrome give arguments for their involvement during auto-immune diseases (AID).
Aims: Taking advantage of splenectomy as part of the treatment of immune thrombocytopenia (ITP), we analyzed TFH within a secondary lymphoid organ during a human AID. Our aim was to characterize and localize splenic TFH and to study their interaction with B cells. The effect of rituximab, a B cell depleting therapy, on TFH was also investigated.
Methods: The spleens of 23 primary ITP patients, among which 10 were previously treated with rituximab, were compared to 8 controls. All patients gave a written informed consent before being included. The study was approved by our institutional review board and ethics committee. TFH were characterized and quantified by flow cytometry. Chemokine and cytokine expression was measured by quantitative PCR. The location of TFH within the spleen was determined by immunohistochemistry. TFH were also quantified in the blood of 10 ITP patients before and after rituximab. Results are given by median with interquartile range. Mann-Whitney *U* test and Wilcoxon's signed-rank test were used as appropriate. Spearman's rank correlation test was used for correlation analyses.
Results: Splenic TFH, defined as $CD3^+CD4^+CXCR5^+ICOS^+PD\text{-}1^+$, displayed a phenotype of memory cells ($CD45RO^+$), were activated ($CD69^+$) and expressed the transcription factor Bcl-6. Upon stimulation, splenic TFH expressed CD154 (CD40 ligand) and were the main producer of IL-21 within the spleen. Consistent with their $CCR7^-CXCR4^+$ phenotype, TFH were located within germinal centers (GC) of secondary follicles. Splenic TFH frequency was increased during ITP (2.04 [1.4-3.2] *vs.* 0.5 [0.3-0.6] % of $CD4^+$ T cells, p=0.003) and associated with an increase in CXCL13 (214 [57.3-418.2] *vs.* 34.9 [13.1-78.8], p=0.02) and IL-21 expressions (12.8 [7.3-17.7] *vs.* 4.4 [3.4-4.6], p=0.009) among $CD4^+$ T cells. ITP patients also displayed higher frequencies of GC B cells ($CD19^+IgD^-CD38^+$; 11.6 [9.9-13.3] *vs.* 4.8% [3-6.2] of B cells, p=0.004), pre-GC B cells ($CD19^+IgD^+CD38^+$; 14.3 [12.3-24] *vs.* 5.7% [3.8-10.6], p=0.002) and plasma cells ($CD19^+IgD^-CD38^{++}$; 2.2 [1.6-2.8] *vs.* 0.7% [0.4-1.3], p=0.003) compared to controls. Interestingly, splenic TFH percentage correlated with the percentage of GC (R=0.7, p=0.003), preGC B cells (R=0.8, p=0.0002) and plasma cells (R=0.7, p=0.003). *In vitro*, the differentiation of splenic B cells into plasma cells together with antibody secretion depended on the use of a CD40 agonist in presence of IL-21, pointing out the involvement of TFH that both expressed CD154 and IL-21. Although they represented only a minor part of $CD4^+$ T cells, circulating TFH were increased during ITP (0.09 [0.04-0.13] *vs.* 0.01% [0.004-0.04] of $CD4^+$ T cells, p=0.005). Blood and splenic B cell depletion following rituximab therapy was associated with a decrease in TFH frequency in the blood (0.09 [0.04-0.13] *vs.* 0.05 [0.02-0.07] % of circulating $CD4^+$ T cells, p=0.04) and in the spleen (0.13 [0.05-0.23] % of $CD4^+$ T cells in rituximab-treated *vs.* 2.04 [1.4-3.2] in ITP patients, p<0.0001).

Summary and Conclusion: Our results showing an increase in splenic and circulating TFH strongly support a role for TFH in the differentiation of pathogenic B cells during ITP. Interestingly, rituximab treatment is associated with a decrease in splenic and blood TFH.

P1215

SUSTAINED RESPONSE AFTER THROMBOPOIETIN RECEPTOR AGONISTS DISCONTINUATION IN 5 PATIENTS AFFECTED BY PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: The efficacy of the thrombopoietin receptor agonists (TPO-RAs) in the treatment of primary immune thrombocytopenia (pITP) patients (pts) has been already demonstrated. The two available TPO-RAs for the treatment of pITP, Romiplostim and Eltrombopag, have been approved by EMA for the treatment of chronic and refractory (post-splenectomy) pITP pts. Although it is well known that these drugs have to be chronically administered to maintain a platelet response, in the last few years case reports on sustained responses off treatment in pITP pts have been reported.
Aims: To retrospectively report our experience of sustained response after TPO-RA discontinuation in pITP patients.
Methods: Data were examined for patients who received a TPO-RA and maintained a response after discontinuation of treatment in the absence of other concomitant medications. Response was defined as a platelet (plt) count ≥50x10^9/L and at least a 2-fold increase of the baseline value; complete response (CR) as a plt count ≥100x10^9/L; sustained response as a plt count ≥50x10^9/L and at least a 2-fold increase of the baseline value, off treatment. Period in response during TPO-RA treatment was expressed as the percentage of weeks in response/weeks on treatment. Phases of pITP were defined in relation to the diagnosis as follows: acute, within 3 months; persistent, between 3 and 12 months; chronic, more than 12 months.
Results: Since February 2009, 39 pITP patients (25 F, 14 M) resistant to one or more therapy lines have been treated with TPO-RAs. Globally, 30/39 (77%) patients obtained a response without concomitant treatment. Five/30 (16.6%) responder pts obtained a sustained response after TPO-RA discontinuation. They were 4 females, 1 male. The median number of therapy lines prior to the start of the TPO-RA administration was 3 (2-5): prednisone, dexamethasone, azathioprine, rituximab, splenectomy, interferon. Two patients started the TPO-RA while still in the acute phase. They were severely symptomatic and resistant to high dose steroids and intravenous immunoglobulins. The other 3 patients were in a chronic phase and they started the TPO-RA at 2.4, 15.1 and 38.7 years from pITP diagnosis, respectively. Two patients had been previously splenectomized 36.9 and 0.25 years before the start of the TPO-RA. They have been all treated with Romiplostim. The characteristics at the start of the TPO-RA were: median age 50 years (41-77); median plt count 11x10^9/L (2-13). Steroids were a concomitant treatment in all patients. The median number of weeks on TPO-RA treatment was 18 (5-133), the median time to obtain a response in absence of concomitant medications was 7 weeks (4-11) and the median Romiplostim dosage was 4 µg/dose (1-6). The median percentage of weeks in response/weeks on treatment, was 64% (40-100). They all stopped TPO-RA treatment having achieved a CR and there was no further indication to continue TPO-RA therapy. The median period in response off treatment is 2.3 years (0.8-3.4). No patient experienced side effects or severe adverse events.
Summary and Conclusion: Five/30 (16.6%) pITP pts treated with Romiplostim have maintained a sustained response after therapy discontinuation. They were either acute or chronic pts. So far, few literature data are reported about sustained response after TPO-RAs discontinuation in pITP pts. Large controlled retrospective and prospective follow-up studies are required.

P1216

NOVEL MUTATIONS AT GPIIB/GPIIIA GENES FROM TURKISH PATIENTS WITH GLANZMANN THROMBASTHENIA SYNDROME

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Background: Glanzmann Thrombasthenia (GT) is a congenital hereditary hemorrhagic disorder, characterized by a defective platelet integrin alpha IIB, beta3 receptor. The autosomal recessive bleeding disorder caused by qualitative or quantitative abnormalities of platelet glycoprotein IIB/IIIA leading to excessive bleeding. The integrin receptor is a heterodimer consisting of alpha IIa and beta 3 subunits found in an inactive state in resting platelets. When platelets are stimulated, in the transmembrane and extracellular domain occurs conformational change then activated integrin alpha IIB/IIIA binds to fibrinogen and von Willebrand factor and some other molecules such as vitronectin, fibronectin. vWf or fibrinogen bring about crosslink between GpIIB/IIIA complex and multipl platelets and this suggesting the formation of the platelet plug at the damaged site. GT has autosomal recessive inheritance and glycoprotein IIB and glycoprotein IIIA genes are determined as associated with this disease. These genes are closely located at chromosome 17q21.31-32. While GpIIB consist of 30 exon and encoding α chain, GpIIIA has eight exon and encoding β chain. Up to now, according to Human Gene Mutation Database 151 mutations at GpIIIA gene, 92 mutations at GpIIB gene have been indentified and large proportion of these mutations constitute missense and nonsense mutations.
Aims: In this study, we aimed to analyze mutations GpIIB and GpIIIA genes in Turkish population as a clinically diagnosed with Glanzmann Thrombasthenia Syndrome.
Methods: A total of 20 Glanzmann Thrombasthenia patients diagnosed at Selcuk University Hematology Department sent to our laboratory for the evaluation of the gene. Peripheral blood was collected from patients and a written informed consent for genetic analysis was obtained from parents. DNA was isolated from by proteinase K and phenol/chloroform extraction. GpIIB and GpIIIA genes were screened by polymerase chain (PCR) reaction. After then, PCR products were cleaned with DNA Purification Kit (Roche DNA Purification Kit) and then samples was sequenced using an automatic DNA Sequencer (Beckman Coulter, USA).
Results: Screening the exons of the GpIIB gene from Turkish patient, we detected five novel mutations in three different region and 2 mutations defined previously within the gene (Figure). These changes are at exon 4; c.438 G>T alteration, at exon 13 c.1277 T>A, c.1291 G>T alterations, at exon 19 c.1921A>G alterations. And from the start point of exon 14, behind 107 base, we detected a heterozygous alteration at Thymine to Guanine. According to Polyphen Database program and NCBI Multipl Aligment Tool Database; five transitions are conserved at evolutionary process, so we can say that these transitions are novel mutations. c. 507T>G alteration at exon 4 and c. 1378 T>A alteration was reported to HGMD (Human Gene Mutation Database) previously. Screening the exons of the GpIIIA gene from the same patient groups, we reported a novel missense mutation at exon 5, at nucleotide 680. At the protein level, the resulting missense mutation leads a change of Leucine to Isoleucine at codon 221 of the GpIIIA gene, also involved a highly conserved aminoacid residue.

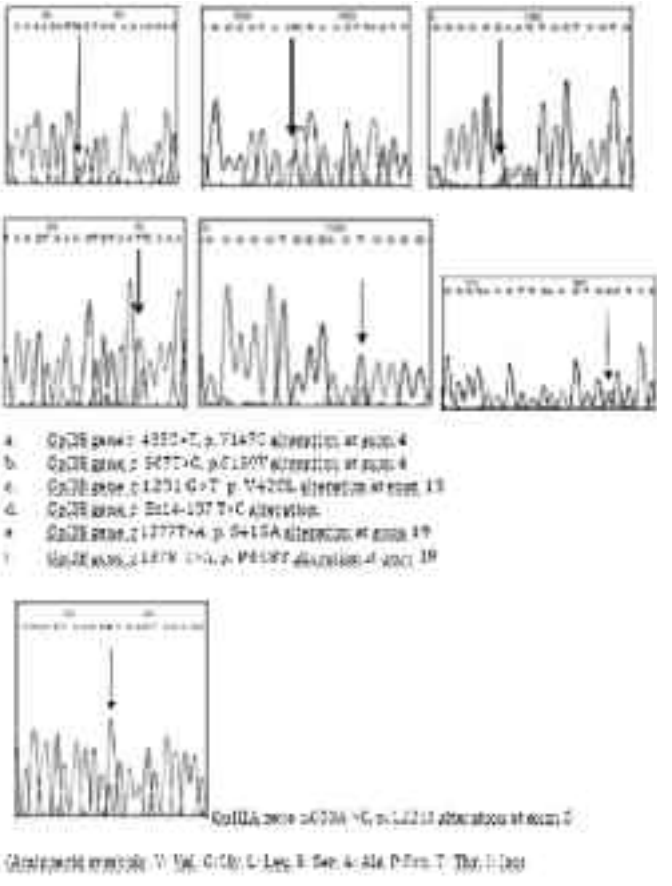

Figure 1.

Summary and Conclusion: These mutations were described for the first time in Turkish population and all novel mutations are not defined HGMD previously. Further collaborative studies are needed for the population point of view.

P1217

NEXT GENERATION SEQUENCING OF NBEAL2 GENE MUTATIONS IN GRAY PLATELET SYNDROME

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Background: Gray platelet syndrome (GPS) is an inherited platelet disorder characterized by variable thrombocytopenia, large platelets, absence of α-granules in platelets and autosomal recessive inheritance. Three simultaneous papers in July 2011 identified biallelic mutations of the *NBEAL2* on chromosome 3p21 as the genetic basis of GPS.
Aims: We report *NBEAL2* gene mutation detection in a Chinese patient with GPS by next generation sequencing (NGS).
Methods: A 49-year old female presented with a history of thrombocytopenia for 10 years without bleeding symptom. She complained of left upper quadrant pain and was found to have splenomegaly of 3 cm below the left costal margin, which showed diffuse enlargement on computed tomography. Complete blood counts showed: hemoglobin 11.6 g/dL, MCV 85.8 fL, white cell count 3.25 x 10^9/L, platelets 48 x 10^9/L and reticulocytes 1.13%. Blood smear review showed large and hypogranular platelets. The bone marrow aspirate was a dry tap. Trephine biopsy showed hyperplastic marrow in association with active trilineage haemopoiesis and reticulin fibrosis. Electron microscopy of the blood platelets showed absence of α-granule but preservation of dense granules and other organelles. The complete *NBEAL2* gene from exons 1 – 54 was subject to Fluidigm access array amplification followed by 454 sequencing, and variants were confirmed by Sanger sequencing.
Results: A heterozygous novel nonsense mutation was identified at exon 23 (c.3226C→T p.Gln1076*) with over 800 fold coverage. Two variants of unknown significance (VUS) were also identified at exon 12 (c.1271G→T p.Arg424Leu) and exon 33 (c.5350C→T p.Arg1784Cys) respectively. The exon 12 VUS was annotated to the ARM domain. *In-silico* analysis showed that the exon 12 VUS was likely benign while the exon 33 VUS was likely damaging. These three variants were all present in the DNA from buccal mucosa cells and of germline nature. The mother and two elder siblings of the patients were alive without bleeding history and family study was being arranged.
Summary and Conclusion: We confirm the pan-ethnic nature of *NBEAL2* gene defect in GPS. Furthermore, since the *NBEAL2* gene does not account for all cases of phenotypic GPS and an autosomal dominant form involving the *GFI1B* gene is recently described, we illustrate that the NGS approach is a clinically useful and practical option for the genetic diagnosis of GPS and a gene panel can be developed.

P1218

IS HIGHER IL-21 LEVEL PREDICTIVE OF RELAPSES IN IMMUNE THROMBOCYTOPENIA AND IS IT ASSOCIATED WITH ACTIVATION OF THE COMPLEMENT SYSTEM?

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Background: Immune thrombocytopenia (ITP) is a disease the pathogenesis of which involves multiple mechanisms. There is contradictory data on whether the antigen-antibody complexes activate the complement system or not.
Aims: We evaluated complement activation and its relationship with recently defined T cell markers. Anti-complement 1q antibody (anti-C1q), complement factor H (CFH), complement fragments Bb, plasma stromal-derived factor (SDF-1), and interleukin-21 (IL-21) levels were determined. C1q plays a role in the activation of the classic complement pathway and is associated with autoimmune diseases, like lupus nephritis. CFH inhibits C3b convertase which takes part in the activation of the alternative complement pathway. Complement fragments Bb show activation of the alternative pathway. Plasma SDF-1 level is associated with lectin pathway. It was recently shown that helper T cells from ITP patients synthesized IL-21.
Methods: We included 92 ITP patients (66 females, 26 males, mean age: 47.3±7.5) and 48 controls (33 females, 15 males, mean age: 44.9±17.6). Ethical committee approval and written informed consent were obtained. Patients' demographic and clinical data were retrieved from medical files. Anti-C1q, CFH, complement fragments Bb, SDF-1, and IL-21 levels were determined in ITP patients' plasma when platelets were<100000/mm3 and also in controls with normal platelets. The same parameters were evaluated in 61 ITP patients whose platelet counts became >100000/mm3 after treatment.
Results: Sixtyfour (69.6%) ITP patients had bleeding symptoms. In 22 (23.9%) patients, platelet counts were<30000/mm3. ITP patients had significantly higher IL-21 [(median and range: 165.8 pg/ml (0-422) *vs*. 83.2 pg/ml (0-2396), p=0.027)], and anti-C1q [(median and range: 3.97 ng/ml (1.07-8.81) *vs*. 2.99 ng/ml (1.06-9.92), p=0.022)] levels than controls. On the other hand, CFH [(median and range: 2.78 ng/ml (1.15-9.97) *vs*. 5.3 ng/ml (1.13-9.68), p<0.001)], and complement fragments Bb [(median and range: 0.16 µg/ml (0-17) *vs*. 0.23 µg/ml (0.1-0.65), p=0.012)] were significantly lower in the ITP group. SDF-1 levels were similar in ITP and control groups (p=0.12). When the values of the evaluated parameters in ITP patients with low (<100000/mm3) *vs*. normal platelet counts (>100000/mm3) were compared, it was observed that IL-21 level decreased significantly (median: 156.9 pg/ml *vs*. 101.9 pg/ml); CFH (median: 3.83 ng/ml *vs*. 5.22 ng/ml) and SDF-1 (median: 247.9 pg/ml *vs*. 456.1 pg/ml) levels incresed significantly (all p values<0.001). There were no changes in anti-C1q and complement fragments Bb levels after therapy. We also compared the initial values of all parameters in 46 ITP patients who obtained either a partial (platelets >50000/mm3) or a complete response (platelets >100000/mm3) with first-line therapy with the initial values of 23 ITP patients who did not respond to first-line therapy; and found no significant differences. Initial IL-21 levels in ITP patients who relapsed after first-line therapy were significantly higher than those who did not relapse (p=0.03).
Summary and Conclusion: Our results suggest that complement activation might be playing a role in the pathogenesis of ITP. It was interesting that CFH and SDF-1 levels decreased significantly after therapy. Our results confirmed the increase in IL-21 level in ITP, and this increase persisted after therapy. There was no association between IL-21 level and complement activation; however, high IL-21 level at initial diagnosis might be used as a marker to predict relapses in ITP.

P1219

BASELINE CHARACTERISTICS, PLATELET COUNT PROGRESSION AND OCCURRENCE OF COMORBIDITIES AMONG ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP): UPDATED RESULTS FROM THE UK ITP REGISTRY

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Background: Primary immune thrombocytopenia (ITP) is a rare, autoimmune blood disorder that manifests itself with bleeding symptoms which is usually associated with a drop in platelet count (<100×10^9/L). The United Kingdom ITP (UKITP) Registry continues with its role in providing contemporary research findings on the epidemiology of ITP, natural progression of the disease, including the development of comorbidities and platelet count changes, and ITP treatment patterns and their effectiveness. Such data from large, representative population is sparse and the Registry is achieving its objective of conducting its research using larger sample size, on which this present study is based.
Aims: In this updated analysis, we described the cohort at baseline (at initial ITP presentation) and platelet count progression following ITP diagnosis, and estimated the rate of certain comorbid occurrences after diagnosis of ITP.
Methods: The registry, based at the Royal London Hospital, collects extensive demographic and clinical data (historical and prospective) on its enrolees through a network of 46 collaborating haematology regional centres. Apart from describing the ITP cohort at initial ITP presentation, the Registry is able to estimate the rate of occurrence of specific comorbidities and platelet progression at or following ITP diagnosis.
Results: The 822 enrolees of the UKITP Registry had a median age at ITP diagnosis of 51.8 years old and 57.7% were females. The majority of enrolees were found to be of European ancestry (79%) followed by African ancestry (3.2%). Prevalence of thromboembolism (TE) was 6.6% where arterial TE was more prevalent than venous TE in the entire cohort (5.4% *vs*. 1.2%). Males were found to have more events of arterial TE whereas females had more venous TE. About 2% of the cohort was found to have cataract prior to ITP diagnosis. Prevalence of cataracts was higher among males 2.3% *vs*. 1.7% among females. As for haematological malignancies, about 1% of the cohort had such a history prior to diagnosis of ITP. The mean and median minimum platelet count for the enrolees at ITP presentation (7days before and 1 day after ITP diagnosis) was 38.3 and 20.0 x 10^9/L, respectively. About 30% of enrolees had their minimum platelet count between 0 and 9 x 10^9/L at the time of diagnosis. The mean platelet count rose above 100 x 10^9/L over the next 6 months but dropped just below this threshold at around 2 years to 86.9 x 10^9/L and 3 years to 99.0 x 10^9/L. In the period of time after the diagnosis of primary ITP, rates of events per 10,000 person-years was 104.8 (95% CI: 75.6-145.3) for TE, 68.3 (95% CI: 45.4-102.8) for cataracts. The rate of haematological malignancies was higher among patients 45 years of age or older [23.6 (95% CI: 7.6-73.3)] compared to patients less than 45 years [9.7 (95% CI: 2.4-38.9)].
Summary and Conclusion: This population based registry provides more knowledge and evidence on the natural history of primary ITP than previously known. Prior to or at the time of the diagnosis of primary ITP, about 7 out of every 100 patients had experienced a thromboembolic event and a lower prevalence of having had cataract and haematological malignancies were found in this cohort.

P1220

CLINICO-PATHOLOGIC FEATURES OF COMMON VARIABLE IMMUNODEFICIENCY (CVID) PATIENTS MANIFESTING CYTOPENIAS

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Background: Common variable immunodeficiency (CVID) is a primary immunodeficiency disorder characterized by recurrent infections and multiple immune defects. The full range of diagnostic problems is poorly described.
Aims: We aim to outline the clinical and laboratory features of patients with cytopenias encountered in a CVID cohort in a single centre.
Methods: An established database was used to identify patients over a 15 year period who had one or more cytopenias (Hb<135g/L in men and<115g/l in women, neutrophils<2.0x10*9/L, platelets<140x10*9/L) that could not be explained and that persisted for more than 2 weeks. The outcome of haematology assessment and bone marrow biopsy were noted.
Results: Of 183 CVID patients 64 (median age 49.5; range 32-86, M:F 0.8:1) were noted to have a cytopenia (55 thrombocytopenia; 28 anaemia; 19 patients had both). Regarding thrombocytopenia (median count 98; range1-136) idiopathic thrombocytopenia purpura (ITP) was presumed to be the cause in 34.6%. Two thirds of ITP patients with CVID experienced a recurrence of thrombocytopenia. Hypersplenism was considered to be contributory to thrombocytopenia in 50%. Two patients had a lymphoma diagnosed following identification of thrombocytopenia. Eleven had a bone marrow aspirate and trephine biopsy (BMAT). Eighteen required intervention - steroids in 13, rituximab 7, variation in immunoglobulin administration 7, splenectomy 6, anti-D immunoglobulin 1. There were 17 bleeding complications reported. Anaemia was reported in 28 patients and a cause identified in 24- autoimmune haemolytic anaemia (AIHA) in 8, pernicious anaemia in 3 and iron deficient erythropoiesis in 11 (of which 5 were secondary to CVID enteropathy-related malabsorption). A BMAT was performed in 6 patients with anaemia. Of the patients with AIHA, 6 required intervention in the form of steroids, rituximab and/or IV Immunoglobulin and 6 required one or more blood transfusions.
Summary and Conclusion: Cytopenias have a high prevalence in CVID patients and can be expected to present in haematology practice where CVID patients are managed. The majority of patients are managed without bone marrow biopsy. ITP and AIHA have well-characterised causal links with cytopenias in patients with CVID. However, other pathologies such as hypersplenism, malabsorption and pernicious anaemia that are common in this group of patients must be excluded.

P1221

PRIMARY VERSUS SECONDARY ITP IN ADULTS; A COMPARATIVE ANALYSIS OF CLINICAL AND LABORTORY ATTRIBURTES IN NEWLY DIAGNOSED PATIENTS IN AN ASIAN POPULATION

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Background: Immune thrombocytopenic purpura (ITP) is a hemorrhagic diathesis, resulting in escalated platelets destruction alongside impaired production in bone marrow. Patients from Asian regions often exhibit distinctive characteristics in comparison to the patients from the western countries. We accomplished this study to determine the spectrum of ITP in an Asian population.
Aims: The aim of this study is to evaluate the prevalence of primary *versus* secondary ITP along with comparative analysis of both the disease groups. The secondary objective is to determine the etiological spectrum of secondary ITP in Asian patients.
Methods: We illustrate the results of a prospective study in a population based large cohort of newly diagnosed adults (≥13 years) with platelet count<100x10^9/l at presentation. The study extended from January 2009 till December 2013. Complete blood counts, peripheral smear examination, HbsAg, Anti HCV, ANA and stool for Helicobacter pylori were analyzed by standard laboratory methodology. HIV, TSH, double stranded DNA, RA factor, Antiphospholipid antibodies and direct coombs test were done where indicated clinically. Bone marrow examination was carried out above 60 years and in those where it was deemed necessary.We computed percentages for the prevalence of primary and secondary ITP. Independent sample t- test were applied for comparative analysis of age and hematological parameters. We also computed pearson Chi-square correlation at 5% level of significance to identify association between categorical data.
Results: A total of 417 patients were incorporated with a mean age of 40.95±14.82 years. Primarily disease is observed in 3rd decade of life. Male to female ratio is 2:3 with a bimodal peak in females. The mean hemoglobin was 11.68±1.75g/dl while mean platelets count was 46.21±27.45x109/l. At diagnosis 43.16% (n=180) patients had hemorrhagic manifestations whilst 56.8% (n=237) were asymptomatic at presentation. Cutaneous bleeding was appreciated in 37.4% patients while epistaxis, gum bleeding and mennorhagia were detected in 23%, 18.7% and 5.7% respectively. None of patient presented with visceral, retropharyngeal or intracranial bleed. 24.4% had platelets<20x109/l, while 33.9% and 41.7% had platelets count between 21-50 and >50 respectively. Severe thrombocytopenia (<20x109/l) was significantly associated with amplified bleeding tendency (P<0.001). The prevalence of secondary ITP was substantially higher(65%) as compared to primary ITP (35%). Secondary ITP was predominantly seen in HCV reactive patients (24%), followed by helicobacter pylori infection (11%). Nevertheless 17% patients had underlying autoimmune disorders (SLE, RA, Evans syndrome and others). Drug induced thrombocytopenia and lymphoproliferative disorder (CLL & NHL) were detected in 5%&2% respectively. Providentially no study subject was found to be HIV reactive. Comparative analysis of primary *versus* secondary ITP revealed statistically significant difference in bleeding tendency and platelets counts(P<0.05). Also secondaryITP was exceedingly distinctive and divulged positive association with advancing age(P<0.05).

Table 1.

Parameters	Primary ITP(n= 147)	Secondary ITP(n= 270)	P- value
Age	37.52±14.82	42.83±14.56	0.043
Hemoglobin	12.21± 1.47 gm/dl	11.38 ±1.82 gm/dl	0.01
Platelets	51.56±21.94 x 10⁹/l	54.11± 26.96x 10⁹/l	0.000
Dry/wet purpura	57.1% / 49%	26.7% / 25.6%	0.001 / 0.008

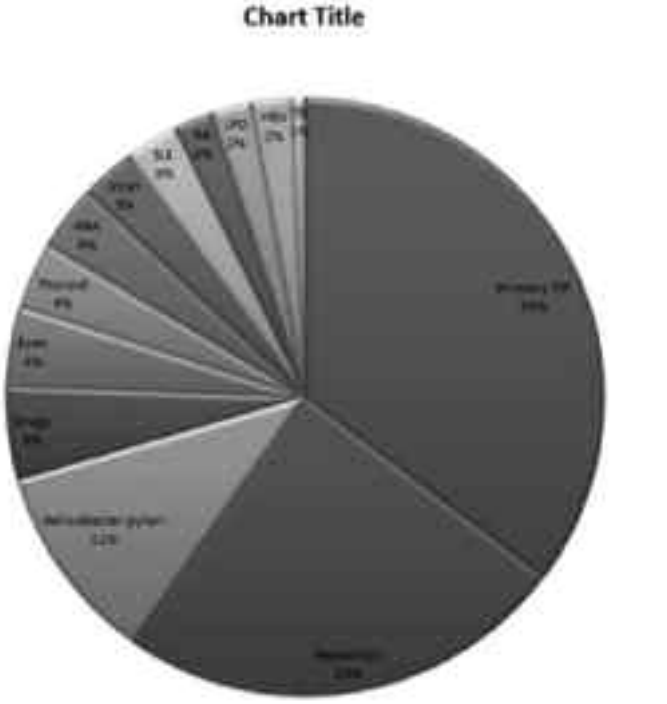

Figure 1.

Summary and Conclusion: Our study revealed predominance of secondary ITP with preponderance of older population. However bleeding manifestations and degree of thrombocytopenia were high in primary ITP cases. Infectious etiology (HCV, H.pylori, HBV,Tuberculosis) followed by autoimmune disorders are mainly implicated for secondary ITP in our setting.

P1222

ROUTINE BONE MARROW EXAMINATIONS MAY NOT BE NECESSARY IN THE EVALUATION OF PATIENTS WITH INMUNE THROMBOCYTOPENIA

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Background: The role of bone marrow examination (BME) in adults with immune thrombocytopenia (ITP) is not well established. The great majority of international guidelines recommend that the BME should be performed in patients who present with additional cytopenias or other hematological abnormalities, in patients with systemic symptoms or in those whom splenectomy or other second-line therapies are considered. Despite these recommendations, there are few studies performing comprehensive analysis of BME results in patients with ITP.
Aims: The objective of this study was to analyze bone marrow findings and to determine the usefulness of BME for the diagnosis (patients with isolated thrombocytopenia) and prognosis stratification in adult patients with ITP.
Methods: We included all consecutive ITP patients diagnosed of ITP in our

hospital between January 2010 and December 2013. Bone marrow aspirate was performed and we described bone marrow cellularity and morphology, and special attention to megakaryocyte number and morphology. Normal limits were established following the Wintrobe method. Cytogenetics and flow cytometry was performed in some patients as physician discretion.
Results: A total of 161 patients; median age was 61 years (range 16-90), 55.9% female. Eighty patients (49.7%) had newly diagnosed ITP, 11 (6.8 %) had persistent and 70 (43.5%) had chronic ITP at the time of the study. Median survivor´s follow-up was 16 months. At last follow-up 77 patients (47.8%) had undergone BME after a median of 2 months (range 0-44) from the first medical visit. The median platelet count at the time of BME was 27 x 109/L. Patients older than 60 years of age (64.9%), cases in which splenectomy or other second lines were considered (28.6%), and cases had hematologic abnormalities (6.5%) were the reasons why BME was performed. The morphology of megakaryocytes and bone marrow cellularity are listed in Table 1. Abnormal findings were evidenced in only 2 of the cases. In one additional case bone marrow was not assessable because the sample was limited. Of the 2 patients with pathological bone marrow, one patient had acute myelogenous leukemia (AML) and the other one a myelodysplastic syndrome (MDS). The AML patient had an isolated thrombocytopenia with a normal peripheral blood examination, but in a week developed a severe pancytopenia. Patient with MDS developed monocytosis during follow up. Flow cytometry study was performed in 66 patients (85.7%); it was normal in 58 patients (70.1%). Cytogenetics was performed in 39 patients (50.6%) and it was normal in 36 patients. Both techniques were helpful in the diagnosis of AML (See Table 1). BME findings implied a different diagnosis in 2.6% of patients who underwent the procedure and 1.24% of the entire cohort. None of the other patients developed any other hematological disease during the follow-up.

Table 1. Myelogram features and additional testing

Summary and Conclusion: Bone marrow examination is rarely informative in patients with isolated thrombocytopenia but proved to be useful in patients in whom there are other abnormalities in peripheral blood. Flow cytometry was useful to find clonal population B, undetectable by morphology in the bone marrow aspirate.

P1223

RITUXIMAB SALVAGE THERAPY IN ADULTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: EFFICACY AND SAFETY
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Background: Chronic immune thrombocytopenia (ITP) treatment recommended after corticosteroid failure remains splenectomy because of a large experience of its efficacy and a long-term follow-up. Despite this, post-surgery complications and risk of infection lead to the arrival of new agents such as Rituximab.
Aims: The purpose of this study is to assess efficacy and tolerance to Rituximab, a monoclonal anti-CD20 antibody.
Methods: We conducted a retrospective study in University Hospital of Caen of all patients treated with Rituximab therapy because of refractory or in relapse ITP from January 2004 to August 2013.
Results: Thirty-five patients with a median age of 49 years old (range 20-82) were enrolled. They all had active disease in relapse or refractory to a first line therapy and received 375mg/m^2 Rituximab dose weekly, for four weeks. Median time from diagnosis to first infusion was 16 months (range 1-362) and median time observation was 33 months (range 5-114).
Efficacy: Overall response rate (ORR) was about 54% of patients one and two years after the first infusion, with 26 and 27% of complete response, respectively, with median time response duration of 24 months (range 1-104). About 45,7% of patients are still maintaining durable and significant platelet response, with a median of 32,5 months (range 5-104) from the first infusion, 81% of them for more than 1 year. Ten of 35 patients (29%) had undergone splenectomy, 4 of them before Rituximab, one of them had responded to Rituximab and still maintained partial response at 17 months from the initial infusion. Durable response after Rituximab was observed in 78,6% of patients in second line therapy (11/14) against 35.7% of patients in third line therapy (5/14) (p=0,05; odds ratio 6.1[1; 51.7]$_{95\%}$).
Toxicity: Two patients suffered from pneumonia. Seven of 17 patients (41%) declared hypogammaglobulinaemia after Rituximab therapy, without clinical infectious consequence. One patient suffered from Guillain-Barre syndrome 18 months after Rituximab therapy. Four patients declared malignancies.
Summary and Conclusion: Rituximab can be considered as an alternative to splenectomy for 54% of patients who respond after more than 1 year and 27% for 5-year follow-up. Its safety profile should lead us to choose this medical option therapy before surgery. This encouraging data should be confirmed by prospective randomized trials and by most retrospective studies with long-term follow-up.

P1224

INTERNATIONAL ITP REGISTRY WITH FOCUS ON THE ASIA PACIFIC REGION: PRELIMINARY FINDINGS OF EPIDEMIOLOGICAL AND CLINICAL DATA
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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by auto-antibody induced platelet (PLT) destruction and reduced PLT production, leading to a low PLT count (<100 x 10^9/L). The availability of robust epidemiological and clinical data outside of Europe and the United States for ITP are very limited. Additionally, there is little uniformity in regards to the treatment of ITP leaving physicians to make treatment decisions based on their experience and institutional protocols. The International ITP-Registry is a prospective cohort study which seeks to collect real-world data on the epidemiological and clinical data of adult ITP predominantly from the Asia-Pacific region.
Aims: The aims of the study are: 1. To compile an anonymous centralised database of demographic, ITP-specific, and co-morbid disease information on ITP patients; 2. To collect data on the presentation and complications of the disease; 3. To document local treatment practices across multiple countries and regions and responses to treatments; 4. To develop and test a prognostic model for major bleeding among adult patients with ITP; 5. To identify factors that predict life-threatening bleeding.
Methods: Recruitment began in 2011. Patients ≥ 18 years old with a recent diagnosis of primary ITP (≤ 6 months since initial diagnosis) are enrolled prospectively upon provision of informed consent. The primary data collected are: patient demographics, laboratory investigations, co-morbidities, bleeding events, blood products used, interventional procedures, pharmacotherapeutic agents administered, response to treatment and adverse events. Data is collected at baseline, after months 6 and 12 and then annually.
Results: As of 31 August 2013, 140 patients had been enrolled at 24 sites across 7 countries in the Asia-Pacific Region and Middle East. At the time of data analysis, 80 patients had reached 6 month and annual follow-up time points with the remaining 60 patients having been enrolled for less than six months from baseline data collection. Sixty per cent were female with a median age of 48 years (range 18 – 96 years). Mean PLT count at baseline was 24 x 10^9/L (range 0 – 94 x 10^9/L). Haematology, coagulation and biochemistry tests were within normal ranges. Prior to enrolment 77 patients had bleeding events recorded. This decreased to 10 events in the follow-up period. Corticosteroids were the most frequently used first-line treatment (59% of patients) achieving

a partial (PR; ≥ 50 x 10^9/l but ≤ 100 x 10^9/l & 2 x pre-treatment) or complete (CR; ≥ 100 x 10^9/l & x 2 pre-treatment) PLT response (82%) and cessation of bleeding (100%) during the 6 month follow-up period. IVIg was also reported as a first line treatment in 14% of patients, achieving a PLT response in 47% and cessation of bleeding in 82% treated. Second line therapies used included splenectomy (n=3; PR=1, CR=2), TPO-receptor agonists (n=2; PR=1, CR=1). 22 patients received Immunotherapy, 14 had a PLT response.
Summary and Conclusion: Enrolled patients have a female predominance with median age of 48 years. Bleeding appears frequently at diagnosis (36%) with symptoms abating with first line treatments. Corticosteroids are the most frequently used first line treatment (59% of patients treated). Splenectomy appears uncommon and was performed in only 3 patients (14% of cohort) with immunotherapies the most often used second line therapy. Data collection and analysis for this study is ongoing.

P1225

CMV INFECTION DOES NOT HAVE AN IMPACT ON BLEEDING MANIFESTATIONS OR PROGNOSIS OF IDIOPATHIC THROMBOCYTOPENIA

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Background: Human cytomegalovirus (CMV) modulates host immunity, and CMV-infected patients often develop signs of immune dysfunction; Several reports have implicated cytomegalovirus (CMV) in the pathogenesis of ITP.
Aims: Our Study aimed to evaluate frequency of CMV positivity among pediatric patients with acute and chronic ITP and its impact on severity of bleeding, response to treatment and development of chronic disease.
Methods: A cross-sectional study was conducted including 40 patients with ITP diagnosed and/or following at Ain-Shams university pediatrics hospital hematology unit in the period between August 2012 and April 2013. Recording of clinical presentation was done for manifestations suggestive of viral infection *e.g.*pneumonitis, hepatitis, petechial rashes, mononucleosis-like syndrome, hepatosplenomegaly, elevated liver enzymes, atypical lymphocytosis, any persistent fever, morbilliform rash; bleeding signs and and severity were assessed using Edslev *et al.* 2007**,** Treatment details and response were included in the study and CMV-PCR was done for all patients.
Results: CMV-PCR was positive in 72% of studied patients with ITP, (85% of patients with acute ITP and 60% of chronic ITP). No significant difference was found in median age at diagnosis according to CMV status (P>0.005). 72.7% of patients with CMV- PCR positive had a chronic ITP compared to 41.4% in CMV -PCR negative patients (p=0.077), and no gender difference. No difference in the initial clinical presentation or bleeding scores according to CMV-PCR status. Two patients with CMV-PCR positive had intracranial bleeding, however, no significant difference were encountered in other bleeding manifestations. Refractory cases formed 36.4% of CMV-PCR negative patients compared to 17.2% in CMV-PCR positive patients (P=0.29) with no significant difference in outcome. No statistically significant difference between patients by CMV PCR positivity as regards first-line of treatment, response to first-line treatment, and refractoriness to treatment. No statistically significant difference in WBC count, lymphocytic count,neutrophil count or platelet count at sampling according to CMV-PCR status. Sensitivity of serology of CMV in comparison to CMV-PCR was 20.69 %, specificity 72.37 % with positive predictive value 66.67% and negative predictive value 25.81%.
Summary and Conclusion: CMV-PCR positivity formed 72% of patients with ITP; CMV IgM serology was not a good indicative of CMV infection. CMV-PCR positivity did not have a significant impact on clinical presentation, bleeding manifestations or outcome of ITP patients

P1226

THE RISK OF CANCER IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP): A NATIONWIDE POPULATION-BASED STUDY

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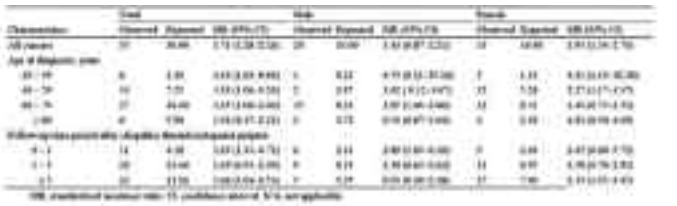
Background: Patients with immune thrombocytopenia (ITP) may have increased risk of develop solid malignancy.
Aims: To evaluate the risk of cancer among patients with ITP.
Methods: A population-based, retrospective cohort study was conducted that included patients diagnosed with ITP. These patients were registered in the National Health Insurance Research Database in Taiwan between 1997 and 2010. Standardized incidence ratios (SIRs) of solid malignancy were calculated to compare the cancer incidence of these patients with those of general populations. Risk factors for cancer development were analyzed by Cox proportional hazards models adjusting for competing mortality.
Results: During the 14-year study period, 53 cancers developed among 816 recruited ITP patients, with a follow-up of 5,396.88 person-years. The SIR for all cancers was 1.71 [95% confidence interval (CI) 1.28–2.24]. The SIRs of follow-up periods were 2.63 (95% CI 1.31–4.71), 1.49 (95% CI 0.91–2.30), and 1.66 (95% CI 1.04–2.51) at 0 – 1 years, 1 – 5 years, and ≥ 5 years, respectively. After exclusion of cancer within 1 year of diagnosis of ITP, a significant higher SIR of cancer was seen in cancers of the digestive (SIR 1.72; 95% CI 1.02–2.71). Multivariate analysis showed that age ≥50 years (hazard ratio [HR] 2.24; 95% CI 1.18–4.24) and diabetes mellitus (HR 2.01; 95% CI 1.00–4.03) were significant risk factors.

Table 1. Standardized incidence ration according to gender, age at diagnostic and duration of immune thrombocytopents



Summary and Conclusion: Patients with ITP are at increased risk of developing solid malignancy. Age ≥50 years and diabetes mellitus are independent risk factors.

P1227

HELICOBACTER PYLORI INFECTION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Eradication of Helicobacter Pylori (HP) infection has been proposed as a therapeutic alternative in the management of immune thrombocytopenia (ITP). Even it is postulated its possible use as an adjuvant therapy in patients with ITP without HP infection, due to immune-modulator effect of some antibiotics.
Aims: This study analyses the possible association ITP-HP and the eventual rol of eradication therapy in the increasement of platelet count.
Methods: 217 patients with primary or secondary immune thrombocytopenia are evaluated: 140 women and 77 men. Age median 56 years old (18-90) and platelet count median 31 x10e9/L. In 64 patients we perform 13C-Urea breath test for the detection of HP infection as a part of the first diagnostic work up. We use the classic eradication scheme OCA (omeprazole, claritromycin, amoxycilin). We check eradication of HP and platelet count increasement up to one month after treatment. The chi-square test and the Student's t-test were used for comparison of proportions and means respectively.
Results: 33 of 64 assessable patients (52%) have a positive 13C-Urea breath test. 31 of them receive eradication therapy. 6 (19%) achieve platelet response; they all with primary ITP, platelet count median 49 x10e9/L *vs* 20 x10e9/L in non-responders (p=0.03), age median 72 years *vs* 57 years in non-responders (p=0.04) and digestive symptoms. Despite the breath test is negative in 31 of 64 patients, 7 receive eradication therapy, without any platelet response. 19 patients receive eradication therapy without performing previous breath test for high clinical suspect. In none of them, platelets increase. In conclusion, 19% patients with positive breath test achieves platelet response after eradication therapy *vs* 0% patients with negative or not performed test. Breath test is repeated in 21 patients who received OCA therapy to confirm eradication of HP. It is negative in 16 of them, including 6 responders.

Table 1.

Platelet response after OCA	Responders (n=6)	Non-responders (n=25)	p-value
Sex (male/female)	2/4	6/19	0.84
Age (median/range)	72(47-85)	57(23-84)	**0.04**
Diagnosis (Primary/secondary IT)	6/0	19/6	0.10
Platelet count (median/range), x10e9/L	49(16-100)	20(1-100)	**0.03**
Digestive Symptoms (yes/no)	6/0	5/20	**0.0002**

Summary and Conclusion: In our series, patients with ITP and HP infection and who are responders to eradication therapy have common features concerning age, platelet counts and digestive symptomatology. According to these results we propose to mark this profile as high pre-test clinical probability of ITP-HP association, performing breath test in these patients and eradication therapy if test is positive.

P1228

RITUXIMAB IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA: A SARDINIAN SINGLE CENTER EXPERIENCE

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is an acute, life-threatening disease characterized by thrombocytopenia associated to microangiopathic anemia and often signs of organ dysfunction with neurologic, cardiac, renal or abdominal symptoms. TTP required a prompt diagnosis and immediate treatment with plasma exchange (PEX). Though the majority of patients recovered after standard PEX and steroids therapy a minority of them needs adjuvant therapy. In the last decade, being immunological origin postulated, the monoclonal antibody anti CD 20 have shown efficacy in the management of refractory and relapsed TTP.
Aims: Since August 2010 to January 2014 a total of 9 (4 males and 5 females) patients with TTP received Rituximab in our Institution. Median age at diagnosis was 39 years (range 19-56), median age at infusion of Rituximab was 44.5 y (19-62). Five patients had Rituximab during a relapse, the other 4 underwent immunotherapy in their first episode of disease because of refractoriness to standard therapy (lack of response after seven days, exarcebation or progression of symptoms or transient response). Rituximab has been administered at standard dose (375 mg/m^2 weekly for 4 total infusions). In refractory patients, Rituximab was started in association to PEX; in the relapsed and PEX-responder patients, was given after the last PEX as "consolidation" therapy.
Results: All the patients showed response to Rituximab, the median time to response was 5 days (range 0-27). All the 4 patients who had Rituximab as part of the first line therapy are still in complete remission showing a median duration of continuous disease free survival of 27.6 months (range 4.4-30.4). At the time of Rituximab administration 2 of the relapsed patients were in their 10th and 6thepisode of TTP respectively, while 3 of them were in the 5th one. Four of the 5 relapsed patients (80%) experienced a new recurrence of disease after Rituximab and 3 of them received a other course of Rituximab when the episode resolves. The median time between relapses of TTP before Rituximab administration in the 5 relapsed patients was respectively 11(3-25), 16(2-24), 12(12-21), 22.5(13-26) and 17(7-41) months. The first 4 patients relapsed again after Rituximab and the duration of response was respectively 30, 22, 12 and 33 months, so in 3 out of 4 relapsed post immunotherapy the interval between relapses was longer compared to previous ones. One out of 5 is still on remission after 36 months from first Rituximab infusion. Only in 1 patient, also affected by Kabuki Syndrome, we observed a serious side effect namely a pneumonia with respiratory failure and a severe hypogammaglobulinemia recovered after antibacterial and intravenous immunoglobulin infusions. At the moment of the writing all the 9 patients were in remission.
Summary and Conclusion: Our experience confirms the data regarding the efficacy and safety of Rituximab in the management of TTP. In particular shows that Rituximab can extend the time of remission in relapsed patients and can be combined successfully with PEX in refractory cases.

P1229

CLINICAL ANALYSIS OF EFFECT OF HELICOBACTER PYLORI ERADICATION IN PATIENTS WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Idiopathic thrombocytopenic purpura (ITP), an autoimmune disease caused by sensitization of platelets by autoantibodies leading to platelet destruction, has been associated with some infectious agents, including Helicobacter pylori. Eradication of Helicobacter pylori was reported to increase the platelet counts in some H. pylori-positive patients with chronic idiopathic thrombocytopenic purpura (cITP). However, the efficacy of the eradication was quite different and the mechanismwas not very clearly according to the previous reports.
Aims: To analysis the effect of Helicobacter pylori eradication in patients with chronic idiopathic thrombocytopenic purpura and to investigate the possible pathogenic mechanisms and to predict the platelet response after eradication of H. pylori. in each cITP patient.
Methods: 86 patients with cITP underwent gastroscopy and Pathological examination, 67(78%) of them were detected H. pylori infection positive. 37 patients with H. pylori infection positive had received the treatment of eradication of H. Pylori, the other 30 patients with H. pylori infection positive had not.The eradication group received a standard triple antibiotic therapy for H. pylori. platelet-associated immunoglobulin G (PAIgG) levels were detected by flow cytometry,and Anti-CagA IgG antibody titer of each patient's serum was measured by ELISA
Results: Of the 37 ITP patients with H. pylori infection positive, 30 (81.1%) of them achieved pylori and eradication. 17 (56.7%) of which showed increased platelet counts within the 4 months following treatment. It was significantly different compare with non-eradication group (0%). Completely responsive patients also showed significant declines in platelet-associated immunoglobulin G (PAIgG) levels after eradication therapy. We also detected the H. pylori cytotoxin-associated gene A (CagA) protein in each patients, we found that The titers of anti-CagA antibodies in the responders were significantly higher than those in the nonresponders,but the levels of anti-CagA antibody declined after eradication therapy.
Summary and Conclusion: H. pylori eradication treatment was an effective therapeutic option for H. pylori-positive patients with cITP. the lever of serum anti-CagA antibody may be a good predictor of platelet recovery, Moreover, the Cross-reactivity between PAIgG and H. pylori CagA protein suggests that molecular mimicry by CagA plays a key role in the pathogenesis of a subset of cITP patients.

Thrombosis and Vascular biology

P1230

A SMALL PROTEIN THAT INHIBITS COMPLEMENT ACTIVITY PROVIDES A POTENTIAL NOVEL ANTI-THROMBOTIC AGENT

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Background: A recombinant protein molecule (16.7kDa) known as coversin, is derived from a salivary molecule from the *Ornithodros moubata* tick where it assists the parasite to feed without provoking a host immunological response. It binds complement C5 and leukotriene B4 (LTB4) independently and with high affinity. It is not an antibody and can therefore be manufactured in prokaryotic cells such as *E. coli* leading to a potential reduction in manufacturing costs compared to monoclonal antibody production. It is considered to be a promising therapeutic approach to thrombotic and haemolytic diseases due to disordered complement activation including atypical haemolytic uraemic syndrome (aHUS), other thrombotic microangiopathies, catastrophic anti-phospholipid syndrome and paroxysmal nocturnal haemoglobinuria (PNH).
Aims: We aimed to perform initial *in vitro* titration experiments of coversin using PNH cells, before commencing a Phase I clinical trial in healthy normal subjects.
Results: Using PNH cells, the maximum inhibition of *in vitro* haemolysis compared to control occurred at a coversin concentration of approximately 10mcg/mL. Further experiments showed the effectiveness of coversin in blocking *in vitro* haemolysis in PNH cases that had both type III (complete GPI-deficiency) and type II (partial GPI deficiency) red cells. Using flow cytometry to demonstrate the deposition and accumulation of significant amounts of C'3d on both type II and type III PNH red cells, these experiments showed therapeutically effective inhibition of *in vitro* haemolysis by coversin. Coversin 10mcg/mL was found to be equivalent to eculizumab 50mcg/mL suggesting a 1:1 binding of the former to C5 and a 1:2 binding of the latter. Coversin has completed a Phase I clinical trial in healthy normal subjects, with informed consent, in which total blockade of complement C5 was achieved following s.c. injection at a dose of 0.57mg/kg. Activity fell below 5% and remained below 50% for 48 hours suggesting that dosing every 2 days is feasible. Additional studies with repeated daily s.c. dosing, at a lower maintenance dose will follow. No drug related clinical or routine laboratory side effects were noted.
Summary and Conclusion: The ability of patients to self-inject, together with potentially lower manufacturing costs and fewer antibody-like adverse reactions makes coversin a promising therapeutic agent for complement-associated thrombotic disorders. Phase II/III multicentre clinical trials are planned, initially with PNH and aHUS patients.

P1231

IMPROVED SURVIVAL WITH ONGOING ECULIZUMAB VERSUS SUPPORTIVE CARE IN PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare and life-threatening condition associated with chronic uncontrolled complement activation and thrombotic microangiopathy (TMA). Prognosis for patients with aHUS is poor; they face progressive organ damage due to TMA and are at constant risk of sudden death. aHUS patients reaching ESRD tend to be confined to long-term dialysis due to high renal graft rejection. The 5-year overall mortality rate is estimated to be 61% for all patients on chronic dialysis. In addition, data from aHUS and TMA registries report mortality rates for defined aHUS patients of 8% (1 year follow-up), 13% (mean follow-up, 17.8 months) and 32% (median follow-up, 4.4 years), despite the provision of supportive care, including PE/PI. The patients enrolled in these registries were not receiving eculizumab treatment. Pivotal prospective clinical studies demonstrate that sustained eculizumab treatment is associated with clinically meaningful improvements in hematologic and renal outcomes in patients with aHUS; at the same time, it has not been demonstrated if these outcomes translate into survival benefits for these patients.
Aims: To evaluate whether ongoing eculizumab treatment improves survival in patients with aHUS enrolled in pivotal studies of eculizumab.
Methods: A Markov model was developed to track progression through three stages of chronic kidney disease (CKD; estimated glomerular filtration rate ≥60, 15–59 and<15), plus transplant and death. Eculizumab treatment outcomes in the model were estimated using observed data from the eculizumab treatment period in prospective studies. These were compared with the outcomes on supportive care, which were estimated by an unbalanced panel fixed effects regression model with robust standard errors clustered at the patient level, using data from the pre□eculizumab treatment period in the same studies. It was assumed that all patients with aHUS and CKD5/ESRD have a mortality rate consistent with patients in the UK Renal Registry (1-year age-adjusted survival, 89.8%). The model assumes three causes of mortality: age, ESRD/dialysis and TMA-related causes other than renal disease.
Results: Pre-treatment (supportive care) data were available for 37 patients; the mean number of days of observation was 352 (range, 1–702). During supportive care, the estimated glomerular filtration rate (eGFR) declined by 5.5 mL/min/1.73m^2 (p<0.01) every 6 months. In contrast, there was a mean increase in eGFR in patients treated with eculizumab at 6 months (32 and 6 mL/min/1.73m^2 for patients with progressing TMA and long disease duration of disease, p=0.001 and p<0.001 respectively). Using the Markov model, the estimated mortality rate for patients receiving supportive care was 8.6% at 1 year and 25.6% at 3 years. During eculizumab treatment, there was one reported death at a median follow-up of 37 months, corresponding to an annual mortality rate of 1.4%. Eculizumab reduced the risk of mortality by 83% and 89% at 2 and 3 years, respectively, compared to the estimated mortality on supportive care only; relative risks were 17% (95% CI, 2–132%) at 2 years and 11% (95% CI, 1–83%) at 3 years (p=0.107 and p=0.014, respectively).
Summary and Conclusion: In the current analysis, eculizumab substantially improved survival in patients with aHUS compared with predicted outcomes for patients receiving long-term supportive care only. By completely blocking uncontrolled complement activity, ongoing eculizumab treatment reduced the mortality rate by 89% at 3 years.

P1232

REDUCTION OF BIOMARKERS RELATED TO THROMBOTIC MICROANGIOPATHY IN PATIENTS WITH AHUS TREATED WITH ECULIZUMAB

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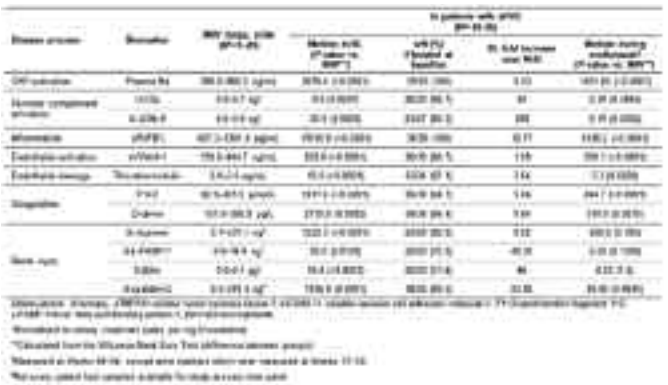
Background: aHUS is a genetic, life-threatening disease of chronic, uncontrolled complement activation, leading to systemic thrombotic microangiopathy (TMA) and organ damage. Eculizumab (Ecu) has been shown in prospective clinical trials to block terminal complement (TC), inhibit TMA, normalize platelets and improve renal function in patients (pts) with aHUS.
Aims: To evaluate the effect of eculizumab on biomarkers of complement and endothelial cell (EC) activation, inflammation, thrombosis and renal injury in pts with aHUS.
Methods: Adult pts (≥18 years; N=41) with aHUS were treated with Ecu in a single-arm, open-label, multicentre trial (NCT01194973). Biomarker levels were measured prior to Ecu (baseline; BL) and at regular intervals during one year of Ecu treatment in pts and compared with levels in normal healthy volunteers (NHV).
Results: Biomarker data were available for 9-20 NHV and 26-38 pts with aHUS treated with eculizumab. At BL, all biomarker levels were significantly elevated in the majority of pts with aHUS (Table), including in pts with prior plasma exchange/infusion (PE/PI), or those with normal BL platelet, lactate dehydrogenase or haptoglobin levels. After treatment with Ecu, TC markers (C5a, sC5b-9) and all renal injury markers normalized (Table). Markers of inflammation (sTNFR1), endothelial damage (thrombomodulin) and coagulation (F1+2 and D-dimer) significantly decreased by up to 99%. Ba and sVCAM-1 levels, which reflect complement alternative pathway (CAP) and EC activation, decreased by 30% and up to 60%, respectively, but remained elevated relative to NHV (Table).

Table 1. Biomarker levels in patients with aHUS compared with normal healthy volunteeter (NHV)



Summary and Conclusion: At baseline, complement activation and elevated biomarkers of inflammation, coagulation, endothelial activation, and renal injury

were evident in the majority of pts with aHUS, including those with prior PE/PI or normal clinical laboratory values. Ecu treatment normalized TC markers, potently reduced markers of EC damage to near-normal levels, markedly reduced inflammatory and thrombosis markers and eliminated signs of ongoing renal injury. These benefits were sustained with ongoing Ecu. Ecu reduced but did not normalize plasma Ba and sVCAM-1, reflecting ongoing CAP and EC activation that was not pathogenic in the presence of Ecu. These data underscore the chronic complement dysregulation and ongoing risk of systemic TMA and organ damage in pts with aHUS, and the requirement for continued TC blockade with Ecu, even when clinical laboratory values have improved.

P1233

DIAGNOSTIC CHALLENGES IN THROMBOTIC MICROANGIOPATHIES (TMAS): DATA FROM A TARGETED ANALYSIS OF THE AUSTRALIAN / NEW ZEALAND TMA REGISTRY

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Background: Advances in the understanding of the pathogenesis of TMAs have led to recognition of distinct clinical entities: TTP (thrombotic thrombocytopenic purpura), STEC-HUS (Shiga-like toxin producing E.Coli hemolytic uremic syndrome) and atypical HUS. Where once precise classification of TMAs made little difference to the management plan, specific treatment strategies are now available for each disease, and consequently it is critical that a rapid and accurate diagnosis is made. This can be achieved, for example, when pre-plasma exchange ADAMTS13 activity levels are found to be<10%, diagnosing TTP. However, many patients with a TMA remain diagnostically challenging, including those with aHUS (primary complement dysregulation) and secondary TMAs (*e.g.* due to drugs, infections). Potential pitfalls to accurate diagnosis include the overlapping clinical symptomology of the syndromes and the lack of readily available diagnostic tests for aHUS; in particular, genetic analysis of complement mutations may not be rapidly available. Treatment as for aHUS (*e.g.* with eculizumab) in all TMA cases with ADAMTS13 activity >10% until another cause is identified has been suggested, but would incur substantial costs. Further study of the spectrum of TMAs presenting clinically is required to target areas where practice can be improved and develop viable strategies.
Aims: To analyze data from a multicenter TMA registry to identify patients without low ADAMTS13 activity at presentation, and examine their clinical course through investigations, diagnosis, management and outcomes.
Methods: Monash University in Melbourne, Australia hosts a national multicenter TTP Registry that was expanded in 2012 to incorporate all TMAs. In 2013, senior clinicians with hematology or nephrology backgrounds undertook validation of cases in the registry targeted at 13 sites that had entered patients with ADAMTS13 activity levels of >10%, excluding patients positive for STEC.
Results: A total of 68 cases were examined in detail: 37 patients had ADAMTS13 activity of >10% at presentation, 14 had no ADAMTS13 result, and 17 had low levels<10%. Comparing patients with TTP to those without low ADAMTS13 results, 82% *versus* 50% had neurological involvement, and 35% *versus* 70% had renal impairment. Excluding patients with low ADAMTS13, 85% had a possible secondary cause for the TMA identified by the validator, 90% received plasma exchange, 41% dialysis and two thirds immunosuppression. A diagnosis of aHUS was rarely made by treating physicians, with TTP or 'TTP-HUS' the most common categories; the validator disagreed with the original diagnosis in approximately 30% of cases. Subsequently, 25% had died at a mean 3 months and 27% had ongoing renal impairment or treatment needs.
Summary and Conclusion: The TMA Registry data demonstrate a marked heterogeneity, particularly in those cases without low ADAMTS13 activity, underlining the diagnostic challenge in these patients. Applying published criteria using platelet and creatinine thresholds is not helpful in this group. Secondary TMA cases outnumber likely aHUS presentations, although the difference between a precipitating event and a secondary TMA is poorly defined, and early decisions on which patients might benefit from eculizumab are therefore difficult. Despite great advances in understanding of TMAs, accurate diagnosis in 'real world' clinical settings remains challenging, with important consequences for clinical management and healthcare costs.

P1234

THE EFFICACY OF VINCRISTINE IN THE TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY THROMBOTIC THROMBOCYTOPENIC PURPURA

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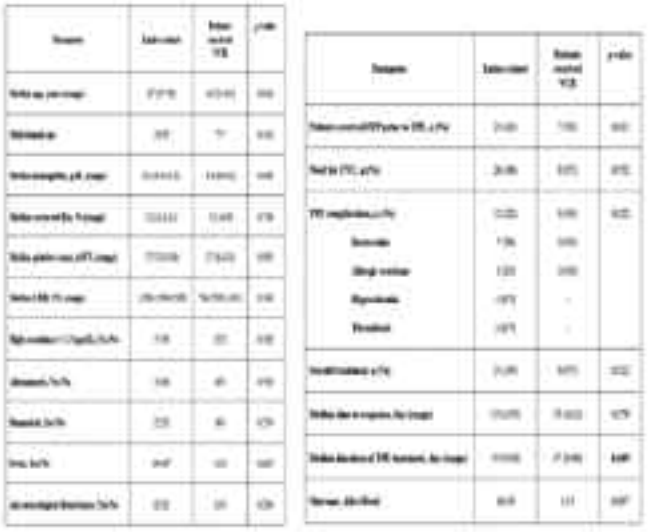
Background: Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease, unless it is recognized promptly and treated agressively. Therapeutic plasma exchange (TPE) therapy has dramatically improved prognosis of TTP, decreasing the mortality rate from 90% to less than 20%. However, a subset of patients with acquired TTP requires long-term TPE to prevent fatal outcome and to achieve a sustained remission. In these patients complementary treatments, including various immunosuppressive agents including vincristine (VCR) have been proposed. Despite all therapeutic options, however, recurrent acute episodes occur in approximately 40% of patients with acquired ADAMTS13 deficiency.
Aims: The aim of this study was to evaluate the efficacy of VCR when used as an adjunct to TPE in patients with relapsed/refractory TTP. And to compare the characteristics of patients who received TPE plus VCR with the entire cohort.
Methods: Fifty-four patients who were diagnosed as TTP between October 1991 and January 2014 were enrolled in the study. Patients' demographics, treatments, complications, follow-up periods and outcomes were noted from the patients' files retrospectively.
Results: The study cohort consisted of 54 patients (male/female: 19/35), and the median age was 37 years (range, 17-78 years). The patients' demographics were displayed in Table 1. Forty-eight patients had a single episode whereas 8 had relapsed (6 cases had two episodes, and two had 3 episodes) giving a total number of sixty-four acute TTP episodes. Fourteen patients (26%) [male/female: 7/7, median age 34 years (range, 21-65 years)] had received VCR as an adjunct to TPE in 17 acute episodes. In eleven of these patients, VCR was administered due to refractory disease in the first episode, and the remaining 6 had received VCR in the recurrence (in one patient with 2 relapses, VCR was used in both recurrences). The treatment modalities, complications, and outcomes were shown in Table 2. Peripheral (n:1) and autonomic (n:1) neuropathies, and constipation/paralytic ileus (n:2/n:1) were observed in patients receiving VCR. Among 6 patients, TTP was diagnosed during pregnancy, and in two VCR was administered which induced a durable remission but the pregnancies were terminated. There was no history for any medication that might play a role in the pathogenesis of TTP among patients who received VCR. There were five patients in the entire cohort with an autoimmune disease (Behçet's disease n;1, Hashimoto's thyroiditis n;1, ulcerative colitis n:1, scleroderma n:1, systemic lupus eritematosus (SLE) n:1). VCR was administered in the patient with SLE, and a durable remission was gained. Among the fourteen relapsed/refractory patients who received VCR as an adjunct to TPE, 11 had achieved a remission, but three of them died due to TTP.

Table 1.



Summary and Conclusion: Although the prognosis of TTP has improved with TPE, there are still relapsed/refractory patients, which can be difficult to manage. In patients with relapsed/refractory disease, immunosuppressive agents including VCR can be a reasonable choice with minimal toxicities when used as an adjunct to TPE which might induce durable remissions.

P1235

RISK OF THROMBOEMBOLIC STROKE IN PATIENTS WITH OVARIAN CANCER: A NATIONWIDE POPULATION-BASED STUDY IN TAIWAN

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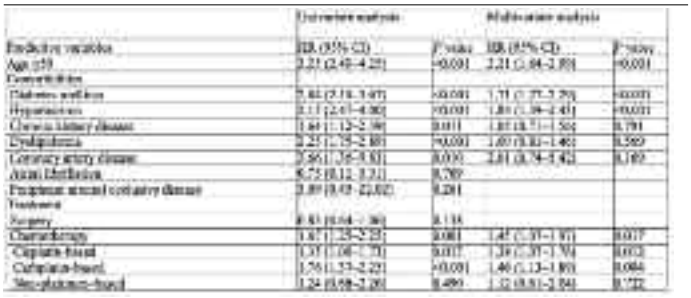
Background: Cancer patients are at risk of thromboembolism. Ovarian cancer is the most common cause of cancer death from gynecologic tumors, and more than 225,000 new ovarian cancers are diagnosed each year. The presence of disseminated intravascular coagulation in ovarian cancer patients may indicate a hypercoagulative status. However, studies investigating the relationship between ovarian cancer and thromboembolic stroke (TES) are lacking.
Aims: This study aimed to assess the association between ovarian cancer and TES, and to determine the predictive risk factors.
Methods: Ovarian cancer patients aged ≥20 years, without antecedent TES events, and who were followed up for >1 year between January 1, 2003 and December 31, 2011 were recruited from the Taiwan National Health Insurance database. Hazard ratios (HRs) of TES risk for ovarian cancer patients compared with an age- and comorbidity-matched cohort were calculated by Cox proportional regression analysis. The difference in cumulative TES incidence between the two cohorts was analyzed with the Kaplan-Meier method and tested with the log-rank test.
Results: Each cohort (ovarian cancer and matched cohort) consisted of 8,810 individuals, with a median age of 49 years. Between the two groups there was no difference in TES-related comorbidities as listed in Table 1. In the ovarian cancer cohort, 6,160 patients (69.9%) received surgery, and 6,590 patients (74.8%) received chemotherapy including cisplatin- (n=3,095; 35.1%) or carboplatin-based (n=5,041; 57.2%) regimens. We found that the TES incidence was 1.38-fold higher in the ovarian cancer cohort than in the matched cohort (9.4 *vs.* 6.8/1000 person-years), with an age- and comorbidity-adjusted HR of 1.49 (*P*<0.001). The TES risk imposed by ovarian cancer was more prominent in patients<50 years (HR 2.28; *P*<0.001) compared to patients ≥50 years (HR 1.33; *P*=0.005). Significant risk factors predicting TES development were age ≥50 years (HR 2.21; *P*<0.001), hypertension (HR 1.84; *P*<0.001), diabetes mellitus (HR 1.71; *P*<0.001), and treatment with chemotherapy (HR 1.45; *P*=0.017), especially platinum-based regimens (Table 1).

Table 1. Analyses of risk factors for thromboembolic stroke (TES) in patients with ovarian cancer



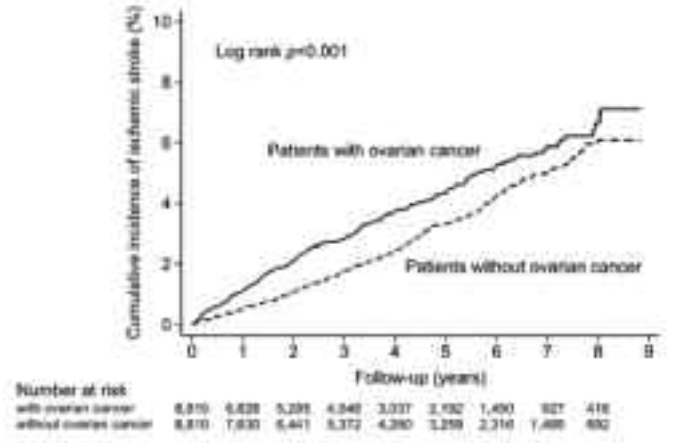


Figure 1.

Summary and Conclusion: Ovarian cancer patients were at an increased risk of developing TES. Age, hypertension, diabetes, and chemotherapy treatment were independent risk factors.

P1236

THE EFFECT OF MORBID OBESITY ON THROMBIN GENERATION AND SENSITIVITY TO THE ANTICOAGULANT ACTIVATED PROTEIN C IN PREGNANT WOMEN

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Background: Obese pregnant women have greatly increased risk of venous thromboembolism (VTE). However, the precise mechanisms underlying this increased risk are poorly understood. To address this, plasma samples were obtained with consent from pregnant women of varying body mass index (BMI) and analysed using an assay that characterises pro-and anticoagulant pathways.
Aims: To characterise the effect of obesity on thrombin generation and sensitivity to activated protein C in pregnant women.
Methods: Blood samples were collected in citrated tubes containing corn trypsin inhibitor (to inhibit contact-mediated coagulation activation) and platelet poor plasma was prepared. Tissue factor (TF)-stimulated thrombin generation was determined by measuring cleavage of a thrombin-specific fluorogenic substrate using Thrombinoscope™ software. Thrombin generation was also assessed in the absence of an exogenous TF stimulus.
Results: 13 women of similar third trimester gestation (BMI 20-29 kg/m^2, n=6; 30-39 kg/m^2, n=4; >40 kg/m^2, n=3) and 2 non-pregnant volunteers were recruited. Mean endogenous thrombin potential (area under the thrombin generation curve; 0.5 pM TF stimulus) was significantly higher in BMI>40kg/m^2 (2056 +/- 88 nm*min) compared with 20-29 kg/m^2 (1350+/-97 nm*min; p=0.003), 30-39 kg/m^2 (1259+/-253.4nm*min; p=0.049) and non-pregnant volunteers (1084+/-264.8 nm*min; p=0.0235). Peak thrombin generation was also greater in the BMI >40kg/m^2 group (158.6+/-11.61nm) than that observed in the BMI 30-39kg/m^2 group (100+/-29.39nm) and in the BMI 20-29kg/m^2 group (95.68+/-22.11nm) and was significantly greater than that measured in the non-pregnant controls (46.17+/-10.33nm, p=0.0069). In addition, a tendency toward shorter lag time to initiation of thrombin generation and to peak thrombin generation were observed in the BMI>40kg/m^2 group compared to the other test groups. To address the hypothesis that circulating plasma TF activity contributed to these observed differences, thrombin generation was initiated in the absence of a TF stimulus. No significant thrombin generation was seen in any group. Characterisation of the anticoagulant protein C pathway was performed by incubation with activated protein C (APC; 5nM; 1pM TF trigger). Remarkably, while APC resistance was observed in all pregnant plasma samples, those of BMI >40 kg/m^2 were most resistant to APC- induced attenuation of thrombin generation compared with patients of BMI 30-40 and 20-30 kg/m^2. In these three groups, pre-incubation with APC attenuated ETP by 20%, 57% and 70% respectively.
Summary and Conclusion: VTE is a potentially life-threatening complication of pregnancy. Morbidly obese pregnant women have pro-and anticoagulant pathways variations that may represent potential mechanisms underlying the observed high VTE risk in these patients.

P1237

HYPOXIA CONTRIBUTES TO PROCOAGULANT ACTIVITY OF BREAST CANCER CELLS

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Background: Venous thrombosis is one of the main causes of morbidity and mortality in cancer patients. This common complication ofcancer is triggered by tissue factor (TF), which also plays an important role in angiogenesis and metastasis. TF pathway inhibitor(TFPI) is the endogenous inhibitor of TF induced coagulation, and has been shown to exhibit anti-angiogenic and anti-metastaticeffects.Hypoxia microenvironment, resulting from aberrant hemoperfusion and high oxygen consumption, is a commonfeature of solid tumors. As an important microenvironment factor, hypoxia facilitates tumor metastasis and propagation. Hypoxiainducible factor 1a (HIF1a) is the main mediator of hypoxia and regulates many gene transcriptions to promote tumor cell survival,which is closely related to tumor propagation and poor prognosis.The correlation between thrombosis and hypoxia is not clearly understood. We focused on the effect of hypoxicmicroenvironment on the procoagulant activity of breast cancer cells and putative mechanisms.
Aims: Our aim was to detect the procoagulant status of breast cancer cells grown in an hypoxix environment and the mechanisms of hypoxic gene regulation.
Methods: Breast cancer cell lines, SKBR3 and MCF7, were either treated with cobalt chloride to mimic hypoxia or cultured in 1% oxygen tension. Cells grown in normoxic conditions (21% oxygen tension) were used as control. Expression of TF and TFPI was detected by RT-PCR, western blot and ELISA. HIF1a and HIF2a expression were measured by western blot. TFPI promoter activity was measured by luciferase reporter assay. The luciferase plasmids were constructed by cloning TFPI promoter region into the pGL3 basic vector.
Results: TF mRNA and protein level was up-regulated in a time- and dose-

dependent way in hypoxia or after CoCl2 treatment. TFPI mRNA and protein level was down-regulated in a time- and dose-dependent way in hypoxia or after CoCl2 treatment. The effects of hypoxia on TF and TFPI expression were impaired when HIF inhibitor was used. The luciferase reporter assay showed reduced luciferase activity of TFPI promoter following hypoxic stimulation. The reduction of luciferase activity of TFPI promoter was also observed when HIF1a was overexpressed.
Summary and Conclusion: The hypoxic microenvironment may contribute to the procoagulant in breast cancer, and HIF1a may be new candidates for the treatment of thromboembolic complications in cancer.

P1238

IMPACT OF RECOMBINANT THROMBOMODULIN ON THE OUTCOME OF NEONATES WITH DISSEMINATED INTRAVASCULAR COAGULATION DIFFERS ACCORDING TO APGAR SCORE

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Background: Recombinant thrombomodulin (rTM) was developed as a new type of treatment for disseminated intravascular coagulation (DIC), which inhibits coagulation factors Va and VIIIa by activating protein C. Since July 2009, we have used rTM in newborns with DIC as front-line therapy. To date, however, few data are available in neonatal medicine. Because the etiology of DIC in newborns is quite different from that in other children and adults, more evidence is needed before rTM can be approved as a standard treatment in this age group.
Aims: To elucidate predictive factors for outcome in newborns with DIC treated with rTM as front-line therapy.
Methods: This retrospective study included 23 newborns (13 boys, 10 girls; median age, 1 day; range, 0–13 days). Median gestational age was 37 weeks (range, 23 weeks 6 days–41 weeks 1 day). Median birth weight was 2296 g (range, 708–3853 g). Diagnosis of DIC was made according to the diagnostic criteria of the Japanese Ministry of Health, Labour, and Welfare. rTM (380 U/kg) was administered daily (median, 5 days; range, 2–16 days) as front-line therapy. Antithrombin (AT) was used concomitantly in 15 patients. Flesh frozen plasma and platelet transfusions were also given to 19 patients. The underlying diseases were hematological disorders (n=5), infection (n=3), asphyxia (n=7), and other (n=8). Treatment failure was defined as death or serious bleeding within 28 days of rTM treatment.
Results: Although 20 patients (87%) were alive 28 days after rTM treatment, pulmonary or intracranial bleeding was observed in 7 (30%). Consequently, failure-free survival was 65%. Median Apgar scores at1 and 3 min in the failure-free group were significantly higher than in the treatment failure group (8 and 9 *versus* 5 and 6.5; p=0.040 and p=0.027, respectively). Other factors including gestational age, birth weight, DIC score, platelet count, prothrombin time, fibrinogen, fibrin and fibrinogen degradation products, D-dimer, and AT activity were not associated with treatment response.
Summary and Conclusion: The incidence of bleeding events was extremely high in neonates. rTM-based treatment is extremely promising for the subgroup with high Apgar scores. In contrast, additional treatment to prevent life-threatening bleeding must be explored in those with a low Apgar score. This concern remains to be solved in this rTM era.

P1239

RESISTANCE TO ACTIVATION OF ENDOGENOUS PROTEIN C ASSOCIATED WITH ANTI-PROTEIN C ANTIBODIES MAY BE A MARKER OF A MORE SEVERE THROMBOTIC PHENOTYPE IN ANTIPHOSPHOLIPID SYNDROME

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Background: Antiphospholipid antibodies (aPL) may interfere with the anticoagulant activity of activated protein C (APC) to induce APC resistance (APCr) and this phenomenon has been proposed as a mechanism of thrombosis in antiphospholipid syndrome (APS).
Aims: We investigated the frequency and characteristics of APCr using exogenous APC and activation of endogenous protein C (PC) with Protac, in a thrombin generation (TG) system in thrombotic APS patients with venous thromboembolism (VTE).
Methods: Resistance to exogenous human APC (rhAPC) and Protac® activated, endogenous PC was studied in 51 APS and 51 non-APS VTE patients on warfarin and 51 normal controls (NC), using the Calibrated Automated Thrombogram® (CAT) system with 5pM tissue factor. Patients with heritable thrombophilia or patients/NC receiving oestrogen were excluded. INR values were comparable (median [range]): 2.4 (1.8-4.2) in APS and 2.3 (1.8-4.3) in non-APS patients. For TG, plasma was mixed 1:1 with PNP to correct factor deficiency. APC resistance (APCr) was calculated as % inhibition of TG by dividing the ETP with rhAPC or Protac® by that for buffer, normalising with PNP (lower % values indicate greater APCr). PC antibodies were measured by ELISA and their avidity was assessed by increasing the salt concentration (NaCl 0.1-6M) in sample dilution buffer.
Results: APS patients showed greater resistance to rhAPC (median [range]: (90% [18-109] [p=0.0006]) than non-APS patients (99% [47-131]) and NC (94% [56-132]), mainly due to a subgroup of 6 patients with little inhibition of thrombin generation with APC. Both APS (median [range] (72% [4-98]) and non-APS (81% [12-136]) patients showed greater APCr with Protac® (p<0.0001) compared to NC (100% [63-141]), but resistance was greater in APS than in non-APS patients (p=0.0034). PC antibodies were more frequent in APS (25/51) than non-APS patients (5/51, p<0.0001). Investigation of the avidity of antibodies demonstrated two clear groups of patients. Ten APS patients had low avidity PC antibodies and 15 had higher avidity antibodies. The percentage of maximum binding to PC in these two groups reached statistical significance at 1M NaCl (mean residual binding 39.7% for the higher avidity samples, compared to 16.5% for the low avidity (p<0.0001). Patients with higher avidity PC antibodies had greater APCr with Protac® than those with low avidity PC antibodies (median [range] 43.3% (3.7 -58.1) and 74.1% (60.9-90.93), Fisher's exact test p<0.0001). Six APS patients with marked APCr using both rhAPC (17.6-35.2%) and Protac® (3.7-33.9%) also had higher avidity PC antibodies and exhibited more severe thrombotic disease *i.e.* arterial as well as VTE or recurrent thrombosis whilst on therapeutic anticoagulation.
Summary and Conclusion: In conclusion, thrombotic APS patients have greater APCr using both exogenous APC and activation of endogenous PC. Those patients with greatest resistance to activation of endogenous PC had higher avidity PC antibodies and a more severe clinical course. The presence of higher avidity PC antibodies might therefore provide a marker for a more severe thrombotic phenotype.

P1240

HIGH INCINDENE OF CEREBRAL LEISIONS IN ADULT PATIENTS WITH BETA-THALASSEMIA MAJOR

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Background: Survival of beta thalassemia major patients has improved significantly over the past few decades as better treatment became available. Subsequently, previously undescribed complications have been recognized, including thromboembolic complications such as cerebral thrombotic events. Incidence as high as 27-60% of asymptomatic silent cerebral infarctions (SCI) have been already demonstrated by MRI studies in patients with beta thalassemia intermedia (TI) (*KM Musallam, et al. Thromb. Res. 130:695-702, 2012*). However, less data is available for the incidence of SCI in transfusion dependent (TD) beta thalassemia major (TM).
Aims: Determination of SCI incidence in TD patients with beta TM.
Methods: The study included 21 patients with TD beta TM, 12 males and 9 females, 21-42 years old. All patients except 2 were splenectomized (SPX). Patients with diabetes mellitus, history of any thromboembolic event or on aspirin or anticoagulation were not included in the study. After obtaining a signed informed consent, brain MRI studies were performed prior to receiving blood transfusion, using 3T Ingenia (Philips) system in SET1 TSET2 diffusion SWI (5mm slice) and 3DFLAIR (1.1 mm slice).
Results: Average pre-transfusion hemoglobin level was 10.0 gm/dL (range 8.7-11.0). Average platelet count in SPX patients was 578 K/micl (range 270-1100 K), and in 2 non SPX 242 K/micl (164-320K). Average ferritin level was 1800 ng/ml (range 251-7431). Thrombophilia complete workup, including lupus anticoagulant and anti cardiolipin antibodies, was normal in all, except 4 cases heterozygous for Factor V Leiden mutation. Fourteen (67%) out of 21 patients had focal bright lesions in the cerebral white matter. Twelve SPX patients had 1-73 (mean 14) lesions, while 2 non SPX patients had 1 and 53 lesions. Seven SPX patients had no lesions. Most of the lesions were in frontal lobes and bilateral, maximal diameter up to 7 mm. All lesions were negative in diffusion with no susceptibility artifacts. No significant correlation was found between MRI findings and all evaluated clinical and laboratory parameters. However, ferritin levels, *i.e.* median level of 1117 ng/ml and mean level of 1925 ng/ml (range 251-7431) among patients with SCI lesions compared to 875 ng/ml and 1551 ng/ml (range 295-4294), respectively, among patients without lesions, were observed. Moreover, 6 (60.0%) out of 10 patients with ferritin levels<1000 ng/ml had a mean number of 5.2 lesions, while 8 (72.7%) out of 11 patients with ferritin level >1000 ng/ml had a mean number of 23.9 (4.6 fold) lesions.
Summary and Conclusion: The results demonstrate an incidence as high as 67% of asymptomatic SCI in TD TM patients. Accounting for the technical differences, the lesions were similar in size and anatomical location to those already observed in TI. These findings may suggest that the increased number

of pathological circulating RBC's in TI may not be the only pathophysiologic factor in the etiology of SCI. In addition to increased number of activated platelets in SPX patients, other factors, such as severity of iron overload demonstrated by higher ferritin levels, may also play a role in the etiology of hypercoagulability in thalassemia. In conclusion, in addition to effective iron chelation therapy, anti platelet drugs, such as low dose aspirin, should be given routinely also to TD beta TM patients.

P1241

PLATELET THROMBUS FORMATION UNDER FLOW CONDITION IS INCREASED IN MYELOPROLIFERATIVE NEOPLASMS (MPNS)

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Background: Essential Thrombocythemia (ET) and Polycythemia Vera (PV) are MPNs at high risk for arterial and venous thrombotic events. Previous studies report on platelet (PLT) abnormalities in these patients, but little data are available on PLT thrombus formation capability in these MPNs.
Aims: In this study we wanted to evaluate whether and to what extent PLT interactions with vessel wall components, assayed in a dynamic system, are altered in ET and PV patients. The influence of V617F JAK2 mutation, cytoreductive therapy, hematological parameters and von Willebrand Factor (vWF) levels on PLT adhesion was also considered.
Methods: Eighteen ET (6M/12F; age range 37-81 years) and 12 PV (6M/6F; 58-87 years) patients were enrolled into the study upon informed consent. Thirteen healthy subjects (CTR; 6M/7F; 35-55 years) acted as control group (CTR). Peripheral venous whole blood was withdrawn in sodium citrate, recalcified with heparin and perfused over a collagen matrix for 4 minutes at 1,000 s^{-1} shear rate using a parallel flow chamber connected to EVOS (AMG) fluorescence microscope. Adherent PLTs were then stained with anti-P-selectin-FITC antibody as PLT activation index, and with annexinV-AlexaFluor647 as a measure of procoagulant phosphatidylserine exposure. Then, phase contrast and fluorescence images were taken in random fields by EVOS. Results are reported as mean±SD of the % of area covered by PLTs, or as % of adherent PLTs positive for either P-selectin or annexinV. Plasma vWF antigen and activity levels were measured by ELISA. Statistical analysis has been performed by SPSS software package.
Results: In ET and PV patients, PLT adhesion was significantly greater compared to CTR ($p<0.001$) (ET: 49.1±12.0%, PV: 51.6±12.5% and CTR: 33.7±8.4%). The adhesion capacity was related to V617F JAK2 mutational status, with greater values found in the homozygous subjects (60.7±4.5%) compared to the mutation-negative ones (43.8±9.0%; $p<0.01$). Patients on hydroxyurea (HU) therapy (n=17) tended to have a lower PLT adhesion (47.6±12.6%) as compared to non-HU-treated patients (n=11; 55.0±9.6%, p=n.s.). The percentage of PLT adhesion was significantly correlated with both PLT ($r=0.508$, $p<0.001$) and leukocyte ($r=0.404$, $p<0.01$) counts, but not with vWF plasma levels. PLT count and V617F JAK2 mutation resulted to be significant determinants of PLT adhesion in the multivariate analysis of the data, adjusted for age, sex and HU therapy. Despite the greater adhesion, the exposure of phosphatidylserine (potentially procoagulant) on adherent PLT was reduced in ET (10.1±6.1% positive platelets) and PV patients (11.3±7.1%) compared to CTR (19.4±7.5%, $p<0.01$ *vs* either ET and PV).
Summary and Conclusion: This study shows that blood from MPN patients produces more platelet thrombi on collagen surface at high shear rate flow conditions compared to controls, and that this is significantly influenced by the V617F JAK2 mutation burden and platelet count. Of interest, adherent PLTs of patients form thrombi that express less phosphatidylserine than controls, suggesting that they may be less capable to generate procoagulant activity. Further studies are warranted to investigate the correlation between PLT thrombus formation potential and thrombotic risk in MPN patients.

P1242

CDC42 REGULATES PROPLATELET FORMATION NOT BY WASP BUT VIA N-WASP

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Background: To produce platelets, the highly specialized bone marrow megakaryocyte (MK) precursor cells, elaborate long branched cytoplasmic extensions, called proplatelets (PPTs). Platelet release essentially relies on cytoskeleton changes, including actin dynamics. We have previously shown that the Rho/Rock pathway is a negative regulator of the proplatelet formation (PPF) through MLC phosphorylation during human megakaryopoiesis.
Aims: Cdc42 and Rho/Rock have been shown to have opposite effects in many cellular processes. Thus, we focused our study on the role of Cdc42 in human MK differentiation.
Methods: $CD34^+$ cells were cultured in serum-free liquid medium in the presence of TPO ± Casin, a specific Cdc42 inhibitor provided by Yi Zheng, Cincinnati, OH, USAi). Cdc42 activity is studied by G-Lisa technique and MKs migration study is performed in the presence of SDF-1. Invalidation of N-WASP and PAK2 were obtained by shRNA strategy. The overexpression of N-WASP was obtained by a lentiviral vector encoding N-WASP cDNA with a SNP not recognized by the shRNA.
Results: We show that Cdc42 activity increases in late stages of the MK differentiation. The Cdc42 dominant-negative construct and the specific chemical Cdc42 inhibitor Casin, both led to a decrease in PPF, indicating that Cdc42 activation could stimulate PPF. To determine the molecular pathway involved in Cdc42-induced PPF, we knocked down its main effector PAK2 by a shRNA strategy and showed that it was not involved in the PPF. The same results were obtained with two chemical inhibitors (IPA-3 and Pak18) targeting both PAK1 and 2. We next investigated the role of the WASP family. We previously showed that WASP did not play a role in PPF, and thus we focused on its family related member N-WASP. Phosphorylation of N-WASP increased during MK differentiation. Its knocked down by shRNA led to a sharp decline in the formation of the PPs despite the fact that MKs express much lower level of N-WASP than WASP. This was associated with an increase in the formation of stress fibers, as revealed by confocal microscopy. Altogether, these results suggest that Cdc42 plays a positive role in the formation of the PPs by inhibiting Rho *via* the N-WASP pathway. These results were reinforced by rescuing the formation of PPs in N-WASP knocked down MKs by re-expressing N-WASP. Interestingly, Cdc42 inhibition by Casin leads to a decrease in N-WASP phosphorylation (pN-WASP^{Y256}) and both invalidation of N-WASP and Casin treatment leads to pMLC2^{Ser19} increment. Furthermore, inhibitors of Src family kinases (PP2, Dasatinib and SU6656) have opposite effects on PPF and pN -WASP^{Y256}. While SU6656 increases PPF, PP2 or Dasatinib inhibit its by inducing an increase or an inhibition of pN -WASP ^{Y256} respectively, suggesting that yet unidentified members of Src family kinase play a crucial and opposite role in this regulation.
Summary and Conclusion: In conclusion, our results show for the first time in human that the Cdc42/N-WASP pathway positively regulates the PPF and suggest a possible retro-regulation of the Rho/Rock pathway *via* N-WASP. It also shows that WASP and N-WASP are not two fully redundant molecules in the MKs by mechanisms that remain to be determined, but which may be related to their differential regulation of the phosphorylation by members of the Src kinase family.

P1243

INCREASED FIBRINOGEN LEVELS AND TESTOSTERONE DEFICIENCY HAVE AN ADDITIVE DETRIMENTAL EFFECT ON CAROTID WALL THICKNESS IN ESSENTIAL HYPERTENSIVE PATIENTS

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Background: Increased fibrinogen levels and testosterone deficiency have an additive detrimental effect on carotid wall thickness in essential hypertensive patients.
Aims: We investigated the combined effect of fibrinogen and testosterone on carotid atherosclerosis in hypertensive subjects.
Methods: Fibrinogen and total testosterone levels were measured in 127 untreated hypertensive men. All participants underwent B-mode *ultrasonographic* measurements of the carotid intima media thickness (I*MT)* which is a marker of early atherosclerosis.
Results: Patients with high compared with those with low fibrinogen exhibited greater IMT (by 0.13 mm, $P<0.001$), whereas patients with low compared with those with high testosterone had higher IMT (by 0.08 mm, $P<0.01$). Regression analysis revealed that age, systolic BP, fibrinogen and testosterone were independent predictors of IMT. The distributions of fibrinogen and testosterone were split by the median (290 mg/dL and 4.06 ng/mL, respectively) and accordingly subjects were stratified into those with high and low values. A significant interaction between fibrinogen and testosterone on IMT is reflected by the finding that the subgroup of high fibrinogen/low testosterone exhibited the higher IMT compared with the subgroups of high fibrinogen/high testosterone, low fibrinogen /low testosterone, and low fibrinogen/high testosterone ($P<0.001$).
Summary and Conclusion: In hypertensive males, elevated fibrinogen and low androgen level exert an additive detrimental effect on peripheral vascular structure, accelerating the atherosclerotic process.

P1244

FUNCTIONAL EVALUATION OF TISSUE FACTOR IN FLOW-DEPENDENT THROMBUS FORMATION ON IMMOBILIZED VON WILLEBRAND FACTOR

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Background: Mural thrombus formation at injured vessel wall is fundamentals for both physiologic haemostasis and pathological thrombosis. Although tissue factor (TF) is up-regulated upon vessel wall damage and plays a pivotal role in this process, little is known about its functional relevance under physiologic blood flow conditions.
Aims: Using an *in vitro* perfusion chamber system, we studied the relevant role of TF on von Willebrand factor (VWF)-dependent thrombus formation, illustrating the functional link between soluble or immobilized TF and VWF under whole blood flow conditions with varying shear rates.
Methods: Recombinant human TF (Innovin; Dade) were co-coated with VWF onto a glass plate to prepare 'surface-immobilized TF/VWF complex'. Surface density of immobilized TF, evaluated by the ELISA-based assay using an anti-TF monoclonal antibody, increased in a concentration-dependent and saturated manner by soluble TF (1-100 pM) added on a plate.Citrated whole blood, recalcified with 8 mM $CaCl_2$ prior to perfusion, was perfused over a VWF-surface in the presence or absence of surface-immobilized TF. Platelet adhesion and aggregation were evaluated by the surface coverage of thrombi in a defined area after 5-min perfusion. The mural thrombi formed on VWF-surface was double-stained with fluorescently labeled anti-fibrin and anti-fibrinogen antibodies. Fibrin generation was evaluated by confocal laser scanning microscopy as a ratio of fibrin relative to fibrinogen fluorescence within mural thrombi.
Results: Surface-immobilized TF significantly augmented flow-dependent fibrin generation as a function of increasing surface density of TF under both low (250 s^{-1}) and high (1500 s^{-1}) shear rate conditions. In this regard, soluble TF, when added to sample blood, similarly increased intra-thrombus fibrin generation in a dose-dependent manner in the absence of immobilized TF. However, coagula formation in sample blood was enormously amplified by soluble TF during perfusion, as judged by the flow-path occlusion time. In addition, immobilized TF significantly up-regulated VWF-dependent platelet adhesion and aggregation under high shear rate conditions, albeit with no appreciable effects under low shear rate conditions.
Summary and Conclusion: With the present experimental approach, we evaluated thrombogenic potentials of two distinct forms of (soluble or surface-immobilized) TF in flow-dependent thrombus formation on the VWF surface. Our results suggest that surface-immobilized TF plays a role in concert with VWF on mural thrombus formation under high shear rate conditions, with a lower risk of systemic hypercoagulability such as disseminated intravascular coagulation which may be caused by circulating soluble TF.

P1245

CLINICAL SIGNIFICANCE OF THE NEUTROPHIL-LYMPHOCYTE RATIO IN VENOUS THROMBOEMBOLISM PATIENTS WITH LUNG CANCER

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Background: The neutrophil-lymphocyte ratio (NLR) has been identified as a potentially useful marker for predicting clinical outcome in patients with cardiovascular disease, diabetes, and various malignancies. The aim of this study was to determine whether NLR at the time of venous thromboembolism (VTE) diagnosis is a prognostic factor for the response to anticoagulation and survival in lung cancer patients treated with anticoagulation for VTE
Aims: The aim of this study was to determine whether NLR at the time of venous thromboembolism (VTE) diagnosis is a prognostic factor for the response to anticoagulation and survival in lung cancer patients treated with anticoagulation for VTE.
Methods: We retrospectively analyzed the clinical characteristics, laboratory parameters, and NLR in 114 lung cancer patients newly diagnosed with VTE, among 991 patients pathologically confirmed for lung cancer between July 2008 and August 2013.
Results: High NLR was significantly associated with high hematocrit (p=0.028), high C-reactive protein (p=0.002), and low albumin (p=0.001). Compared with the low NLR group, stage IV non-small cell lung cancer (NSCLC) at the time of VTE diagnosis (55.6 *vs*. 74.6%, p=0.055), central nervous system metastasis (5.8 *vs*. 25.8%, p=0.004), and cancer progression (14.3 *vs*. 38.8%, p=0.008) at the time of VTE diagnosis were also significant in the high NLR group. Moreover, the poor response to anticoagulation was statistically correlated with patients with NSCLC (p=0.037), high NLR (p=0.004), and low albumin (p=0.029).
Summary and Conclusion: The results demonstrate that the NLR at the time of VTE diagnosis could be a useful biomarker for predicting the response and prognosis following anticoagulation in patients with lung cancer and VTE.

P1246

PATTERNS OF DIFFERING THROMBIN GENERATION AND SENSITIVITY TO THE ANTICOAGULANT ACTIVATED PROTEIN C IN PATIENTS WITH MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Patients with multiple myeloma are at an increased risk of venous thromboembolism, however the precise mechanisms underlying this prothrombotic phenotype are poorly understood. Monoclonal gammopathy of undetermined significance (MGUS), a related condition, represents a pre-malignant state in which abnormal production of a monoclonal immunoglobulin arises from a plasma cell clone in the absence of end-organ pathology. To date, the identification of molecular mechanisms potentially associated with a prothrombotic state in MGUS have not been characterised in detail.
Aims: To characterise plasma thrombin generation in plasma from patients with newly diagnosed or newly relapsed myeloma, MGUS and healthy volunteers.
Methods: Blood samples were collected in citrated blood tubes from patients with myeloma (prior to commencement of chemotherapy), MGUS and age and sex-matched healthy volunteers. Platelet poor plasma was prepared from whole blood by centrifugation. Calibrated automated thrombography was used to determine parameters of thrombin generation following incubation of plasma in the presence of phospholipid and soluble tissue factor (TF). Analysis of thrombin generation parameters was performed using Thrombinoscope™ software. Statistical analysis was performed using the statistical software package, Prism™.
Results: Eight patients with newly diagnosed or newly relapsed myeloma were identified and recruited with consent. Age and gender-matched patients with MGUS and healthy volunteers were also identified and recruited (n=8 per group). Thrombin generation was assessed following initiation of coagulation by soluble TF (0.5pm). Peak thrombin generation was significantly lower in plasma from healthy volunteers (276.7+/-20.8nm) compared with myeloma patients (383.4+/-33.4nm, P<0.005) and compared with MGUS patients (353.4+/-16.59nm, P<0.05). A non-significant trend towards higher endogenous thrombin potential (ETP), shorter time to initiation of thrombin generation and shorter time to peak thrombin generation was observed in both myeloma and MGUS patients compared with healthy controls. A non-significant trend towards higher ETP and peak thrombin generation was also observed in myeloma patients compared with MGUS patients. Characterisation of the anticoagulant protein C pathway was performed by incubation of plasma with human activated protein C (APC; 10nM; 1pM TF trigger). While ETP was attenuated in all groups, the ETP observed in the healthy volunteer group (ETP 228.6+/-44.32nm*min) was significantly lower than that in either the myeloma group (ETP 866.2+/-241.3nm*min, p=0.009) or the MGUS group (627+/-91.49nm*min, p=0.0025). In these three groups, APC attenuated ETP by 88% (healthy volunteers), 68% (MGUS patients) and 59.27% (myeloma patients) respectively.
Summary and Conclusion: Plasma thrombin generation is significantly enhanced in both myeloma and in MGUS in comparison to healthy controls. Activated protein C resistance may contribute to the prothrombotic phenotype in both conditions. The precise mechanisms underlying activated protein C resistance in these related conditions has yet to be determined.

P1247

EVALUATION OF THE THROMBOSIS TENDENCY IN THALASSEMIA MAJOR PATIENTS WITH THROMBIN GENERATION TEST, PROCOAGULANT PHOSPHOLIPID ACTIVITY AND ENDOTHELIAL MICROPARTICLES LEVELS

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Background: There are increasing evidences that there is a thrombosis tendency in thalassemia like diseases going on with chronic hemolysis. Although a lot of etiological factors have a role in this tendency, it is thought that the vascular endothelial activation under the effect of chronic oxidative stress existing in these patients has the most effect on this tendency.
Aims: Our aim was to evaluate in thalassemia major (TM) patients the procoagulant activity of endothelial microparticles (EMP), which are generated by the vascular endothelial activation.
Methods: Subjects of 31 TM patients who were regularly transfused and chelated, had known not to having in his/her and/or family history

hemorrhagic/thrombotic diseases or other conditions known to alter the hemostatic balance, had not used ever any oral anticoagulants or other antithrombotic drugs or oral contraceptives, had not used any drug that interferences with hemostasis during the last month of transfusion and had not had a splenectomy were included to our study with 50 healthy controls who sustained the same criterias. Procoagulant phospholipid activity tests and thrombin generation tests were done which are the most advisable tests for the evaluation of the *in vivo* hemostasis and endothelial microparticles levels were calculated in both groups. The results of TM patients are compared with the results of the healthy controls.
Results: Endothelial microparticle levels of the TM patients (191,7 ± 108,9 / µl) were statistically significantly higher than the healthy controls (86,6 ± 8,2 /µl) (p=0.0001) and there were statistically significant positive correlation between ferritin and EMP levels (r=0.357, p=0.048). Procoagulant phospholipid activity of TM patients (65.6 ± 12.3 sn) were statistically significantly longer than the healthy controls (59.5 ± 15.2 sn) (p=0.049). Endogenous thrombin potentials of TM patients (1129,8 ± 187,4 nM thrombin.min) were statistically significantly lower than the healthy controls (1260,1 ± 267,7 nM thrombin.min) (p=0,012). In addition, aPTT (34,8 ± 7,8 sn) and PT levels (15,4 ± 2,8 sn) of the TM patients were statistically significantly longer than aPTT (31,1 ± 5,7 sn) and PT levels (13,2 ± 0,8 sn) of the healthy controls (p=0,016; p=0,0001, respectively), but there were not difference in terms of platelet and fibrinogen levels between groups. Again there were statistically significant positive correlation between ferritin and aPTT and INR levels (r=0.417, p=0.019; r=0.466, p=0.009, respectively). These results have shown that there is not a procoagulan activity, in contrast there is an anticoagulan activity in regularly transfused, chelated, nonsplenectomized TM patients.
Summary and Conclusion: In recent years, the existence of an EMP dependent anticoagulant and profibrinolytic compensatory mechanisms has been demonstrated. These compensatory mechanisms may counterbalance their classical known procoagulant phenotype. In conclusion, these results of non-splenectomized, regularly transfused and chelated children with TM in our research have shown that, the thrombosis tendency in the nature of this illness may be compensated via EMP dependent anticoagulant mechanisms and also may be via profibrinolytic compensatory mechanisms.
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P1248

HAEMATOLOGICAL MORBIDITY IN OBSTETRIC PATIENTS WITH CARDIAC DISEASE

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Background: Cardiac disease is the leading cause of maternal mortality in the United Kingdom. An increased risk of venous thromboembolic (VTE) and haemorrhagic events in pregnant women with cardiac disease has been described, however little published evidence exists to guide the management of these competing risks, particularly in the setting of operative delivery. Currently published guidelines on optimal mangement of these patients recommend that a haematologist participates in the care of this high risk group. The Rotunda hospital is a large, maternity university hospital in Dublin, Ireland, accommodating over 9000 deliveries per year. Within this institution, the multidisciplinary management of pregnant patients with cardiac disease includes collaboration between obstetricians, obstetric haematologists, cardiologists, anaesthetists and paediatricians. In addition to formulating individualized management plans, this multidisciplinary service also facilitates the emergency management of acute bleeding or thrombotic complications and streamlines management of blood transfusion.
Aims: The aim of this study was to evaluate the prevention, incidence and management of haematological morbidity in pregnant women with congenital and acquired cardiac disease.
Methods: Patients were identified from a database compiled by the obstetric cardiology multidisciplinary service that listed all pregnant patients referred for specialist cardiology assessment to the Rotunda hospital during the period 2004 to 2011. Patients with underlying cardiac disease of moderate to high complexity as defined by international consensus guidelines and those at high risk due to the presence of mechanical heart valves were included. Baseline information collected from the database included age, medical history, nature of the underlying cardiac disease, regular medications and obstetric history. Further data were obtained from the medical and obstetric case notes relating to antenatal complications, details of perinatal care and postnatal complications.
Results: From 2004-2011, 451 patients with cardiac disease were referred for obstetric care to the Rotunda hospital. 59 patients were identified as having moderate to high risk cardiac disease. Each of these patients was discussed regularly at a formal multi-disciplinary team meeting, where an individualized care pathway was formulated and an agreed, written delivery plan recorded in the medical notes. Nine patients were receiving treatment with anticoagulant or antiplatelet agents pre-conception, including two patients receiving warfarin and one patient receiving therapeutic dose low molecular weight heparin (LMWH). During the peripartum period, 3 women were receiving therapeutic dose LMWH/unfractionated heparin, 12 were receiving prophylactic LMWH and 1 woman was taking an antiplatelet agent. The mean estimated blood loss at delivery was 371 (100-2000) ml and the rate of postpartum haemorrhage (approx. 5%) was similar to that reported in the general obstetric population. Regional anaesthesia was utilized in 54 cases with no local bleeding complications. No VTE events occurred. Moreover, during the 8 year period, there were no maternal deaths among this cohort.
Summary and Conclusion: Few previously published case series have focused upon haematological complications in pregnant patients with cardiac disease. Within our institution the multidisciplinary approach to management facilitates decision making among senior clinicians at an early stage in pregnancy, allowing pre-emptive measures to be taken against the anticipated risks in each pregnancy as well as producing care plans to provide guidance to junior colleagues. This is of particular importance in scenarios where there is limited published evidence to guide management. In the absence of further additions to the evidence base in this area, improvements in the provision of obstetric care to patients with cardiac disease will likely be dependent upon close co-operation between clinicians and other stakeholders involved in delivering care to this challenging patient group.

Venous thrombosis

P1249

PREVALENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: A DANISH FOLLOW-UP STUDY

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Background: Venous thromboembolism (VTE); *i.e.* deep venous thrombosis (DVT) and pulmonary embolism (PE) is a critical and common complication in patients with cancer. However, the information on VTE among patients with haematological malignancies such as diffuse large B-cell lymphoma (DLBCL) is sparse.
Aims: The aim of this study was to evaluate the incidence of VTE in patients with DLBCL in a two year period from date of lymphoma. Furthermore the aim was to evaluate the quality of registry data on VTE among DLBCL patients.
Methods: We conducted this singlecenter study to investigate the incidence of VTE among patients with a first time diagnose with DLBCL at the Department of Haematology Aalborg University Hospital from January 1, 2007 to December 31, 2013. The follow-up of patients were from date of DLBCL until two years later, date of death or November 1, 2013 whatever occurred first. The entire medical records were reviewed for all DLBCL patients and data on lifestyle factors, medication with anticoagulation and confirmed episodes of VTE by imaging were retracted. Furthermore we retracted data on VTE risk factors, platelet count, haemoglobin and leukocyte count at date of VTE. We further evaluated the data quality on VTE discharge diagnosis in the Danish National Registry of Patients among DLBCL patients. We retracted register data on VTE discharge diagnosis among all patients included in our study. These data were compared with information from medical records on VTE events.
Results: A total of 296 patients were identified with a first time diagnosis of DLBCL at the Department of Haematology Aalborg University Hospital during the study period. Of the 296 DLBCL patients 32 (10.8%) had a VTE confirmed by imaging within a two-year follow up. This included 19 cases (59%) of DVTs and 13 cases (41%) of PEs of which three were found on routine CT scan whereas 10 were symptomatic. Of the 19 DVTs, seven were located at the upper extremities (six were associated with central vein catheter). The platelet count was subnormal in 44% of patients and 12% of VTE patients had a platelet count below $50*10^9$ per L at time of VTE. In the discharge register 20 of the 296 patients had a diagnosis of VTE during a two years follow-up period of which 17 were confirmed by review of the medical records. The positive predictive value of a VTE discharge diagnosis among patients with DLCBL was 85%. Only 17 of the 32 confirmed VTE cases were registered in the discharge register. The sensitivity of a VTE discharge diagnosis was 53% which means that the incidence of VTE among DLBCL patients is underestimated by using register data.
Summary and Conclusion: We found an incidence of 10.8 % of VTE among DLBCL patients during two years of follow-up from date of DLBCL. Register data on VTE discharge diagnosis is valid among patients with DLBCL with a positive predictive value of 85%. The sensitivity of a VTE discharge diagnosis among DLCBL patients is only 53%.

P1250

CONSENSUS PAPER ON: "PLATELET CUT-OFF FOR ANTICOAGULANT THERAPY IN CANCER PATIENTS WITH VENOUS THROMBOEMBOLISM AND THROMBOCYTOPENIA"

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Background: Cancer-related Venous Thromboembolism (VTE) requires treatment with Low Molecular Weight Heparin (LMWH), which is more effective and safer than warfarin; however, the risk of major hemorrhage still remains clinically relevant (up to 5%). This rate is even higher in case of impaired hemostasis, such as it may occur during thrombocytopenia (due to myelosuppression or chemo-therapy) where the bleeding risk is directly related to the values of platelet count. At the present, no guidelines are available regarding the best management of patients with acute or non-acute cancer-related VTE during thrombocytopenia.
Aims: To develop a consensus about the platelet cut-off for safely administering LMWH in cancer patients with acute (no lather then 1 month) and non acute VTE and thrombocytopenia, based on RAND/UCLA Appropriateness Method (RAM).
Methods: A systematic review of the literature was performed via electronic databases. Topics and research terms were cancer, venous thromboembolism, platelets, risk of bleeding, anticoagulant drugs, low-molecular-weight heparin and treatments. A questionnaire of appropriateness of the use of different doses of LMWH, according to the platelet count, was produced. A panel of experts was identified; the literature review and the list of indications were sent to all members of this panel. For each indication, the panel members rate the benefit-to-harm ratio of the procedure on a scale of 1 to 9, where 1 means that the expected harms greatly outweigh the expected benefits, and 9 means that the expected benefits greatly outweigh the expected harms. A middle rating of 5 can mean either that the harms and benefits are about equal or that the rater cannot make the judge for the patient described in the indication.
Results: The panel of expert reached the following consensus.
Appropriate:
- In acute VTE (including catheter-related VTE): Full dosage of LMWH if platelets >50,000<100,000/mm^3 **(9/9)**; Reduced dose to 50% of full dose, if platelet >30,000<50,000/mm^3; The discontinuation of LMWH treatment if platelets<30,000/mm^3 **(6/9)**; and, in case of DVT of the lower limbs, the positioning of IVC filter **(6/9)**.
- In non-acute VTE (including catheter-related VTE): LMWH reduced to 75% of full dosage, if platelets >50,000<100,000/mm^3 **(7/9)**; The discontinuation of LMWH treatment if platelets<30,000/mm^3 **(7/9)**.
Uncertain:
- In acute VTE (including catheter-related VTE): Full dose of LMWH if platelets >30,000<50,000/mm^3 **(7/9)**.
- In non acute VTE (including catheter-related VTE): LMWH reduced to 75% of full dosage if platelets >30,000<50,000/mm^3 **(7/9)**; LMWH discontinuation and insertion of IVC (in case of DVT), if platelets<30,000/mm^3 **(5/9)**; LMWH reduced to 75% of full dosage if platelets >30,000<50,000/mm^3 **(6/9)**.
Inappropriate:
- In acute VTE (including catheter-related VTE): Full dose of LMWH if platelets<30,000/mm^3 **(9/9)**; The discontinuation of LMWH if platelets count >30,000<50,000/mm^3 **(6/9)**.
- In non acute VTE (including catheter-related VTE)**:** Reduced dose to 75% LMWH if platelets<30,000/mm^3 **(7/9)**; The discontinuation of LMWH if platelets count >30,000<50,000/mm^3 **(5/9)**.
Summary and Conclusion: This is the first expert opinion based on RAM to establish the safe platelet cut-off to administer LMWH therapy in patients affected by acute and non-acute VTE. The present panel of expert suggests as appropriate the use of dose-adjusted LMWH according to platelets count. Further investigations by means of well designed prospective clinical trials are needed to establish the best management of cancer-related VTE in patients with thrombocytopenia.

P1251

A SINGLE CENTRE RETROSPECTIVE ANALYSIS OF THE INCIDENCE OF MAJOR BLEEDING IN OVER 1000 PATIENTS COMMENCED ON RIVAROXABAN FOR NON-VALVULAR ATRIAL FIBRILLATION AND VENOUS THROMBOEMBOLISM

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Background: The novel oral anticoagulant rivaroxaban has been NICE approved in the UK for anticoagulation in patients with Non-Valvular Atrial Fibrillation (AF) since May 2012, Deep Vein Thrombosis (DVT) since July 2012 and Pulmonary Embolism (PE) since June 2013. Despite published randomised trial data comparing rivaroxaban to warfarin, questions remain as to the incidence and pattern of major bleeding in patients on rivaroxaban in the post authorisation setting. Furthermore there is no clearly identified mechanism to reverse its action in the event of major bleeding. In our institution we have approved use of a single dose of Prothrombin Complex Concentrate (PCC) in the event of major, life threatening and ongoing bleed. In addition we have recommended not commencing rivaroxaban in patients with an estimated Glomerular Filtration Rate (eFGR) of<30ml/min.
Aims: To ascertain the incidence and pattern of major bleeding in over 1000 unselected patients commencing rivaroxaban in our institute. To contrast this by collecting data on the rate and pattern of major bleeding requiring reversal with PCC in the >6400 patients taking warfarin who are monitored by our institution.
Methods: We retrospectively analysed clinical and laboratory data for episodes of major bleeding in 1002 patients commencing rivaroxaban in our institution between 8th of November 2012 and the 18th of February 2014. Basic demographics were collected and the ISTH criteria for major bleeding (including >=2g/dl fall in Haemoglobin and/or the transfusion of >=2 units of packed red blood cells) was used. Haemoglobin at presentation of major bleeding was compared to Haemoglobin at commencement of rivaroxaban. The site and outcome (fatal *versus* non-fatal) of major bleeding was recorded. Use of PCC was recorded in both the rivaroxaban and warfarin groups.
Results: 1002 patients were commenced on rivaroxaban in this time frame. 845 patients were commenced on rivaroxaban 20mg od and 157 patients were commenced on rivaroxaban 15mg od due to eGFR being<50ml/min. The indication for anticoagulation was AF in 886 patients and DVT/PE in 116 patients. The median age was 75 years (range 21-100 years). In the rivaroxaban group we did not identify any intracranial haemorrhage requiring reversal of anticoagulation with PCC. However 1 patient presented with an intracranial haemorrhage at 3 days following their last dose of rivaroxaban with normal renal function and thus did not require attempted reversal of anitcoagulation with

PCC. The outcome of this event is non-fatal at the time of authorship. 10 patients were identified who fulfilled the major bleeding criteria. One of these major bleeding events had a fatal outcome where a genitourinary bleed was a contributory factor to death. However this patient did not receive PCC. Four of these major bleeding events were confirmed as Upper Gastro-Intestinal (GI) bleeds, though only one of these events required reversal with PCC. There was one further suspected, though not proven GI bleed. In the remaining 4 events no bleeding source was identified. Within the same time frame PCC was administerd to 86 patients in the warfarin group. There were 28 deaths within this group, 17 of which were due to intracranial haemorrhage. In total 34 of the 86 patients receiving PCC had had an intracranial haemorrhage.
Summary and Conclusion: Our analysis is in keeping with published randomised trial data in respect to the incidence of major bleeding, including intracranial haemorrhage, for patients taking rivaroxaban. Furthermore this contrasts positively to our own experience (as well as historical published data) with respect to bleeding events on warfarin, in particular the rate of intracranial haemorrhage. The authors suggest that limiting use of rivaroxaban to patients with eGFR of >=30 in their institution, plus the routine counselling and standardised information provided to patients and primary care physicians when commencing rivaroxaban, may have contributed to the favourable outcome of this analysis.

P1252

ANTICOAGULANT MANAGEMENT OF PREGNANT WOMEN WITH MECHANICAL HEART VALVES: A RETROSPECTIVE COHORT STUDY

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Background: The anticoagulant management of pregnant women with mechanical heart valves is challenging since they face a high risk of valve thrombosis and death of systemic embolism. Unfortunately, no trials are available, and only limited data on the risk associated with different anticoagulant regimens are available.
Aims: The aim of this study was to determine maternal and fetal outcomes in pregnant women with mechanical heart valves managed with anticoagulation at a tertiary referral hospital in the Netherlands.
Methods: We conducted a retrospective cohort study of pregnant women with mechanical heart valves receiving different anticoagulant regimes. We identified patients using laboratory, cardiology and obstetric databases of our tertiary referral center and captured all women treated between 1995 and 2013. We studied maternal thromboembolic and hemorrhagic complications, pregnancy and fetal outcomes.
Results: We included 26 pregnancies in 13 women (Table). Sixteen pregnancies (62%) continued after 20 weeks gestation. In 6 of these 16 (38%) pregnancies, low-molecular-weight heparin (LMWH) was the predominant anticoagulant used throughout pregnancy. In 10 (63%) of these pregnancies, women received mainly vitamin K antagonists (VKA), with LMWH given only in the first trimester and peri-delivery or unfractionated heparin (UFH) given only peri-delivery. Five (19% of all pregnancies) thromboembolic complications (TEC) occurred, 4 in women with a mechanical valve in the aortic and 1 in a woman with a valve in the pulmonalis position. Three TEC occurred during LMWH treatment. One woman underwent valve replacement. There were no deaths. Antenatal and postpartum maternal hemorrhagic complications occurred in three (12%) and seven (27%) of all pregnancies, respectively. Of pregnancies continuing beyond 20 weeks, 83% (5 of 6) of women treated with LMWH during pregnancy had a surviving infant compared to 90% (9 of 10) of those treated with VKA. There were five preterm births before a gestational age of 37 weeks, four in pregnancies managed with VKA.

Table

Summary and Conclusion: In conclusion, the risk of thromboembolic and hemorrhagic complications in pregnant women with mechanical heart valves was high, regardless of the type of anticoagulant. Close monitoring of treatment is essential.

P1253

MICROPARTICLE-ASSOCIATED TISSUE FACTOR ACTIVITY IN PATIENTS WITH ACUTE UNPROVOKED DEEP VEIN THROMBOSIS AND DURING THE COURSE OF ONE YEAR

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Background: Tissue factor (TF) is the main *in-vivo* initiator of blood coagulation. Microparticles (MPs) are small procoagulant membrane vesicles. Elevated TF-bearing MPs have been found in different prothrombotic conditions and MP-associated TF activity may contribute to the pathogenesis of unprovoked deep vein thrombosis (DVT).
Aims: To determine MP-TF activity levels at diagnosis of DVT and at four additional time points during the course of one year in a well- defined group of patients with unprovoked DVT of the lower limb.
Methods: In this study, 41 patients with acute unilateral symptomatic and unprovoked DVT of the lower limb were included and followed for 1 year. Venous blood samples for determination of MP-TF activity were drawn at diagnosis of acute DVT, and 1-, 3-, 6-, and 12 months later. In addition, 10 young and healthy control subjects were included.
Results: The median MP-TF activity was 0.06 pg/mL (25^{th}-75^{th} percentile: 0.0-0.53) in patients with acute DVT and 0.18 pg/mL (0.07-0.33) in healthy controls, and did not differ significantly (p=0.35). No significant changes in MP-TF activity were found in the follow-up measurements. MP-TF activity did also not differ significantly between patients with proximal- or distal DVT and between those with- or without residual DVT after 6 months.
Summary and Conclusion: MP-TF activity is low at the acute event in patients with unprovoked DVT of the lower limb and remains unchanged during the course of the disease. Our data do not support the hypothesis that TF-bearing MPs play a determining role in the pathogenesis of unprovoked DVT.

P1254

DEVELOPMENT OF A PHARMACOGENETIC-BASED WARFARIN DOSING ALGORITHM IN BRAZILIAN PATIENTS

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Background: In 2007, the Food and Drug Administration added pharmacogenetic information to the warfarin product label and consequently trials have recently been performed to test whether knowledge of *CYP2C9* and *VKORC1* variants improve the outcomes for patients.
Aims: To develop a pharmacogenetic-based warfarin dosing algorithm from a Brazilian patient cohort with stable dose and to perform comparisons with other cohorts from patients without stable dose. and *VKORC1* variants improve the outcomes for patients.
Methods: This retrospective cohort study included 1037 patients treated with warfarin for at least 12 months at the Heart Institute (Incor), University of São Paulo, São Paulo, Brazil. The development was based on patients on stable warfarin dose, *i.e.*, achievement of the therapeutic target defined as three consecutive values of INR between 1.8 and 3.2 (including the current test). Dose-prediction algorithmic model was performed with 10,000 randomized analyses in a derivation cohort formed for patients with stable dose. A testing cohort formed for patients who had not stable dose were analyzed. In a multiple linear regression were included the variables age, gender, weight (Kg), height (cm), self-declared "race/color", amiodarone use, enzyme inducers use, *VKORC1* genotypes, and predicted phenotypes due to *CYP2C9* polymorphisms (EM, IM or PM). were analyzed.
Results: The algorithmic model was developed with a derivation cohort formed for patients on stable warfarin dose. The construction of the model achieved a moderate multiple R2 including 9 variables. Also, a moderate correlation between predicted dose by IWPC algorithm and predicted dose by our algorithm was observed (r=0.54, p<0.001). Mean warfarin dose was significantly lower in patients carrying IM+PM predicted phenotypes (25.8±0.8 mg/week) compared to EM patients (29.7±0.5 mg/week) (p<0.001). For the *VKORC1* polymorphism, patients carrying GG genotype had higher mean warfarin dose (32.3±0.6 mg/week) compared to patients carrying AA genotype (21.5±1.2 mg/week) (p<0.001).
Summary and Conclusion: These information is a step towards implementation of a genotype-guide dosing of warfarin and it could be very useful to identify or not improvement in the initial anticoagulation therapy in a multi-ethnic population.

P1255

CURRENT MANAGEMENT AND OUTCOMES OF VENOUS THROMBOEMBOLISM IN AUSTRALIA: INITIAL RESULTS FROM THE VENOUS THROMBOEMBOLISM COHORT STUDY

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Background: Venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common and important cause of morbidity and mortality.
Aims: The VTE Cohort Study is a prospective observational registry established by Monash University to describe VTE epidemiology, current therapeutic strategies and patient outcomes in Australia.
Methods: Between July 2011 and February 2014, data from diagnosis to last follow-up were prospectively collected on patients, aged 18 years or older, at two Haemostasis and Thrombosis Clinics in Melbourne, Australia. Data collection was undertaken by clinicians and included the patients' clinical presentation, acute and long-term therapy and outcomes, including VTE recurrence.
Results: A total of 236 presentations of VTE in 235 patients (1 patient had a second presentation) were registered. Median age was 54 years [IQR 39-68], 48% were male and 88% were of European descent. The majority of patients (62%) presented with an isolated lower extremity DVT (calf alone=29%; proximal alone=32%), 4% presented with upper limb thrombosis, 1% with cerebral vein thrombosis, 24% with PE alone, and 9% patients with both DVT and PE. A history of VTE (6%), obesity (BMI≥30) (52%), major surgery (within 4 weeks) or bedridden (27%), oral contraceptive pill (17%), active cancer (7%), and inherited thrombophilia (7%) were reported risk factors. Post-thrombotic syndrome (PTS) was reported in 17.5% of patients. Of these, 68% had mild, 23% had moderate and 9% had severe PTS according to the Villalta scale. The presence of an ulcer was reported in 1 patient. Median time of PTS onset was 1 month (IQR 1-5; range=0-13) after diagnosis. In the acute phase, treatment consisted of low molecular weight heparin (LMWH) (93%) for a median duration of 7 days [IQR 7-10]. Rivaroxaban was prescribed for acute therapy in only 3.3% of patients. Few patients had an inferior vena cava filter inserted (3%) or had thrombolytic therapy (1.8%). Long-term therapy included warfarin (68%), LMWH (19%) or rivaroxaban (5.8%). Stockings were prescribed for 47 % of patients with DVT. Bleeding occurred in 6.8% of patients of which 23% was considered a major or life-threatening bleed and 77% a minor bleed. Pulmonary hypertension was reported in 4.5% of patients with a PE with or without a DVT. No deaths were reported in the study period.
Summary and Conclusion: Prospectively collected data from the VTE Cohort Study are contributing to documenting and understanding factors that influence clinical outcomes of VTE to better define optimal management. *This study is supported by the Australian Centre for Blood Diseases, Monash University and Bayer Australia*

P1256

IN-VIVO REVERSAL OF THE ANTICOAGULANT EFFECT OF APIXABAN WITH 4-FACTOR PROTHROMBIN COMPLEX CONCENTRATE

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Background: The ability of non-specific prohaemostatic agents to reverse the anticoagulant effect of apixaban has never been assessed in humans. Previously, 50 U/kg of 4-factor prothrombin complex concentrate (PCC) restored endogenous thrombin potential (ETP) and prothrombin time (PT) to baseline values in rivaroxaban treated healthy volunteers. Animal models suggest that lower PCC doses are equally effective for reversal of rivaroxaban as well as for apixaban.
Aims: In this experiment, we assessed whether infusion with doses of 37.5 and 25 U/kg of PCC (off label use) are able to reverse the effect of a high dose of apixaban.
Methods: In a randomized, double blind, placebo-controlled, crossover study, 6 healthy volunteers took apixaban 10 mg twice daily. Three hours after the last dose of apixaban they received either a single bolus of PCC 37.5 U/kg, PCC 25 U/kg or placebo, with a wash-out period of 18 days in between infusions. Outcome was the effect of PCC on ETP (15 min after the PCC/placebo infusion and over 24 h) and PT (at 15 min). All volunteers gave written informed consent.
Results: Fifteen min after 25 U/kg PCC, ETP increased from 44% ± 12% to 51% ± 15% (p=0.03). After 37.5 U/kg PCC, ETP increased from 41% ± 11% to 56% ± 23% (p=0.06). Placebo did not increase ETP (45% ± 10% to 44% ± 11%, p=0.8). Over the 24 h observation period, ETP was significantly higher in 25 U/kg and 37.5 U/kg PCC doses compared with placebo (p=0.04 and p<0.001, respectively). Both 25 U/kg and 37.5 U/kg of PCC lowered the PT to baseline values after 15 min (p=0.001 and p=0.000 respectively), whereas placebo did not (p=0.442).

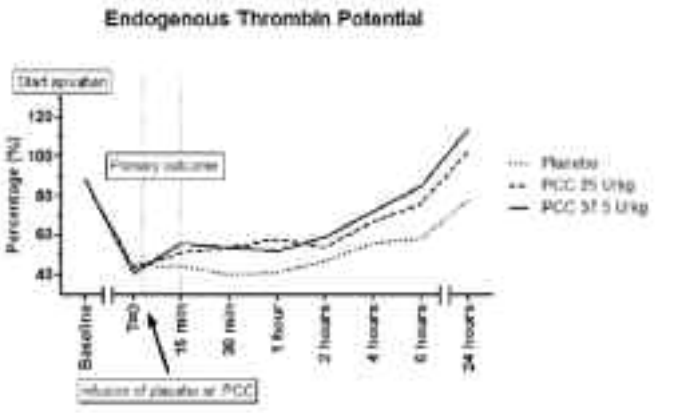

Figure 1.

Summary and Conclusion: PCC in a dose of 25 U/kg and 37.5 U/kg increases ETP and normalises PT in patients treated with a high dose of apixaban, indicating partial reversal of the anticoagulant effect.

P1257

EFFICACY OF REPEAT DUPLEX ULTRASOUND IN PATIENTS WITH SUSPECTED DEEP VENOUS THROMBOSIS

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Background: About 300 patients a year are referred to our outpatient clinic at Orbis Medical Centre for suspected DVT. Referring general practitioners (GP's) use a primary decision rule combined with D-dimer testing to stratify patients who should undergo a duplex ultrasound to safely exclude a deep vein thrombosis (DVT). In all patients with a negative duplex ultrasound but elevated D-dimer test and/or high clinical suspicion, guidelines advice to perform a second duplex ultrasound.
Aims: In this study, we investigate prevalence of DVT after GP referral and the effectiveness of repeat ultrasound in case of elevated D-dimer test in these patients.
Methods: We performed a single centre, prospective analysis of GP referred patients suspected of DVT. Inclusion was started at July 17th 2012 and still ongoing, a preliminary analysis was performed after inclusion of 150 patients. All patients underwent clinical investigations to determine Wells-score, D-dimer testing and a duplex ultrasound. In case of elevated D-dimer test and/or a persisting clinical suspicion for DVT repeat duplex ultrasound after 5-7 days was performed in all patients.
Results: During a period of 5.5 months, 153 GP referred patients were seen to rule out a DVT. Of these 153 patients effectively 28.8% had a DVT and in 7.8% of cases a thrombophlebitis was diagnosed. Negative duplex ultrasound results for thrombosis were seen in 97 patients. In these patients 56 had a repeat duplex ultrasound 5-7 days later. The repeat ultrasound was positive for DVT in 3.6% (n=2) of cases. All patients with a primary Wells score >4 had a positive duplex ultrasound, in two cases only the second duplex ultrasound. 41 patients had no repeat ultrasound performed; 29.3% had a normal D-dimer test, 43.9% had an alternative diagnosis and 19.5% had low Wells scores in combination with a D-dimer test<750. Two patients refused additional ultrasound.
Summary and Conclusion: At our outpatient clinic 28.8% of GP referred patients had a comfirmed deep vein thrombosis by duplex ultrasound. Deep venous thrombosis was diagnosed in only 3.6% of patients after repeat duplex ultrasound. All patients with a Wells score above 4 had a positive duplex ultrasound, including the patients with a positive second duplex ultrasound. In conclusion a repeat duplex ultrasound seems effective in GP referred patients with high Wells scores (>4) and a primary negative duplex ultrasound. Efficacy and effectiveness of repeat ultrasound will be further analysed in our prospective cohort study

P1258

SPANISH REGISTRY OF THROMBOEMBOLIC DISEASE (TD) RELATED WITH HORMONAL THERAPY, PREGNANCY, OBSTETRICS COMPLICATIONS OR ASSISTED REPRODUCTIVE TECHNIQUES IN WOMEN (ARP): RESULTS OF THE TEAM PROJECT

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Background: There is a lack of information regarding thromboembolism, thrombophilia and clinical management in different specific clinical settings

such as vascular obstetric complication in women in the clinical practice. In this context, we are conducting a national registry of women in those situations, the so-called TEAM Project: a multicenter observational cohort study. (See website: www.SETH.es).
Aims: To obtain information about the clincal management of women and the presence or not of thrombophilia that develop thrombosis during pregnancy or vascular placental complications in the Spanish population.
Methods: From 2009 since now, almost 60 Centers from Spain and also now, Uruguay have been including patients. The inclusion criteria are women with hormonal therapy or pregnancy-related thrombosis disease, vascular placental complications (VPC) or ischemic placental disease or ART and women with thrombophilia, that will undergo or not thromboprophylaxis during pregnancy or ART The study has been approved by the Ethical Committee of each Center. Among the primary outcomes, the investigation will focus on the incidence, prophylaxis and treatment of TD, hormonal or antineoplasic therapies or ART, as well as the management of vascular placental complications or thrombosis in those situations.
Results: We have already recruited 329 patients from 20 Centers. 10% were women with thrombosis during pregnancy, 14% were women with VPC, 36% were included for thrombosis prophylaxis during pregnancy, 26% for prophylaxis of VPC during pregnancy and 2% had recurrent implantation failure.The thrombophilia test revealed that the most common risks factor were homozygous for F12 46C/T polymorphism, Factor V Leiden and high levels of Factor VIIIc. In general, 70% women received heparin for prophylaxis, some of them 30,6% received aspirin alone or in combination, of treatments during pregnancy. Heparin was used as treatment (11%) and prevention (89%), being tinzaparin 45% and enoxaparin (30%) the most indicated, along with bemiparin (20,5%) or dalteparin (4.5%). Clinical outcomes, in terms of relapse of thrombosis or VPC, showed only 1 case of thombosis and 9 of CVG.
Summary and Conclusion: The preliminary results from the Team Project reflects the clinical practice, and the differences among investigators in thromboprophylaxis, indication of thrombophilia testing and treatment in the clinical setting. Also new risk biological factors in women's issues such as FVIIIc or homozygous for F12 46C/T polymorphism. The information of this ongoing registry will allow us to make the translation from clinical practice to clinical and biological investigation.

P1259

RETROSPECTIVE AUDIT OF INCIDENCE OF PICC LINE RELATED DEEP VEIN THROMBOSIS IN HAEM-ONCOLOGY PATIENTS
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Background: The use of Peripherally Inserted Central Catheters (PICCs) have become increasingly popular as the procedure is quick and it provides long-term access for the administration of chemotherapy or stem cell rescue in hem-oncology patients. However, concerns have been raised regarding the potential complications associated with this procedure, in particular the development of a deep vein thrombosis (DVT). This can have a detrimental effect on the patient's therapy plan, by delaying further chemotherapy or stem cell transplant, placing them at risk of early disease relapse. Additionally, the diagnosis of a DVT can affect the patient's quality of life through further complications, including infection and pulmonary embolism. As a result, there has been increasing interest in the use of prophylactic anti-thrombotic agents to prevent this complication. Few studies have reviewed the incidence of thrombotic events in this group of patients, and the decision to use prophylactic anticoagulation is usually left to the prescribing clinician. However, there is increasing data to suggest that the benefits of using low molecular weight heparin to prevent a thrombosis outweigh the risk of a bleeding event.
Aims: To estimate the prevalence of thromboembolic events in our hem-oncology population and assess the risk factors associated with this. Additionally we wished to identify the impact of a thromboembolic event on the patient's long-term treatment plan.
Methods: The electronic and paper medical records of all hem-oncology patients who had a PICC line inserted in our day unit between 1st January 2010 to 1st January 2013 were retrospectively reviewed. Data collected included gender; age; underlying hematological malignancy; previous history of thromboembolic event; any concurrent anti-thrombotic medication; when DVT diagnosed through ultrasound; whether any therapy was delayed; any further complications as a result of the thrombosis.
Results: 346 patients were identified. The median age was 49.7 years with a range of 18 to 77 years. The incidence of thrombotic events was 6% (20) with the majority being women 60% (12). Notably the frequency of DVTs in patients was increasing each year from only 4 patients in 2010 to 11 patients in 2012. The most common underlying malignancy associated with DVT was AML 40%, followed by ALL 20%, NHL 20%, HL 10% and Multiple Myeloma 10%. Only 2 patients had previous history of thrombosis with one already established on anticoagulation. All thrombotic events occurred an average 32.8 days following PICC line insertion. The majority of patients developed a thrombosis following treatment. However 3 patients presented prior to their next course of chemotherapy with 1 patient presenting a few days prior to their stem cell transplant. All of these patients had their therapy delayed an average 13.8 days. All patients had complications following diagnosis of a DVT with 80% (16) having their line removed and 45% (9) developing sepsis. One patient required admission to critical care due to septic shock and their line was removed on ICU.
Summary and Conclusion: The prevalence of DVT events was similar to that described in other published studies of this patient population. However, notably the incidence in our hospital was increasing, despite the average number of patients analyzed each year remaining the same. The most likely risk factors associated with a thrombotic event included female gender, age 40-50 years and AML. The complication rate was higher compared to those previously reported. Overall it was suggested that all patients should have a risk assessment prior to PICC line insertion and considered for short-term thromboprophylaxis, since previous studies have shown that, despite thrombocytopenia, the risk of a bleeding event is low. Furthermore, given the delay in diagnosis and management, it was felt that both junior staff and patients need to be re-educated on how to recognize early signs and symptoms of a thrombosis, and the prompt management of this.

P1260

THREE YEAR MORTALITY ASSOCIATED WITH VENOUS THROMBOEMBOLISM (VTE): COMPARISON OF HOSPITAL ACQUIRED, COMMUNITY ACQUIRED AND INCIDENTAL FINDINGS
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Background: Outcome data has previously been produced showing significantly higher one-year mortality associated with hospital acquired thrombosis (HAT) at 29% compared with community acquired thrombosis (CAT) 12% (p-value<0.0001) occurring in 2010. The CAT value for mortality at one year is closer to that from Dr Foster health episode statistics (HES), which describes 15% mortality one year from VTE diagnosis. Data is now presented for the same patient group for mortality at three years from VTE diagnosis. In addition, mortality associated with incidental VTE (IFT) findings over the same timescale was also investigated. The aim being to detect whether differences in mortality are maintained over three years and how the mortality associated with incidental findings compares to other VTE events.
Aims: The aim of this project was to look again at mortality associated with VTE events that were diagnosed in 2010 at three years from diagnosis. Looking at hospital acquired VTE, Incidental VTE findings and community acquired VTE to see if there are differences in mortality associated with each group and if the mortality numbers previously identified after year 1 were continued in the following two years.
Methods: The hospital radiological reporting system (CRIS) was used to identify positive VTE events over 2010, including both targeted VTE investigations and CT scans for incidental findings. The positive cases were then cross-checked with the patient information system to establish whether they met the criteria for HAT. Mortality was derived from the same patient management system for the preceding twelve months to produce data for three consecutive years. This outcome data was then analysed using the SPSS Statistics package to determine any statistically significant trends in mortality data.
Results: Table 1 shows total VTE events for the three thrombotic types in 2010 and the consecutive 12 month mortality figures for subsequent years 1 to 3 for each cohort. HAT has a significantly increased association with mortality over three years when compared with CAT (p-value<0.0001). Both HAT (p-value<0.0001) and IFT (p-value<0.0001) have significantly increased mortality rates at 12 months when compared with all VTE events over the same period. It was not possible to carry out further analysis of IFT events because the very high twelve mortality of 74% did not allow this. The majority of these events were cancer related. Mortality occurs predominantly within the first 12 months for all three VTE cohorts. The odds ratio (OR) for CAT mortality from year one to year two is 0.68 and to year three is 0.61. For HAT over the same periods, 0.65 and 0.52. This demonstrates statistically significant mortality within 12 months of diagnosis for all VTE types compared with subsequent years.

Table 1.

Summary and Conclusion: Both HAT and IFT have significantly greater association with mortality at twelve months, compared to CAT. The majority of

patients did not receive a post mortem so it was not possible to accurately determine the cause of death across all VTE groups. However, the majority of IFR events were cancer associated, so this might explain the relatively high associated death rates. Mortality data for five years from VTE diagnosis is planned to see if the pattern changes.

P1261

INFLAMMATORY RESPONSE AMONG PATIENTS WITH SUSPECTED VENOUS THROMBOEMBOLISM

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Background: Deep venous thrombosis (DVT) is still a diagnostic challenge, and D-dimer in combination with Well's score and ultrasound are used to diagnose or exclude DVT. Recent studies have emphasized the importance of the local inflammatory response mediated mainly by monocyte and neutrophil activation. The serum levels of several inflammatory cytokines are increased in patients diagnosed DVT; however previous studies have examined one or only a few mediators.
Aims: In the present study we investigated 44 different mediators to get a broader characterization of the inflammatory process.
Methods: A cohort of 169 patients with suspected DVT was included in the study after written informed consent. The diagnosis was based on clinical evaluation (*i.e.* Well's score), D-dimer, ultrasound and eventually venography if a leg vein thrombosis was suspected. Plasma samples from the first 89 consecutive patients and 20 healthy controls were analyzed with Luminex multiplex determining plasma concentration of 44 mediators: 13 interleukins, 3 immunomodulatory cytokines, 8 chemokines, 8 growth factors, 4 adhesion molecules and 8 matrix metalloproteases. The last 80 consecutive patients were used as a validation group, and samples were analyzed using ELISA to verify observations from the first group of patients. For statistical analysis we used the Mann-Whitney test, and we used unsupervised hierarchical clustering to examine relationship and cytokine expression patterns.
Results: 34 of 169 (18%) of referred patients were diagnosed DVT. Main differential diagnoses were thrombophlebitis, stasis, infection, inflammatory, traumatic and non-inflammatory or unknown. In the first cohort, mainly referred during winter months, 13 (26%) of 89 patients were diagnosed DVT. In the second cohort, mainly referred during summer months, 13 (16%) of 80 patients were diagnosed DVT. Four different mediators differed significantly when comparing patients with DVT *versus* patients without DVT: P-selectin ($p<0.0001$) and VCAM-1 ($p=0.0009$) show the strongest significance whereas the significance was weaker for hepatocyte growth factor (HGF, $p=0.0415$) and matrix metalloprotease 8 (MMP8, $p=0.0151$). However, for all mediators there were a considerable overlap between patients and healthy individuals. Subgroup analysis show less discriminative differences between the patients with more inflammatory conditions. Unsupervised hierarchical clustering analysis including the first 89 patients demonstrated that DVT patients were mainly included in certain clusters. When comparing DVT patients with healthy controls we observed statistically significant differences for several mediator including both interleukin (IL1ra), growth factors (EGF, HGF and Leptin), chemokine (CXCL10), adhesion molecules (ICAM-1, P-selectin and VCAM-1) and matrix metalloproteases (MMP2/3/7/8/9). HGF ($p<0.0001$), P-selectin ($p=0.0009$), VCAM-1 ($p<0.0001$) and all the MMPs (all $p<0.0014$) showed the strongest significant differences. DVT above the femoral band compared to lower DVT showed increased levels of P-selectin ($p=0.0434$), HGF ($p=0.0193$), MMP8 ($p=0.0213$) and D-dimer ($p=0.0152$), reflecting larger thrombosis.
Summary and Conclusion: Serum levels of several inflammatory mediators (*i.e.* interleukins, chemokines, soluble adhesion molecules, proteases) are increased in DVT patients compared with healthy controls; this is consistent with a DVT-induced inflammatory response reflected in these serum levels. However, there is a considerable overlap in serum levels for DVT patients and other patients admitted to hospital with suspected DVT, and for this reason analysis of single mediators or mediator profiles seems to have a limited value in the differential diagnostic evaluation of patients with suspected DVT.

P1262

REDUCTIONS IN VENOUS THROMBOEBOLISM EVENTS, ASSOCIATED WITH RADIOLOGICAL DATA PROVIDING REAL-TIME FEEDBACK TO CLINICIANS ABOUT HOSPITAL ACQUIRED THROMBOSIS, GIVEN INADEQUATE PREVENTATIVE MEASURES

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Background: Since 2010, metrics for Venous Thromboembolism (VTE) events diagnosed within a large teaching hospital have been produced using the hospital radiological system. All targeted investigations (computed tomography pulmonary angiogram CTPA, Doppler ultrasound scans and ventilation perfusion scans) are included together with incidental findings from CT of thorax abdomen and pelvis. Positive scans are cross-checked against the patient information system to ensure criteria for hospital acquired thrombosis (HAT) are met. This is defined as a VTE diagnosed as an inpatient but not present on admission, or any VTE event within 90 days of hospital discharge.
Aims: The aim was to collect outcome data on VTE events diagnosed within the Trust and how many of these met the established criteria to be termed hospital acquired thrombosis. In addition by undertaking a root cause analysis we wanted to look at whether appropriate preventative measures were used and to reduce the incidence of this.
Methods: All HAT events receive a basic root cause analysis. Including VTE risk assessment, appropriateness of prophylaxis, VTE risk factors, admission speciality and whether appropriate thromboprophylaxis (TP) for VTE took place according to hospital guidelines. These outcome metrics were used to identify areas where HAT was more prevalent, to target education around VTE prevention measures. Feedback about the event is then given to the admitting consultant, highlighting areas of concern. Inadequate TP covers absent, wrong dose or missed prescribed dose of anticoagulant. Clinical teams are often not aware of HAT events occurring in their patients as the event often presents post discharge and treated by other clinicians and teams. In addition HAT events associated with inadequate treatment may be preventable, may cause death and may leave hospitals open to litigation claims.
Results: Results are shared with the Thrombosis Committee (a multi disciplinary group including patient representation), providing monthly reports. Errors identified including inappropriate preventative measures or missed prescribed doses of anticoagulant TP, are included on an incident form, requiring the appropriate team or admitting ward to investigate the error. Reports were submitted to the hospital safe care group when common themes were identified. There is a particular focus on inappropriate TP associated with positive HAT events. Presentations were made to the pharmacy department, consultant meetings and within the annual Trust update for all staff. To further raise the profile of HAT throughout the hospital, a presence from the VTE prevention team was developed in many clinical areas. This included working with the medical admissions unit, where over 95% of medical patients are admitted, to ensure appropriate risk assessment and prescribing of TP. In surgery, targeting the pre-operative assessment unit ensured patients received both written and verbal information about VTE, empowering them to ensure prophylaxis was given. Snap shot audits on all wards across the hospital was undertaken to determine levels of VTE risk assessment and prevention, giving real time feedback to clinical staff. Data on total VTE and percentage HAT events has been determined since 2010 in table 1.

Table 1.

Summary and Conclusion: The overall VTE prevention initiative locally, has led to reductions in total HAT, HAT as a percentage of total VTE and reduction in HAT associated with inadequate TP. In 2011, focus was made on appropriate prescribing which dramatically improved but with a large rise in missed doses that year. In 2012 work was intensified on reducing all inappropriate TP, a theme that continued in 2013, with positive outcomes. This service improvement has been achieved within a relatively short time-frame, using radiological data to provide real-time feedback and work with clinical teams to drive change.

P1263

SCREENING FOR MALIGNANCY IN PATIENTS WITH UNPROVOKED VENOUS THROMBOEMBOLISM

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Background: Whipps Cross Hospital is a district general teaching hospital serving a local population of approximately 400,000 in North East London. Patients newly diagnosed with venous thromboembolism (VTE) within the hospital are referred to a physician-led anticoagulation clinic. Each new patient diagnosed with venous thromboembolic disease is reviewed by a haematology consultant or specialist registrar. The National Institute For Health and Care Excellence (NICE) has recently produced national guidelines for the management of VTE[1] in England. It recommends that patients with unprovoked deep vein thrombosis (DVT) or pulmonary embolism (PE) who are not already known to have an underlying malignancy are offered timely investigations for cancer. This has implications for management with low-molecular-weight heparin rather than a vitamin K antagonist in up to 11% of such patients who

may have an underlying malignancy. A clinical benefit of early cancer diagnosis with screening is also inferred. In addition to a physical examination guided by the history, all patients with unprovoked DVT or PE should have a bloods tests (full blood count, liver function and serum calcium), urinalysis and a chest X-ray performed. Further investigations for cancer with an abdomino-pelvic CT scan (and a mammogram for women) should also be considered in all patients over 40 years with a first unprovoked DVT or PE. This strategy was felt to be cost effective, whereas additional investigations such as abdominal ultrasound and tumour markers were not. The National Health Service (NHS) waiting time target for investigations for people with suspected malignancy is two weeks.
Aims: We evaluated our practice retrospectively against this standard by evaluating clinical records. Over a six month period (June - November 2013) there were 52 (20 male) patients newly diagnosed (45 first episodes, 7 recurrences) with DVT (n=33) or PE(n=19). We will illustrate the age ranges by decade of this cohort. The ages ranged from 27-92 years, with the commonest age group affected between 60-69 years.
Results: The commonest risk factors identified were surgery (13 patients) and recent medical hospital admission (9 patients); see figure. The second largest group identified were patients with unprovoked episodes of VTE (n=12 (22%)), although travel related VTE may have been a factor in a small number. Combining first and recurrent episodes would make this the largest group within our cohort. Only four (25%) of these patients had a documented screen for malignancy carried out by the haematologist reviewing them in the anticoagulation clinic on their first visit. The remaining 75% did have limited investigations (100% had relevant blood tests, 58% a chest X-ray) following diagnosis of VTE. Additional investigations by acute medical teams included abdominal ultrasound scans and tumour markers. The investigations performed in each of the 12 cases and the origin of the requests will be explored further. One patient was subsequently diagnosed with advanced metastatic pancreatic malignancy as a result of self-directed screening and we will present a illustrative case summary of this.

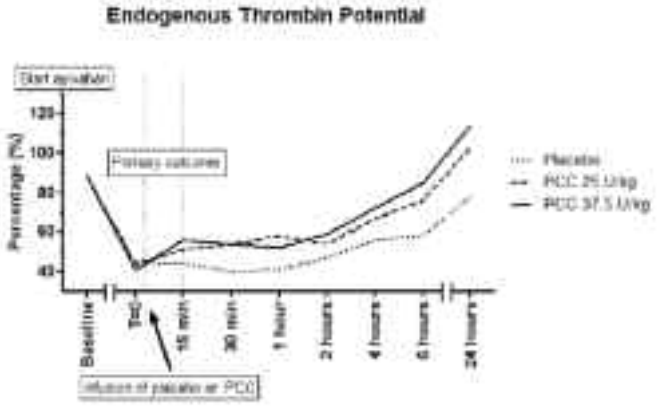

Figure 1.

Summary and Conclusion: This audit has demonstrated the practical difficulties in implementation of the NICE standard. The guideline does not give advice as to where the responsibility for screening lies. Our practice has been varied. Some patients are investigated in-house whilst for others a referral was made to the patient's general practitioner (GP) for this purpose. There has been resistance from some GPs due to limited access to imaging facilities. Some investigations are carried out by the admitting medical team although apart from blood tests this is not on a consistent basis. Our practice suggests that these difficulties may not be an isolated example. It is common practice for anticoagulant services to be led by clinical nurse specialists or pharmacists or to be managed predominantly by community-based staff and a risk of screening being overlooked. A clear joint pathway, with interdisciplinary involvement between general and acute physicians, ambulatory or Emergency Department staff and general practitioners is key in addressing this unmet need which has significant public health and economic implications.

P1264

LONG-TERM OUTCOME OF NON-CENTRAL-VENOUS-LINE-RELATED DEEP VEIN THROMBOSES LOCATED AT LOWER LIMBS IN CHILDREN
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Background: The true frequency of adverse outcomes, such as recurrences and the development of postthrombotic syndrome (PTS), following deep vein thrombosis (DVT) in lower limbs in children have not been broadly studied.
Aims: The aim was to investigate morbidity and outcomes in neonates and children diagnosed with non-central-venous-line-related DVTs in lower limbs in our Centre.
Methods: We retrospectively analyzed the data of 29 children (58.6% males) whereof 3 neonates referred from 1/1999 to 1/2014. Children suffered malignancies and followed at oncology departments of our hospital were excluded from the analysis, due to incomplete data. Ultrasound (US) was used to diagnose DVT while for evaluation of severity of PTS the modified Vilalta scoring system was applied.
Results: The mean age was 7.4 years (2 months- 15 years) for children and 8 days (2-14 days) for neonates. The primary location of DVTs was: left femoral vein (n:10), right femoral vein (n:7), bilateral femoral veins (n:4), left popliteal vein (n:3), right popliteal vein (n:2), bilateral iliac veins (n:3). DVTs extended to inferior vena cava in 9 patients (31%) while concomitant pulmonary embolism was present in three patients (10%), all non-neonate children. The mean follow-up period was 7 years (3-15 y). All patients (100%) survived thrombosis; one patient succumbed during the follow-up period because of an underlying congenital heart disease. Recurrence rates were 27.5% (8/29) in children and zero in neonates. Recurrences at the primary location were present only in two cases. All children who suffered recurrences had either a strong congenital (PS deficiency n:1, FVLeiden heterozygosity n:1) or acquired (Lupus Anticoagulant n:1) thrombophilic factor or an underlying disease predisposing to DVT (n:7). Positive family history of DVT or thrombophilia was reported in 5 cases with no recurrence. PTS was developed in 9 patients (31%) -5/9 were boys- after a median period of 3 months (1-12 months) following DVT. Additionally, one neonate endured amputation of both his limbs due to extensive necrosis. According to modified Vilalta scale, PTS was initially mild in 6 children and moderate in 3. Elevation in D-dimers was present in all nine cases at DVT diagnosis. Extensive occlusion (n=9), presence of thrombophilic factors - especially a natural inhibitor deficiency (n=4) - and recurrence of DVT (n=4) were associated with development of PTS. All PTS patients had been treated upon DVT diagnosis with anticoagulants for a mean period of 24 months (3-60 months), while graduated elastic compression stockings were applied in 8/9 for 1-4 years, soon after PTS development. Appropriate diet was initiated in one obese female adolescent for achieving the optimal body weight. In a mean observational period of 6 years (3-12 y), the outcome of PTS was complete recovery in one boy with moderate PTS, improvement from moderate to mild in one boy with initially diagnosed moderate PTS, while no change on PTS grade was defined in all 6 patients with mild and in one with moderate PTS. There are 11 children still on long-term anticoagulation because of a recurrent DVT or an underlying disease.
Summary and Conclusion: Non-CVL-related DVTs in lower limbs in children are rare events that may be complicated with adverse outcomes affecting the patients' quality of life. Future studies must aim to define a risk-stratified approach to anticoagulation in order to improve long-term outcomes.

P1265

MORTALITY AND INCIDENCE OF VENOUS THROMBOEMBOLISM (VTE) IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND RELATION TO LABORATORY BIOMARKERS: AN UPDATE OF A SINGLE CENTER STUDY
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Background: Venous Thromboembolism (VTE) is a well-recognized complication of cancer, which aggravates the clinical course and leads to additional morbidity and increased mortality. However, primary thrombophrophylaxis in cancer patients is challenging, as the risk of VTE is not equal in all patients, and as anticoagulation in those patients is associated with increased bleeding complications compared with patients without cancer.
Aims: to study whether vascular and inflammatory biomarkers (as well as clinical variables) can be used as predictors of mortality and VTE in patients with hematological malignancies.
Methods: This study is a prospective observational cohort study; it was conducted on a group of 171 patients with malignant hematological conditions. They have been followed up for an average period of 416.82 days with an endpoint of mortality or VTE. **Exclusion criteria included** Overt infection within the last 2 weeks, Venous or arterial thromboembolism within the last 3 months, continuous use of oral or subcutaneous anticoagulants. Hypercoagulability and inflammation were assessed at the initiation of the study by measuring the circulating levels of the following parameters: Coagulation and fibrinolysis activation markers (D-dimer, Fibrinogen, Antithrombin, plasminogen activator inhibitor 1 [PAI-1]); Endothelium and platelet activation markers(von Willebrand Factor [vWF], soluble P-selectin); and inflammation markers (Tumor necrosis factor α, Interleukin-6).
Results: Out of 171 patients, 48 (28.1%) were diagnosed to have Lymphoproliferative disorders, 40 (23.4%) were diagnosed to have Myeloproliferative neoplasms, 38 (22.2%) were diagnosed to have AML (or MDS progressed to AML), 19 (11.1%) were diagnosed to have ALL, 26 (15.2%) were diagnosed to have Paraproteinaemias. Incidence of symptomatic VTE in patients with hematological malignancies was 7%. There were statistically significant

associations between mortality and ECOG performance status (P value:<0.001), presence of co morbidities (P value: 0.047) duration of hospital stay (P value: 0.006), Hemoglobin level (P value: 0.003), platelet count (P value: 0.001), serum albumin (P value: 0.011), ESR (P value: 0.004), Antithrombin (P value: 0.012), vWF (P value: 0.002). For prediction of mortality, ROC Curve of Albumin level showed that a level of 3.35 g/dl showed the highest likelihood ratio (LR) of 1.78 with sensitivity of 62.7% and specificity of 64.8%. ROC Curve of ESR showed that a level of 118 mm/hr showed the highest LR of 1.52 with sensitivity of 57.9% and specificity of 62%. ROC Curve of Antithrombin level showed that a level of 16.25 mg/dl showed the highest LR of 1.34 with sensitivity of 55.9% and specificity of 61%. ROC Curve of vWF level for showed that a level of 1.87mU/ml showed the highest LR of 1.35 with sensitivity of 64.9% and specificity of 52%. There were statistically significant associations between VTE occurrence and CVAD insertion (P value: 0.014), Prothrombin time (P value: 0.037), ESR (P value: 0.05). For prediction of VTE occurrence, ROC Curve of ESR showed that a level of 106.5 mm/hr showed the highest LR of 1.44 with sensitivity of 66.7% and specificity of 53.8%.

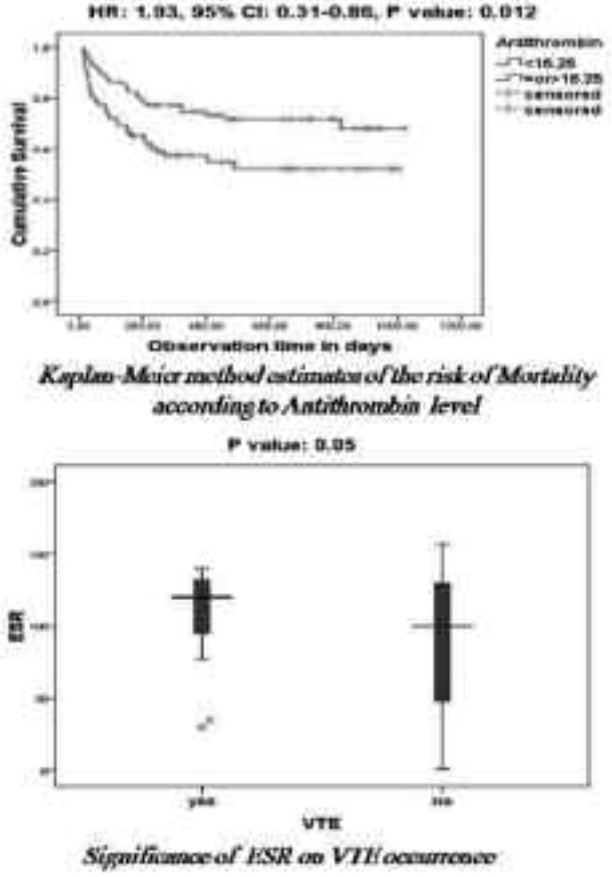

Figure 1.

Summary and Conclusion: Incidence of symptomatic VTE in patients with hematological malignancies was 7%. Initial Poor ECOG performance status, presence of co morbidities, duration of hospital stay, Hemoglobin level, platelet count and initial levels of Albumin below 3.35g/dl, ESR above 118 mm/hr, Antithrombin below 16.25 mg/dl, vWF above 1.87mU/ml are associated with poor outcome with increased mortality. There were statistically significant associations between VTE occurrence and CVAD insertion, Prothrombin time, ESR. ESR above 106.5 mm/hr is associated with increased VTE occurrence.

P1266

GENETIC AND ACQUIRED PREDICTORS OF POSTTHROMBOTIC SYNDROME IN YOUNG PATIENTS WITH DVT IN THE NORTH-WEST REGION OF RUSSIA

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Background: Postthrombotic syndrome (PTS) is frequent and severe complication of deep vein thrombosis (DVT), which leads to a resistant disability. The role of acquired and hereditary risk factors in formation of PTS is studied insufficiently.
Aims: To identify genetic and acquired predictors of severe PTS in young patients with DVT originated from the North-Western region of Russia

Methods: Retrospective «case-control» study included 120 adults up to 45 years old (men − 57, women − 63, middle age − 37.4) with DVT diagnosed during the period from 2000 to 2011. For grading PTS, we used the clinical scale of chronic venous insufficiency ($CEAP_{0-6}$). Sixty seven (55.8%) patients were genotyped for nine polymorphisms: factor I (FI) –455 G/A, FI Thr312Ala, FII 20210 G/A, FV 1691 G/A, FXII 46 C/T, FXIII Val34Leu, PAI-1 –675 4G/5G, TPA 311 bp I/D, EPCR Ser219Gly, that were discriminated by PCR-RFLP method. All results were analyzed with the SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA).The differences in genotype distributions were estimated by Fisher's exact test with calculating odds ratios (OR), their 95% confidence intervals (CI) and p-value. The discriminant function analysis was used to define the predictors of severe PTS
Results: Severe PTS ($CEAP_{4-6}$) was registered in 15 out of 67 patients (22.4%). The risk of severe PTS was increased dramatically in patients with established genetic risk factors, in particular, G20210A mutation (OR=4.3; 95%CI: 1.3-14.1; p=0.01), FV Leiden variant (OR=2.6; 95%CI: 0.9-7.1; p=0.057) and the homozygous FXIII 34Leu/Leu genotype (OR=6.9; 95%CI: 0.7-67.5; p=0.057). However, the discriminant function analysis with inclusion of additional criteria (clinical and acquired) had shown that none of these DNA variations played a significant role in the outcome of DVT: FV 1691GA (p=0.160), FII 20210GA (p=0.501), FXIII 34Leu/Leu (p=0.141). On the contrary, such external factors as recurrence of DVT on the same leg (p=0.075), obesity (p=0.002), varicose veins (p=0.087) and short courses of anticoagulant therapy (less than 3 months) (p=0.030) were associated with the risk of severe PTS
Summary and Conclusion: Genetic variations FII 20210 G/A, FV 1691 G/A and FXIII 34Leu/Leu can increase the risk of severe PTS but, probably, don't play a crucial role in the development of this complication. Instead, short courses of anticoagulant therapy (less than 3 months), obesity, ipsilateral recurrence of DVT and varicose veins may be predictive factors for PTS.

P1267

EFFICACY, SAFETY AND CLINICAL MANAGEMENT OF THE NEW GENERATION ORAL ANTICOAGULANTS (NOAS) IN THE CLINICAL PRACTICE: "THE REAL LIFE COHORT STUDY"

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Background: The NOAs in clinical trials have shown to be at least as effective and safe as warfarin for stroke prevention in non-valvular atrial fibrillation (NVAF).
Aims: In our centre, we have initiated "The Real Life Cohort study" which is an ongoing observational cohort study to analyse the efficacy and safety as well as other clinical aspects such as bridging therapy of NOAs after their introduction in daily clinical practice since they have been approved in Spain.
Methods: Since 2010 we have included consecutively patients under treatment with NOAs. Inclusion criteria were patients with NVAF, with or without a history of stroke. Exclusion criteria are those who do not meet the criteria for the prescription of the Catalan Institute of Health. All of them gave their informed consent. Follow-up consisted in outpatient visits each 3 months and thereafter efficacy and safety data were collected.
Results: Until October 31th 2013, 184 patients have enrolled in our registry. Out of these, 93 (50.5%) patients received dabigatran, 72 (39.1%) received rivaroxaban and 19 (10.3%) received apixaban. Median age of 77 years-old (range: 24-94) with 51.6% females; 82% of the patients had a CHA2DS2-VASc score greater than or equal to 3, the 75% a HASBLED score greater than or equal to 3. During follow-up, in the dabigatran group, ischemic strokes occurred in 4.3% (n=4) and only one of them with 150 mg. Twenty five patients (26.8%) reported bleeding complications (60% minor, 28 % non-major, clinically relevant and 12% major bleeding). For non-major bleedings, mucosal and gastrointestinal bleeding were the most common bleeding sites (72% of all bleedings), in our cohort occurred mostly in patients with doses of 110 mg. Patients with CNS hemorrhage (n=2) received prothrombin complex concentrate with good evolution. The non-hemorrhagic adverse envents were: dyspepsia 15%, diarrhea 2.2%, urticaria 2.2% and peripheral edema 1.1%. As rivaroxaban an apixaban were approved later, results from these cohorts are still under the observational period.
Summary and Conclusion: The preliminary results of the ongoing "REAL LIFE" Cohort study in the dabigatran group showed that are higher incidence of ischemic stroke and total bleeding compared with those published in clinical assays. Although we have no data from other groups, this study will give us some clues about the behavior of these NOAs and their best management in "REAL LIFE".

Health economics

P1268

COST-EFFECTIVENESS OF EDOXABAN, APIXABAN, RIVAROXABAN AND DABIGATRAN VERSUS WARFARIN FOR STROKE PREVENTION IN NONVALVULAR ATRIAL FIBRILLATION

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Background: Non-valvular atrial fibrillation (NVAF) causes one fifth of overall ischemic strokes, therefore, oral anticoagulation with warfarin is recommended since it can prevent about one third of the events. Novel oral anticoagulants (NAO), *i.e.* oral inhibitors of thrombin or factor Xa, compete with warfarin in this setting since a simplified monitoring is required and intracranial beedings are significantly reduced.
Aims: We aimed at assessing the cost-effectiveness of NAOs (dabigatran, apixaban, rivaroxaban, edoxaban), *versus* warfarin, for stroke prevention in NVAF patients with CHADS2 score ≥2.
Methods: We built a Markov decision model including 11 health-states. Quarterly transition probabilities among states were estimated based on data from published randomized trials. Quality of life estimations for each event or health-state were derived from reference studies. For each therapeutic strategy, the model estimated expected quality-adjusted life-years (QALY) in the lifetime horizon. The economic analysis was performed in the perspective of the Italian National Health System. Quarterly drug cost of NAOs was €198 *versus* €3.9 of warfarin. Future life years and costs were discounted by 3.5% per year, according to international guidelines. Incremental cost per QALY gained (ICUR) as compared with warfarin was calculated for each NAO. First- and second-order (probabilistic) sensitivity analyses were run. The model was developed and run by TreeAge SW.
Results: NAOs increased quality-adjusted life expectancy by 0.405-0.753 discounted years, as compared with warfarin. Incremental lifetime health-care costs of patients assigned to NAOS ranged from €3,439 to €4,923. The cost-effectiveness of NAOs *versus* warfarin ranged from €4,567/QALY (80%CI: 2,129-8,993) for apixaban to €12,156/QALY (80%CI: 5,147-33,200) for rivaroxaban. Dabigatran and edoxaban reported intermediate ICUR values: €6,307/QALY (80%CI: 3,034-13,421) and € €7,713/QALY (80%CI: €3,909-€17,963), respectively. Second-order sentivity analysis showed that the results were robust: over 90% of the simulations provided an incremental-cost-effectiveness ratio lower than €50,000/QALY, irrspectively of the NAO being compared with warfarin. The results were sensitive to the time in warfarin therapeutic range, the analysed time horizon, and the impact of anticoagulants onto quality of life. NAO cost variation by 20% induced ICUR variations by about €3,000/QALY.
Summary and Conclusion: NAOs are cost-effective drugs for stroke prevention in moderate-high risk patients with NVAF. Lacking head-to-head studies, the relative cost-effectiveness of one NAO *versus* another one is surrounded by a wide undcertainty. Economic sustainability of widespread NAO adoption in industrialized countries still needs to be assessed, therefore phase 4 studies should investigate clinical and economic outcomes in specific patient subgroups, *i.e.* warfarin-treated patients with a high rate of time-in-treatment-range.

P1269

ECONOMIC VALUE CREATED BY ADDING RITUXIMAB TO CHEMOTHERAPY IN THE UNITED STATES FROM 1998-2013

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Background: Rituximab has become the standard in diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and chronic lymphocytic leukemia (CLL). The incremental cost of adding rituximab to existing chemotherapy and the value of the life years saved have not been estimated at the population level.
Aims: Our objective is to estimate the net economic value of rituximab in the United States for policy decision-makers.
Methods: We constructed a population effectiveness model from 1998-2013. Age group, gender, and year-specific incidence rates for each cancer were estimated from the Surveillance, Epidemiology, and End Results (SEER) program. Incidence rates were multiplied by Census estimates to calculate total diagnosed patient counts. Utilization, survival and cost inputs were estimated using SEER Medicare merged data for patients diagnosed 1999-2009 and followed through 2010, extrapolated through 2013. First-line utilization of rituximab plus chemotherapy (R+Chemo) and Chemo Alone were calculated as a proportion of all diagnosed patients to estimate treated patient counts. Differences in mean survival between R+Chemo and Chemo Alone for each cancer were estimated using flexible parametric survival models. The incremental cost of R+Chemo *versus* Chemo Alone for each cancer was based on Medicare paid amounts for all Part A and B services using regression, accounting for censoring. Costs were computed over 72-months to capture short and long-term effects, and inflated to 2013 US dollars. The value of a life year saved ($90,941 in 2013 dollars) was based on published literature (Lee, *et al.* 2009). Monte Carlo sampling was used to estimate the 95% uncertainty intervals (UI).
Results: Across all three cancers from 1998 to 2013, there were 280,819 cumulative life years saved (95% UI, 269,136-293,345). The additional total costs of care for R+Chemo were $33,525, $23,511, and $31,435 in DLBCL, FL, and CLL, respectively. Across all 3 tumors, the incremental direct medical cost of R+Chemo compared to Chemo Alone was $7.0 billion (95% UI $5.8-$8.2 billion), and the resulting economic value of the life years saved was $25.5 billion (95% UI $11.7-$69.1 billion).
Summary and Conclusion: For DLBCL, FL and CLL patients treated with R+Chemo in the US from 1998-2013, 281,000 life years were saved that created a net economic value of $18.5 billion.

P1270

COST-EFFECTIVENESS ANALYSIS OF BENDAMUSTINE PLUS RITUXIMAB AS FIRST-LINE TREATMENT FOR PATIENTS WITH FOLLICULAR LYMPHOMA. PRELIMINARY RESULTS

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Background: Follicular lymphoma (FL) is the most common type of indolent non-Hodgkin´s lymphomas (NHL), and despite substantial improvement in survival, it is usually incurable in most cases. The disease characteriscally responds well to first-line therapy but typically manifests repeated relapses with the need of recurrent therapeutic interventions, with disease-free intervals. Patients with FL in advanced stages and high-tumour burden usually receive front-line chemoimmunotherapy, rituximab (R) + chemotherapy, during the so called induction phase, followed by maintenance therapy with R in patients who achieve at least a partial response after the induction phase, as it is recommended by several Clinical Guidelines.
Aims: To evaluate the cost-effectiveness of rituximab-bendamustine (R-B) compared with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) as first-line treatment for patients with advanced FL in Spain.
Methods: A Markov model was built to simulate a patient cohort with FL during a time horizon of 25 years (4 weeks-cycle length). Clinical data were adapted from two phase III randomized trials, one with the FL patients analyzed by the German Study group indolent Lymphomas (StiL) which compared R-B with R-CHOP during the induction phase, followed by the data obtained in the PRIMA trial during the maintenance therapy with R. Four health states were considered: progression-free survival (including induction and maintenance), first relapse, second relapse and death. Transition probabilities were obtained from progression-free survival curves available in the literature. Mortality rates were obtained from GLOBOCAN registry and from recently published epidemiological data after the "rituximab era". The Study Perspective was the Spanish National Health System (NHS) and only direct healthcare costs were considered. Resources consumed during patients treatment and follow-up where identified by panel of Spanish experts in FL. Unitary costs were obtained from the Drug Catalogue and e-Salud Database. Drug costs were calculated based on ex-factory prices with mandatory 7.5% rebate. All costs were updated to € 2013. Utilities for each health state were obtained from the literature. The final efficacy measure was quality-adjusted life-years (QALYs). Costs and health outcomes were discounted at a 3% annual discount rate. To check for the robustness of the results, a probabilistic sensitivity analysis was performed with 10,000 Monte Carlo simulations.
Results: The initial treatment and administration costs during the induction phase were higher with R-B (€16,481.63) compared to R-CHOP (€10,793.97). Nevertheless, at the end of the 25 year period, in the deterministic analysis, and considering only the GLOBOCAN registry mortality rate, R-B first line strategy accounted a total cost of €686,848.46 compared to €725,450.80 for R-CHOP. Health benefits measured as QALYs was higher in R-B with 14.25 QALYs than in R-CHOP with 13.95 QALYs. In the probabilistic analysis, R-B was a dominant strategy compared to R-CHOP in 99.9% of the simulations.

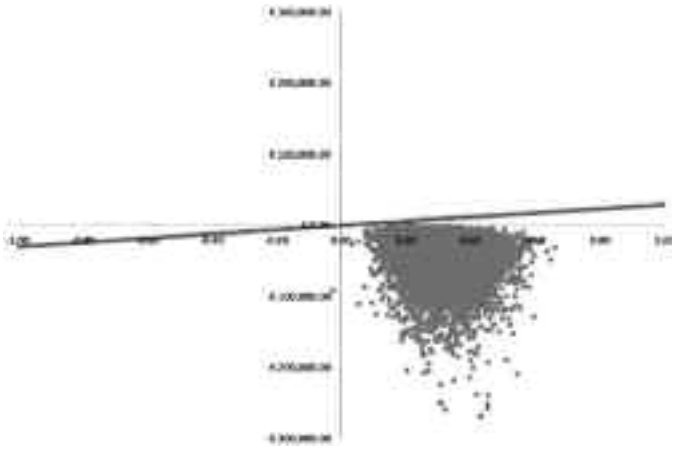

Figure 1.

Summary and Conclusion: First line treatment with R-B in FL patients is a dominant strategy compared to R-CHOP, showing cost savings and higher health benefits for the Spanish NHS, despite its higher initial cost.

P1271

IS OBINUTUZUMAB COST-EFFECTIVE FOR PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA IN THE UNITED STATES?

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Background: Obinutuzumab, also known as GA101 (G), is a novel therapy shown to have improved PFS in combination with chlorambucil (GClb) compared to rituximab + chlorambucil (RClb) in the recent CLL-11 trial for previously untreated CLL. The incremental value of G *vs*. rituximab (R) has not been studied.
Aims: The objective is to evaluate the cost-effectiveness of GClb *vs*. RClb in the United States for previously untreated CLL.
Methods: Patient outcomes were simulated using a 3-state Markov model that included PFS, progression, and death. The patient population was assumed to be analogous to that studied in the CLL-11 trial. Efficacy parameters were 1) probability of progression, and 2) probability of dying after disease progression. The model parameters were fitted to the observed trial data. Drug utilization and adverse events were incorporated based on trial data, and costs were based on Medicare reimbursements and drug wholesale acquisition costs. Sensitivity analyses were conducted to assess uncertainty in the results.
Results: Treatment with GClb led to an increase in average life years (+0.61y) and quality-adjusted life years (QALYs)(+0.56 y) relative to RClb, respectively. The average total costs were similar, with higher drug and adverse event costs for GClb being offset by higher cost of disease progression with RClb.

Table 1.

Outcome	GClb	RClb	Difference
Average life years	5.05	4.44	0.61
Average QALYs	3.36	2.80	0.56
Total drug cost	$37,460	$34,875	$2,585
Drug administration	$1,134	$803	$330
Supportive care	$128	$75	$52
Adverse events	$9,851	$6,766	$3,085
Cost of progression	$40,004	$46,075	-$6,070
Average total cost	$88,577	$88,595	-$18

In probabilistic sensitivity analyses, the difference in QALYs ranged from 0.03 to 1.02, and the difference in total cost ranged from approximately -$53,000 to $56,0000. There was an 89% probability that G+Clb was cost-effective at the $100,000 per QALY threshold.
Summary and Conclusion: Based on the results of the CLL-11 trial, our analysis suggests treatment with GClb compared to RClb is likely cost-effective. Further analyses based on indirect comparisons with other treatment options, as well as updated follow-up data, will help inform coverage and reimbursement policy decisions.

P1272

HOME CARE MANAGEMENT FOR HEMATOLOGICAL PATIENTS: RESULTS OF A SURVEY CONDUCTED ON A REGIONAL SCALE BY THE R.E.D.E.R. NETWORK

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Background: In the last decade home care has achieved a relevant role in the global management of patients with blood malignancies reducing heath care costs, improving quality of life and preventing discomfort due to prolonged hospitalization, driving distances and long waiting times. Owing to the empirical nature of these evidences, few data have been published in this setting of care and some clinical issues remain unexplored.
Aims: In order to collect experiences and promote mutual partnership in the field of home care for unfit and frail hematological patients, the R.E.D.E.R. project ("Rete Ematologica Domiciliare Emilia-Romagna") was informally established in 2013 by six groups operating in the Italian region Emilia-Romagna on behalf of six local no-profit associations directly involved in sustaining domiciliary programs of supportive and palliative care (Bologna=BO, Ferrara=FE, Forlì=FC, Modena=MO, Ravenna=RA, Reggio Emilia=RE).
Here we present the results of a preliminary survey conducted with the purpose to evaluate the main key activity indicators in this setting of care.
Methods: At the beginning of 2014 each consultant hematologist representing a group was requested to fill a multiple-items form divided in two sections: in the first one it was required to indicate the overall number of patients enrolled during 2013 and their outcome; in the second section doctors were asked to "take a snapshot" of their activity and provide an overview at that precise moment focused on clinical features of patients, transfusion requirements, use of central venous catheters and incidence of pain.
Results: During the year 2013 a total of 521 hematology patients (BO=115, FE=50, FC=71, MO=170, RA=26, RE=89) was referred to the home care network. 271 were newly-referred patients, 250 were patients already followed in the previous years. 195 (37.4% of all patients) died as part of the home care cycle, 86 at home (44.1%) and 109 in hospice or in a hospital ward (55.9%). 42 patients (8%) were referred back to the hospital hematology unit. At the beginning of 2014 the total of patients assisted simultaneously was 274. The median age was 78.4 with a 47/53% male/female gender distribution. According to the phase of disease and to the type of treatment 90 patients (32.8%) were still under active therapy, 123 (44.9%) were chronically ill, 61 (22.3%) were terminally ill. According to diagnosis there were 56 patients with myelodisplastic syndromes (MDS=20.4%), 51 with lymphoma (NHL/HL=18.6%), 48 with myeloma (MM=17.5%), 44 with chronic myeloproliferative neoplasms (MPN=16.2%), 31 with acute leukemia (AML/ALL=11.1%), 44 with other blood malignancies or benign conditions (other=16.2%). 107 patients (39.1%) were transfusion-dependent (BO=27, FE=7, FC=13, MO=28, RA=5, RE=27), 34 needing both packed red blood cells (RBC) and platelets concentrats (PC), 70 only RBC, 3 only PC. 14 patients (13.1%) had transfusion requirement of at least 2 RBC units per week. 11 patients (10.3%) were under iron chelation therapy. A central venous line was available for 32 patients (PICC=24, Hickman=6, Port-a-cath=2). 65 patients (23.7%) needed around-the-clock opiod drugs for moderate-to-severe pain management (oxycodone+/-naloxone=37, transdermal fentanyl=13, continuous subcutaneous morphine=12, other=3).
Summary and Conclusion: In Emilia-Romagna home care management for hematological patients is almost entirely financed by no-profit fundraising organizations, without which hundreds of unfit and frail patients would inevitably redound upon hospital wards, day units and outpatient clinics. The R.E.D.E.R. network project, open to new entries and new challenges, was conceived with the primary goal, on a local/regional scale, to update the state-of-the-art, share professional expertise, perform cost-benefit analysis and eventually provide guidelines on best practices (quality-of-life assessment, home transfusions, end-of-life care).

P1273

SENSITIVITY COST ANALYSIS OF TYROSINE KINASE INHIBITORS IN PHARMACOECONOMIC MODELING OF CML TREATMENT

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Background: The use of second-generation tyrosine kinase inhibitors (TKI) in treatment of newly diagnosed chronic myelogenous leukemia (CML) patients showed their advantages over imatinib in respect of tolerability, rate and

promptness of deep molecular responses obtaining. Long lasting complete molecular response (CMR) is a major predisposing factor for successful cessation of TKI. The main obstacle for the wide use of second generation TKIs in first-line setting is their higher cost in comparison with imatinib. The TKI cost may change dramatically in the nearest future due to marketing policy, generic substitution, etc. Pharmacoeconomic modeling of CML treatment can establish limits when various strategies will become cost saving.

Aims: The aim of our study was to conduct sensitivity analysis of TKI costs in CML treatment.

Methods: We have used previously constructed Markov chain models[1] to compare CML first-line treatment strategies with imatinib or nilotinib with subsequent therapy cessation in cases of CMR. The input parameters and transition rates were selected from several clinical trials (IRIS, ENESTnd, DASISION, ENACT, CA180013, STIM, FILMC group), our own data, and experts' opinions. We have chosen the model population size of 800 newly diagnosed CML patients in Russian Federation annually. 20-years' time horizon was used. We calculated total cost for the next twenty-year period (2014-2033) including the expenses for existing and newly diagnosed CML patients. We have recalculated the total cost in Euros to make our results more representative. Simulation model was used for statistical analysis.

Results: The different TKIs prices substitution allowed us to construct the function of cost-saving limits. The function plot presented on fig.1. The first-line treatment with Nilotinib was always cost-saving in case of Imatinib price is more than half-price of Nilotinib.

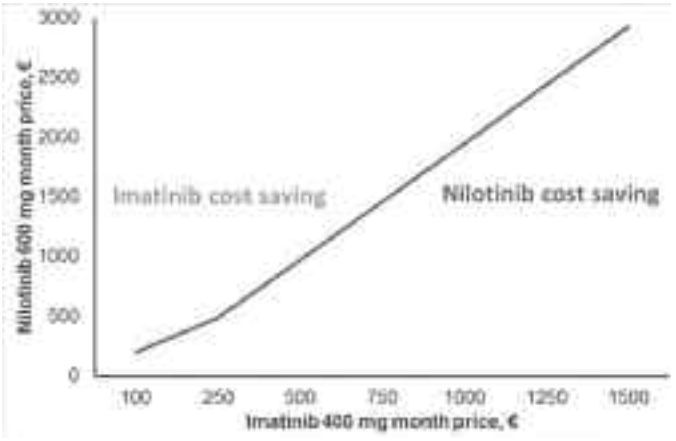

Figure 1. Cost saving function plot for Nilotinib and Imatinib first-line in CML treatment

Summary and Conclusion: Sensitivity analyses in pharmacoeconomic modelling can establish limits when one treatment strategy have some advantages over another one. The results of such analyses could be of value for decision-making process for healthcare authorities.

Reference: [1]Shuvaev V.A. *et al.* ELN Information letter October 2013. – p.14.

P1274

DIRECT COST ANALYSIS ABOUT THE THREE CHELATORS FOR THE TREATMENT OF THALASSEMIA PATIENTS WITH CHRONIC IRON OVERLOAD: AN ITALIAN PERSPECTIVE FROM THE MIOT NETWORK

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Background: In thalassemia major (TM) three iron chelators in monotherapy are available to treat chronic iron overload due to blood transfusions: subcutaneous desferrioxamine (DFO) introduced in the 1960s, oral deferiprone introduced in 1999 and oral deferasirox (DFX) licensed in 2006. Nowadays pharmacoeconomics analysis are frequently required by the health authorities due to the actual economic crisis. The aim should be to ensure to the whole community the sustainability for health care of proved quality.

Aims: The objective of this study was to determine the costs of the three chelators in monotherapy in a cohort of 193 TM patients followed prospectively for 18 ± 3 months.

Methods: Within the MIOT (Myocardial Iron Overload in Thalassemia) network, we evaluated prospectively 193 TM patients who had been received one chelator alone between the 2 Magnetic Resonance scans and we calculated the direct costs (drug, administration and monitoring) for each patient treated with DFX, DFO and DFP. We used the cost values for the year 2007. For the drugs we considered the cost ex-factory. For the oral chelators the administration cost was considered null. For the DFO we calculated the costs for the administration (pump, infusion set, syringes and gauzes) using the tariffs applied in Veneto Region, Italy. For the monitoring costs we considered the exams suggested in the technical sheet for each drug; we considered the tariffs by the Veneto Region, Italy. In Italy Veneto Region was proved to be one of the most upright region in the health costs management. In the analysis we considered the drug cost for the standard dosage reported in the technical sheet: 40 mg/kg/d for DFO, 75 mg/kg/d for DFP and 30 mg/kg/d for DFX. Based on the mean weight of the patients we referred the drug cost to a patient of 60 Kg.

Results: In the clinical practice the dose of DFX was 26±7 mg/kg/d, DFP was 73±13 mg/kg/d and DFO was 41±6 mg/kg for 5.5 d/wk. Excellent/good levels of compliance were similar in the 3 groups (DFX 99%, DFP 95%; DFO 96%, P=0.6). The cost/mg was € 0.006 for Generic DFO, € 0.003 for Ferriprox® (DFP) and € 0.047 for Exjade® (DFX). For 18 months of treatment the total costs for DFO were € 10.465,8 (administration and monitoring costs € 3.965,1 + drug cost 6.500,7), for DFP were € 8.292,9 (administration and monitoring costs € 460,8 + drug cost 7.832,2), for DFX were € 46.461,2 (administration and monitoring costs € 211,14 + drug cost 46.249,8). The details about the administration and monitoring costs for DFO, DFP and DFX are reported in the table.

Table 1.

	Unit Cost (€)	Number /year	Cost/ 18 months (€)
DFO			
Pump	1241	0.25	465.375
Thalaset	4.2	390	2457
Syringes	1.24	390	725.4
Gauze	0.07	365	38.325
Audiometry	18.6	5	139.5
Ophthalmology	18.6	5	139.5
DFP			
Neutrophil count	4.35	52	339.3
Serum creatinine	1.6	12	27.9
AST and ALT	5.2	12	93.6
DFX			
Audiometry	18.6	1	27.9
Ophthalmology	18.6	1	27.9
Serum creatinine	1.55	17	39.5
Proteinuria	1.3	12	22.5
AST and ALT	5.2	12	93.6

Summary and Conclusion: In this analysis, for managing chronic iron overload the direct costs for oral DFP appeared to be the less expensive. The limit of this study is that a cost-utility analysis taking into account efficacy, adverse events and route of administration was not performed.

P1275

TREATMENT PATTERNS OF PATIENTS WITH CLL INCLUDING PATTERNS OF RITUXIMAB USE BY CLINICIANS ACROSS FIVE EUROPEAN COUNTRIES: REAL WORLD EVALUATION UTILIZING IPSOS HEALTHCARE SURVEY

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Background: Chronic lymphocytic leukemia (CLL) is a disease that predominantly afflicts the elderly and represents the most common leukemia in Europe. CLL is considered incurable and is characterized by disease relapses and refractoriness to earlier lines of treatments. Anti-CD20 agents, such as rituximab is commonly used for CLL treatment. In several real world studies, rituximab is frequently used as single agent and in combination therapy with other agents in the US for elderly or unfit CLL patients. In Europe, rituximab is also recommended for use in combination therapy but no real world usage pattern in Europe has been reported.

Aims: We sought to evaluate the real-world treatment patterns of CLL examining the use of treatment agents and regimens in all lines of treatment across five European countries during the years of 2012-2013.

Methods: IPSOS Healthcare Global Oncology Monitor conducted a survey of 509 oncologists across Germany, France, UK, Spain and Italy who completed

patient chart review and provided information on how they managed patients in everyday practice. Of these, 104 physicians provided CLL patient information across first, second and third-line CLL treatments.
Results: Survey data estimated approximately 37,119 patients who received treatment for CLL in a period from July 2012 to June 2013. Patients receiving treatment were predominantly male (62.3%), with a total population mean age of 71 years. Patients treated were of ECOG performance status 0 (33.9%), 1 (51.9%), 2 (10.8%) and 3 (0.65%). The majority of these patients (23.1%) were estimated to have received fludarabine-cyclophosphamide-rituximab followed by bendamustine-rituximab (17.4%), chlorambucil (16.2%), fludarabine (7.2%), single-agent rituximab (5.8%), and chlorambucil-rituximab (5.6%). The data show higher rituximab monotherapy share in later lines of therapy for CLL (first line, 0.3%; second line, 8.2%, third line or more, 22.1%) irrespective of age. In later lines of therapy, rituximab monotherapy (22%) treatment was the second most commonly used treatment option after bendamustine plus rituximab (31%), followed by chlorambucil monotherapy (9%), prednisolone (7%) and ofatumumab (6%). Patients who received rituximab monotherapy had a mean age of 70.2 years. 70% and 26% of these patients were of ECOG performance status 0 and 1, respectively. The most commonly reported co-morbidities were hypertension (24%), diabetes (13%) and cardiovascular disease (12%).
Summary and Conclusion: This survey has shown that there is a wide range of CLL treatment regimens used across Europe, over different lines of treatment. The most commonly used anti-CD20 is rituximab, which is utilized either in combination therapy or as single agent. Single agent rituximab is increasingly more commonly used in later lines of treatment. Real world results such as this may be useful in helping the design of appropriate clinical trial programs or in interpreting their subsequent outcomes.

P1276

PATTERN OF ADVERSE DRUG REACTIONS IN ONCO-HEMATOLOGIC PATIENTS: RESULTS FROM THE ITALIAN PHARMACOVIGILANCE PROJECT NAMED "FARMAREL"

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Background: Adverse drug reactions (ADRs) are an important cause of hospitalization and inpatient complications. Probability of ADRs occurrence in onco-hematologic patients is notable, as they are treated with multiple antineoplastic agents within complex therapeutic schedules. Post marketing spontaneous reporting of ADRs is crucial for a deeper knowledge of drugs safety profile, once they are used in real clinical practice, but it is not widespread.
Aims: The Italian regional project FarmaRel has been implemented to create awareness on the importance of ADR monitoring and to encourage the voluntary reporting culture among onco-hematology health care professionals.
Methods: In 13 Haematology Units, an "ADR monitor" has been designed with specifically allocated grants to promote pharmacovigilance procedures among clinicians and nurses and supporting them in ADRs reporting. A retrospective analysis of all ADRs collected from January 2009 to May 2012, stored in an electronic database**,** has been performed considering various parameters as patient demographics, drug and reaction characteristics, ADRs seriousness, outcome, treatment strategy and detection setting.
Results: The total number of ADRs reports over the audit period was 1060, with a an average of around 25 reports per month. Regarding patient demographics in the reported ADRs, 471 (44%) were female and 589 (56%) were male. Number of patients in young (0–29 years), adult (30-65 years) and geriatric (>65 years) groups were respectively 58 (5%), 518 (49%), 484 (46%). The majority of the ADRs reported involved non Hodgkin lymphoma patients (394; 37%), followed by multiple myeloma (266; 25%) and chronic lymphatic leukemia patients (102; 10%). The drug class most frequently involved in the ADRs was, as expected, antineoplastic agents (ATC L01) and among them, rituximab was the undisputed leader of suspected drugs having collected 276 citations as drug potentially related to ADR; in the list of suspected drugs, cyclophosphamide, doxorubicin and bortezomib were the subsequent. More than half of the reactions (584; 55%) were classified as serious according to the WHO definition: 463 caused hospitalization or prolonged hospital stay, 60 life threatening event, 27 severe or persistent disability, 34 patient death. Overall, in 598/1060 (57%) reports, patient recovered (526 completely; 72 partially) from the reaction, in 260 (24%) only a clinical improvement was observed, in 67 (6%) cases, reaction was defined unchanged or worsened, in 34 reports patient died (in the remaining 101 reports, outcome was not available). Blood and lymphatic disorders were signaled in 190 reports, infections and infestations in 97, skin disorders in 87, but in majority of cases (258) a mix of reactions affecting different system organ class were reported. Regarding ADR management, in 461 cases the suspected drug was stopped, in 62 was reduced, in 489 an additional treatment was prescribed (in some cases more than one action has been adopted). The majority of ADRs (588; 56%) have been detected during scheduled Day Hospital access or outpatient visit, while a small but not negligible number, in emergency room (98; 9%) or during not planned hospitalization (72; 7%).
Summary and Conclusion: The employment of people dedicated to pharmacovigilance activities has proved to be a successful strategy to improve number and quality of ADR reports. The project FarmaRel significantly contributed to reach and overcome, in Italy, the "Gold Standard" for pharmacovigilance, set by WHO in 300 reported cases per 1 million inhabitants.

P1277

PLERIXAFOR IS COST-EFFECTIVE FOR FIRST LINE STEM CELL MOBILISATION: INDIVIDUAL PATIENT COSTING ANALYSIS OF THE LIVERPOOL PHANTASTIC TRIAL

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Background: This report provides results of the first stage of this process by evaluating clinical and cost-effectiveness of plerixafor in enhancing the mobilisation of sufficient additional stem cells to facilitate successful stem cell transplantation.
Aims: Assessing the 'value' of first line plerixafor in improving stem cell mobilisation is a two stage process. First evaluate the extent to which the collection of stem cells is enhanced and at what cost and second evaluate the impact on the quantity and quality of life experienced by patients. Such analyses aim to identify 'marginal' improvements resulting from plerixafor in comparison to standard care. Economic analysis compared cost-effectiveness underlying the PHANTASTIC clinical trial of first line plerixafor (clinical data reported at EHA 2013 [Vithanarachchi U et al] and submitted) with patients mobilised by conventional chemotherapy
Methods: Economic analysis was integrated into the PHANTASTIC trial of first line plerixafor. The primary outcome captured the extent to which an 'adequate' cell harvest had been achieved. 'Adequacy' was interpreted as being ≥4 x 10^6 $CD34^+$/kg in no more than 2 stem cell collection days with no evidence of neutropenia (<1.0 x 10^9 / Litre) in the 3 weeks following initiation of mobilisation. The historic control data set consisted of 151 consecutive patients treated in Liverpool after November 2006. Active treatment arm consisted of 98 patients mobilised by first line plerixafor between April 2010 and July 2012. A detailed Micro-costing analysis identified, measured and valued resources consumed by each patient in both the plerixafor and standard care arms of the study. Outcome data (adequacy of stem cell collection) were directly derived from the clinical trial.
Results: Nature and severity of adverse events differed significantly between the two groups. In the standard care arm nineteen patients experienced a serious adverse event which required some form of hospital treatment. These SAEs included neutropenic sepsis, haemorrhage, urinary tract infections, upper respiratory tract infections and severe chest infections. In the plerixafor group only three patients required treatment due to non-severe adverse events (one patient had low levels of potassium, one had nausea and pain and one required platelets). In terms of treatment efficacy 71% of patients achieved the primary outcome in the plerixafor arm compared to 32% in the control arm. Average cost of patients in the plerixafor arm was £13859 compared to £11763 in the comparator arm. Comparative costs for plerixafor *vs*. control in each subgroup were £11763 *vs*. £4762 for myeloma, £16890 *vs* £18083 for non-Hodgkin lymphoma and £12155 *vs*. £19378 for Hodgkin's disease.
Summary and Conclusion: Plerixafor is an effective means of enhancing stem cell harvests. Economic results emphasize that in many patients these benefits can be obtained at a 'reasonable' cost as significant resource savings occur elsewhere in the patient pathway. However, at this stage, the results obtained should be treated with caution. The analysis was not randomized and although propensity matching techniques were used to obtain 'like with like' comparisons a number of confounding factors may remain. Despite this the economic analysis offers encouraging signs that Plerixafor may be effective at enhancing cell harvests at an acceptable cost. Further work is

under way to investigate the extent to which this enhanced harvest of cells translates into an improved quality and quantity of life experienced by patients in each subgroup.

P1278

NUMBER NEEDED TO TREAT & RELATED COSTS FOR ACHIEVING 1 MR 4.5 (BCR-ABL≤ 0.0032%) UNDER DIFFERENT TKIS IN GREECE

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Background: Innovative treatments have transformed CML into a chronic disease mostly managed in outpatient care. The main reason for hospitalization is due to blast crisis or the management of adverse events.
Aims: This study aimed to estimate the cost for achieving a MR 4.5 (BCR-ABL≤0.0032%) at 4 and 5 years of treatment under different Tyrosine Kinase Inhibitors (TKIs) in Greece.
Methods: The Number Needed to Treat (NNT) approach has been used in order to estimate the number of patients needed to treat to prevent one extra person having one bad outcome.i NNT is calculated as the inverse of the Absolute Risk Reduction (ARR) (NNT=1/ARR)[i]. In the current analysis as ARR the MR 4.5 rate by 48 & 60 months was considered (NNT=1/MR4.5). A NNT of 1 means that the preferable outcome occurs in every patient given the treatment. The higher the NNT, the less effective is the treatment. i-[ii] MR4.5 and Adverse Events (AEs) rates were extracted from the DASISION (Kantarjian 2012[iii]) and ENESTnd (Larson 2013[iv]) clinical trials in newly diagnosed CP-CML patients, by evaluating efficacy and safety. The estimation of each TKI cost of treatment has been performed based on 2014 NHS hospital prices in Euros. The estimation of the cost per MR 4.5 is the product of NNT of each TKI treatment cost. Additionally, the cost of treating AEs has been estimated in order to calculate the overall cost of treatment on a per patient basis. The cost of 5 year treatment with dasatinib wasn't estimated since there is not yet data available.
Results: The cost of 4 and 5 years treatment with nilotinib was estimated at €116.507 and €145.634 respectively, for imatinib €87.236 and €109.045 respectively and for dasatinib was considered at €130.929 for the 4y treatment. In order to achieve 1 MR 4.5 at 48 month interval 2.50 patients need to be treated with nilotinib, 4.35 or 4.76 patients with imatinib and 2.94 patients with dasatinib. At 60 month interval 1.85 patients need to be treated with nilotinib and 3,23 with imatinib. The cost for achieving a MR 4.5 for 48 months nilotinib appears to be less costly (€291,268) than imatinib(€379,286 and/or 415,408) and dasanitib (€385,084) and for 60 months €269.692 for nilotinib and €351.757 for imatinib. Mean hospitalization cost per patient due to the treatment of adverse events is as follows: for anemia €2.193 and neutropenia of €1.312.
Summary and Conclusion: Innovative treatments for CML have resulted in treating patients in outpatient care with lower adverse events and consequently, lower cost. Study findings suggest that greater proportion of CML patients on nilotinib achieve target (MR4.5) in a shorter period of time than dasatinib and imatinib resulting in a significant burden reduction of the National Health Care budget. References: [i] Centre for Evidence Based Medicine, *Number Needed to Treat (NNT)*. Available at<http://www.cebm.net/index.aspx?o=1044>; [ii]The Cochrane Collaboration, Summary Statistics for dichotomous outcome data: Number needed to treat. Available at: [iii]Kantarjian HM, Kim DW, Issaragrisil S, *et al.* Enestnd 4-Year (y) Update: Continued Superiority of Nilotinib *vs* Imatinib in Patients (pts) with Newly Diagnosed Philadelphia Chromosome–Positive (Ph+) Chronic Myeloid Leukemia in Chronic Phase (CML-CP). *Blood* 2012; 120(21) (2012 ASH Annual Meeting - Abstract 1676). [iv] Larson RA, Hochhaus A, Saglio G, *et al.* Nilotinib *vs* Imatinib in Patients With Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): ENESTnd 4-Year Update. J ClinOncol 2013;31 (2013 ASCO Annual Meeting - Abstract 7052).

P1279

PREVENTABILITY ASSESSMENT OF ADVERSE DRUG REACTIONS IN ONCOHEMATOLOGIC PATIENTS: HEMATOLOGY CLINICAL JUDGMENT COMPARED TO SCHUMOCK AND THORNTON EXPLICIT CRITERIA

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Background: It is well known that adverse drug reactions (ADRs) cause considerable morbidity and mortality. Preventable adverse drug events reactions (pADRs) have been reported to be common in both outpatient and inpatient clinical practice. However, the rate of pADRs varies considerably in different studies, even when conducted in the same settings, depending on methods adopted for assessing ADRs preventability.
Aims: Our aim is to analyze the preventability of ADRs in oncohematology patients, comparing two different approaches: the judgment of one or more investigators *vs* the pre-defined explicit criteria of Schumock and Thornton (S&T).
Methods: ADRs collected by the pharmacovigilance project FarmaRel from January 2009 to December 2012 have been included in the study. At time of ADR reporting, the hematologist gave his personal judgment regarding event preventability. Retrospectively, a second preventability assessment was performed by two independent expert reviewers applying S&T criteria, considering ADRs preventable if one or more of the following conditions occurred: patient had a history of allergy or previous reactions to the suspected drug; drug involved or its dose, route or frequency of administration was inappropriate for patient's clinical condition; required therapeutic drug monitoring or other necessary laboratory test was not performed; a drug interaction or poor compliance was involved in the ADR; a toxic serum concentration or a pathologic laboratory monitoring test was documented.
Results: The total number of pADRs applying S&T criteria or hematologist judgment was 115, and the concordance between the two approaches occurred only in 5 cases (5/115; 4%). To note, out of 887 ADRs evaluated, hematologist considered preventable 17 (2%), compared to 103 (12%) applying S&T criteria. In the S&T pADRs, drug interactions were the preventability cause in 39, inappropriate therapy in 3, history of allergy or previous reactions to the suspected drug in 46, and finally in 15 reports more than one criteria of preventability were identified. More than half (70/103; 68%) of the pADRs were serious. Complete, partial resolution or improvement was observed in 78% of the cases (55/70). When considering the not preventable ADRs, they were 799/887 (90%) for hematologist and 752/887 (85%) with S&T criteria. The two methods were in accordance for 680 out of the 871 ADRs judged not preventable by S&T criteria or hematologist judgment (78%)(see attached table for summary data).

Table 1.

	METHODS	
ADR PREVENTABILITY	Schumock and Thornton	Hematologist
Not preventable	752 (85%)	799 (90%)
Preventable	103 (12%)	17 (2%)
Not assessable	32 (3%)	71 (8%)
TOTAL	887 (100%)	887 (100%)

Summary and Conclusion: The high variability of pADRs rate assessed by the two different methods is a burning topic of discussion: it possible that hematologist evaluation underestimates pADRs, or, as well, that S&T criteria may be only partially applicable to oncohematology. Indeed, since in this setting treatments are often life-saving, other criteria should be taken into account: the purpose of therapy administered (disease treatment or palliative care), the possible alternative treatments available, and, finally, the specific drug risk/benefit ratio.

P1280

COMPARATIVE ANALYSIS OF ADVERSE REACTIONS ARISING FROM THE ADMINISTRATION OF CONVENTIONAL CYTOTOXIC DRUGS AND RECENT TARGETED AGENTS, ALONE OR IN ASSOCIATION, IN ONCOHEMATOLOGIC PATIENTS

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Background: In the last decade a large number of new therapeutic agents, as monoclonal antibodies, biological and immunomodulatory drugs, has been introduced in onco-hematology. Because of their fast introduction and spread in clinical use, post marketing safety data should be collected. They are frequently employed in association with conventional cytotoxic agents to enhance the therapeutic efficacy, representing a potential risk factor for cumulative unexpected toxicities.
Aims: To compare adverse drugs reactions (ADRs) arising from the administration of conventional chemotherapeutic agents, recent targeted drugs, and their association, in terms of severity, outcome, detection setting, treatment strategy, organ or system affected.
Methods: We retrospectively analyzed ADRs collected by the pharmacovigilance project FarmaRel. ADRs were divided into three groups according to the suspected drugs: "C" (conventional), "T" (targeted) and "C+T" (in case both a C and a T drug was suspected). In the C group we considered conventional cytotoxic drugs as cyclophosphamide, doxorubicin, cisplatin, while monoclonal antibodies, immunomodulatory drugs and targeted therapies have been classified in the T group.
Results: A total of 1060 ADRs have been considered for analysis. Of these, 424 (40%) have been ascribed to T drugs, 428 (40%) to C agents and 208 (20%) to the combined employment of C+T drugs. Considering seriousness, the highest percentage of serious reactions has been observed in C (285/428; 67%) and in C+T (133/208; 64%) groups, while T drugs resulted associated to serious reactions only in 166/424 (39%) cases. The death rate was respectively 4% in C, 3% in C+T, and 2% in T classes. ADRs causing patient hospitalization were more frequent with C (232/428; 54%) and C+T (108/208; 52%) than with T drugs (123/424; 29%). Differently from seriousness, outcome did not differ significantly among drug groups. Regarding treatment strategy, withdrawal of suspected drug has been applied more frequently for T drugs (227/424; 54%), while dose reduction has been adopted with similar frequencies in all groups. As far as detection setting is concerned, a considerable percentage of reactions related to C+T drugs (35/208; 16%) has been reported in emergency room; the majority of ADRs from T drugs (319/424; 75%) has been collected during scheduled accesses in outpatient or Day Hospital services, while ADRs from C drugs have more frequently been observed during programmed hospitalization (166/428; 39%). Also organ or system affected by ADRs varied significantly among the three classes: for example, infections resulted more frequently reported in C+T class, nervous system disorders in T group. For full data review, see attached table.

Table 1.

		T	C	C+T	Total	χ2 P value
Severity	Not severe	206 (61%)	143 (33%)	75 (36%)	476	
	Death	11 (3%)	17 (4%)	6 (3%)	34	
	Hospitalization	123 (29%)	232 (54%)	108 (52%)	463	< 0.0001
	Life-threatening	20 (5%)	25 (6%)	15 (7%)	60	
	Invalidity	12 (3%)	11 (2%)	4 (2%)	27	
Outcome	Full resolution	211 (50%)	222 (52%)	93 (45%)	526	
	Partial resolution	23 (5%)	31 (7%)	18 (8%)	72	
	Death	11 (3%)	17 (4%)	6 (3%)	34	0.1427
	Improvement	100 (23%)	99 (23%)	42 (20%)	240	
	Unchanged	27 (6%)	20 (5%)	10 (5%)	57	
	Unknown	52 (12%)	40 (9%)	19 (9%)	101	
Treatment	No Withdrawal	197 (46%)	271 (63%)	131 (63%)	599	< 0.0001
	Withdrawal	227 (54%)	157 (37%)	77 (37%)	461	
	No dose change	369 (82%)	407 (95%)	202 (97%)	978	0.0140
	Dose change	55 (18%)	21 (5%)	6 (3%)	82	
Detection	Access Room	28 (6%)	37 (9%)	33 (16%)	98	
	Not progr. access in amb./DH	21 (5%)	33 (8%)	21 (10%)	75	
	Program. access in amb./DH	319 (75%)	153 (36%)	116 (56%)	588	< 0.0001
	Not programmed hospitalization	15 (4%)	39 (9%)	18 (9%)	72	
	Programmed hospitalization	43 (10%)	166 (39%)	18 (9%)	227	
Organ or system affected	Cardiac	15 (4%)	12 (3%)	1 (0.5%)	28	
	Skin	37 (9%)	41 (10%)	9 (4%)	87	
	Emolymphopoietic	83 (20%)	59 (14%)	48 (23%)	190	
	Epatobiliary	6 (1%)	15 (3%)	2 (1%)	23	
	Investigations	13 (3%)	9 (2%)	4 (2%)	26	
	Gastrointestinal	18 (4%)	34 (8%)	8 (4%)	60	
	Infections	13 (3%)	49 (11%)	35 (17%)	97	
	More than one	89 (20%)	110 (26%)	63 (30%)	268	< 0.0001
	Muscular	9 (2%)	16 (4%)	0 (0%)	25	
	Renal	4 (1%)	16 (4%)	0 (0%)	20	
	Respiratory	28 (7%)	21 (5%)	15 (7%)	64	
	Systemic	27 (6%)	15 (4%)	10 (5%)	52	
	Nervous	49 (12%)	21 (5%)	7 (4%)	77	
	Tumor	11 (2%)	5 (1%)	2 (1%)	18	
	Vascular	14 (3%)	3 (1%)	1 (0.5%)	18	
	Other (eye, ear, endocrine)	12 (3%)	14 (3%)	3 (1%)	29	
	Total	424 (100%)	428 (100%)	208 (100%)	1060	

Summary and Conclusion: Our study shows that ADRs arising from the administration of conventional, new drugs and their association, varied significantly for most of the parameters considered. Interestingly, we found that cytotoxic drugs are more often associated with serious events; ADRs from new agents has been more frequently approached with drug suspension, for reasons that need to be clarified, and that accesses to emergency room for ADRs are more frequently reported when targeted and conventional drugs are combined. In conclusion, these data confirmed the importance of post-marketing surveillance of drugs, whether they are new, conventional or employed in association.

P1281

SOCIETAL ECONOMIC BURDEN OF IRON DEFICIENCY INDUCED FATIGUE

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Background: Fatigue is a common symptom in general practice, affecting up to one-third of the population. Similarly, iron deficiency (ID) is a disorder affecting about one-fourth of menstruating women. A deeper understanding of the association between ID, fatigue and its societal burden is important for patients, physicians and health care decision makers.
Aims: The aim of this project was to estimate ID-conditioned sick leaves and their impact on the social burden of the disease among the female working population. In addition, correlations between national GDP (2011) and total costs or annual days of sick-leave were assessed by Spearman's correlation coefficients.
Methods: An online survey was conducted among female consumer panels aged 18-50 years suffering from fatigue (July 2013). Fourteen different countries were included (Australia, Austria, Brazil, Belgium, Greece, Israel, Italy, Netherlands, Portugal, Singapore, S. Korea, Spain, Sweden, Turkey). Participants were included for symptomatic iron deficiency and asked to fill in a standardized questionnaire in regard to demographic information, conditions related to fatigue and to time lost at work due to fatigue.
Results: In total, 1'401 participants completed the survey (mean age: 32 years). Most predominant condition was un-refreshing sleep (81%) followed by lack of concentration/ memory impairment (66%). Across all countries, 1'039 (74%) women were in the workforce. On average, 29% had to take sick-leaves due to fatigue symptoms (range: 7% in Portugal, to 42% in Singapore). The median length of sick-leave per patient ranged from 12 days (Singapore) to 84 days (Turkey) per year. Assuming a 9% prevalence of ID in the female population (CDC, USA) of whom 10% are assumed to show fatigue symptoms (Favrat 2014), the estimated total annual social burden ranged between 4.1M USD (Singapore) to 197.7M USD (Australia) with a human capital approach. Using the friction method (elasticity for annual labor time *versus* labor productivity=0.8), the social economic burden would lead to costs between 3.3M USD (Singapore) and 158.1M USD (Australia) (Tab 1). The total costs were positively correlated with the national GDP (Spearman's rho: 0.793, p=0.001), whereas the number of sick-leaves due to fatigue showed no correlation with GDP.
Summary and Conclusion: The present study has some limitations including the small sample size, the qualitative approach of the survey and the study design, which might be prone for bias. Nevertheless this is a first study analyzing the social burden of sick-leaves among fatigue women in the work force and further studies are needed in this area to validate our results. The potential societal burden of ID-conditioned sick-leaves seems to be very high and should get awareness of employers and payers. It is of uttermost importance that ID in women with fatigue is diagnosed and treated in a timely manner.

P1282

WILL SUBSTITUTION OF TKI GENERIC BRING THE CML TREATMENT FUTURE TO THE PAST? PHARMACOECONOMIC MODELING OF CML TREATMENT WITH OWN GENERIC USE EXPERIENCE

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Background: Shift from imatinib to second-generation tyrosine kinase inhibitors (TKI) in the treatment of newly diagnosed chronic myelogenous leukemia (CML) patients showed the encouraging results with reduction frequencies of adverse events (AE) and substantial improvement of the rate and promptness of deep molecular responses obtaining. At present, the patients with complete molecular responses (CMR) are considered as candidates for therapy cessation in the setting of clinical trials. Exclusive patent protection expiring led to substitution of imatinib with generic in Russia since August 2012. To date, the cost of generics is more than a half lower than original imatinib.

Some patients and doctors fear that more efficient and modern second generation TKIs could be substituted by less expensive imatinib analogues.
Aims: The aim of our study was to compare the costs of first and second generation TKI in first-line CML treatment in era of generic substitution.
Methods: We have used previously constructed Markov chain models[1] to compare CML first-line treatment strategies with imatinib or nilotinib with subsequent therapy cessation in cases of CMR. Government registered and obtained during state procurement TKI's (imatinib generics, nilotinib, dasatinib) costs were used. Imatinib generics' substitution experience was used as scenario for the second generation TKI's. We have chosen the model population size of 800 newly diagnosed CML patients in Russia annually. 20-years' time horizon was used. We have tried to assess the treatment cost for one patient and cumulative budget burden for the entire Russian CML population. The total cost included the direct costs of diagnostic procedures to establish diagnosis, residual disease monitoring, costs of medications (TKI and concomitant drugs for AE management), allogeneic stem cell transplantation. We have chosen the discount rate as 3% per annum. We have recalculated the total cost in Euros to make our results more representative. Simulation model was used for statistical analysis.
Results: The results of our analysis showed that the average total cost per one patient in case of nilotinib use instead of imatinib becomes cheaper in three years of CML treatment. It caused by more frequent successful therapy cessation in spite of the use of imatinib generics. Similarly, total CML budget burden in case of nilotinib 1st line treatment was higher than imatinib during first eight years. Subsequently, more frequent therapy cessation and second generation TKI's generic substitution results in cost saving (fig.1). Importantly that the use of nilotinib in 1st line could save more than 500 CML patients' lives without any additional expenses. The results of our analysis are strongly depend on input parameters values, which could be changed in the nearest future.

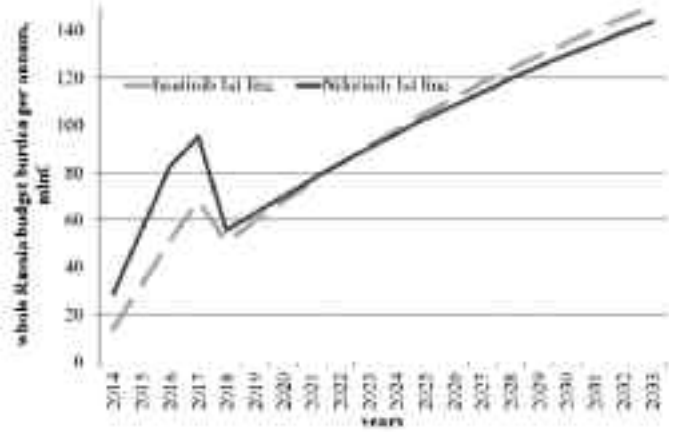

Figure 1. Nilotinib and Imatinib with generic substitution in first-line CML treatment, Russian budget burden

Summary and Conclusion: Pharmacoeconomic modelling can simulate budget burden and its future dynamics on the individual and national level in various economic situations. The results of such modelling could be of value in decision-making process for the national guidelines development.
Reference: [1]Shuvaev V.A. *et al.* ELN Information letter October 2013. – p.14.

P1283

EXAMINATION OF REAL WORLD INDUCTION THERAPY AMONG OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA IN THE UNITED STATES
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Background: Acute myeloid leukemia (AML) is the most common form of acute leukemia in the US, and incidence of the disease increases over age 65. While US guidelines recommend specific treatment options for older patients, including cytarabine-based therapy, hypomethylating agents, clinical trials or supportive care, information on real-world treatment patterns is limited.
Aims: The objective of this study was to examine real-world data on induction anti-cancer therapy received among older AML patients (≥ 65 years old).
Methods: Commercial and Medicare Advantage enrollees with a diagnosis of AML (≥1 medical claim with ICD-9-CM code of 205.0x) between 1/2007 and 7/2013, were identified from a large, national US health plan claims database. The date of the first claim with an AML diagnosis was the index date. Patients were required to be continuously enrolled in the health plan for 6 mos prior to the index date (baseline period) and at least 3 mos after the index date until the earliest of death, disenrollment from the health plan or the end of the study period on 31 July 2013. Patients were excluded from analysis if they had a claim for remission or relapse (ICD-9-CM 205.01 or 205.02) on the index date, or had evidence of AML or other cancer during the baseline period. Patients ≥65 years old who initiated therapy within 3 mos of the index date were examined. The first anti-cancer systemic therapy observed was considered the start of induction therapy and the regimen included all agents received in the 10 days following therapy initiation. Evidence of hematopoietic stem cell transplant (HSCT) and radiation therapy was assessed.
Results: A total of 712 newly diagnosed AML patients were identified; 45% (n=321) were ≥65 years old. Among the older patients, 57% (n=182) were male, 78% (n=251) were Medicare Advantage enrollees, mean (SD) baseline Charlson Comorbidity Index (CCI) was 1.2 (1.5) and mean (SD) follow-up was 371 (333) days. Overall, 16 patients (5%) received radiotherapy and 17 patients (5%) had a HSCT during the follow-up period. A total 157 (49%) patients had evidence of initiating anti-cancer systemic therapy. Among these 157 patients, mean (SD) age was 74 (6) years, 59% were male, mean (SD) baseline CCI was 1.0 (1.4), 13 (8%) received HSCT and 4% (n=7) received initial therapy during an inpatient stay. The most common agents were azacitidine (58%, n=91), decitabine (39%, n=61) and cytarabine (11%, n=18). Monotherapy with azacitidine (n=83, 53%) or decitabine (n=50, 32%) were the most common initial therapy. Patients who initiated therapy with azacitidine (age 76 [SD 6]) or decitabine (age 74 [SD 6]) were older than those who initiated cytarabine-based therapy (age 69 [SD 5]). Time to initial therapy was a mean of 66 days (median 25).
Summary and Conclusion: A substantial portion of the studied population appears not to have received induction therapy for AML. This is similar to the results of a previously published study (Oran et al, Haematologica 2012) which reported only 38.6% of Medicare patients were treated within 3 months of diagnosis. Among treated patients, azacitidine and decitabine were more widely used than cytarabine-based therapy, and time to initiation of any therapy was longer than expected. An important limitation of this data is an inability to capture therapy delivered as part of a clinical trial.

P1284

REAL-WORLD SAFETY DATA FOR TBO-FILGRASTIM
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Background: Medical products registered in the European Union (EU) are subject to Periodic Safety Update Reports (PSURs), which provide comprehensive worldwide safety data assessments and benefit-risk analyses.
Aims: The present PSUR for tbo-filgrastim-containing products within the TEVA group analyzed safety data accumulated between April 1, 2012 and March 31, 2013 in 38 countries.
Methods: Cumulative data were collected from clinical trials and post-marketing sources from September 15, 2008 (initial approval) to the end of the current PSUR period, March 31, 2013. Data were collected from spontaneous reports from healthcare professionals, consumers, scientific literature, competent authorities, and solicited case reports. Adverse reactions were categorized by system organ class (SOC), source, and seriousness. Teva pharmacovigilance requires screening of all adverse reaction reports and antibody assessment for cases of suspected immunogenicity.
Results: From September 15, 2008 (initial approval) to the end of the current PSUR period, March 31, 2013, the estimated cumulative exposure to products containing tbo-filgrastim was ~4,474,929 patient-days. Estimated cumulative exposure to tbo-filgrastim in clinical trials in healthy subjects and patients with cancer was 190 and 22,099 patient-days, respectively. The global safety database processed 254 tbo-filgrastim case reports from initial product approval through March 31, 2013 (~4.5 years), including 61 (24%) from the PSUR period. Post-marketing data sources cumulatively reported 461 adverse reactions, 131 (28%) from the PSUR period. The most commonly occurring preferred terms in association with important identified and potential risks over the cumulative period were allergic type reactions (11), interstitial pneumonia (4), and splenomegaly (2). Eleven new cases were identified that required immunogenicity testing during the PSUR period. The most common reasons for requiring testing were lack of efficacy (n=7) and type II hypersensitivity (n=2).
Summary and Conclusion: No new safety risks were identified for tbo-filgrastim based on PSUR data from the most recent reporting period ending March 31, 2013.

SIMULTANEOUS SESSIONS III

Myeloma and other monoclonal gammopathies - Clinical 2

S1285

PROGNOSTIC VALUE OF DEEP SEQUENCING APPROACH FOR MINIMAL RESIDUAL DISEASE (MRD) DETECTION IN MULTIPLE MYELOMA PATIENTS

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Background: The assessment of minimal residual disease (MRD) is an emerging component of response evaluation in multiple myeloma (MM) patients.
Aims: We compared the prognostic value of traditional response criteria with MRD measured by three different methods: a sequencing-based method, termed the LymphoSIGHT™ platform, multiparameter flow cytometry (MFC) and ASO-PCR of immunoglobulin (Ig) genes in a cohort of 133 uniformly-treated MM patients from the Spanish Myeloma Group trials.
Methods: Bone marrow samples were obtained from 133 patients at diagnostic and post-treatment time points on GEM clinical trials (GEM00 and GEM05). All 133 patients were either in CR or VGPR at the post-treatment time point. Using the sequencing assay, we identified clonal rearrangements of immunoglobulin (*IGH-VDJ, IGH-DJ*, and *IGK*) genes in diagnostic samples. We then assessed MRD in follow-up samples, analyzing concordance between: sequencing, MFC and ASO-PCR of Ig genes MRD results, and comparing the prognostic value of each method with traditional response criteria.
Results: The sequencing assay detected the presence of a myeloma-specific gene rearrangement in diagnostic samples from 121 of 133 (91%) patients. We tested MRD in follow-up time points in 110 of the 121 patients. Of the 110 patients, 80 were positive by sequencing at MRD levels of 10^{-5} or higher and 30 were MRD negative. A high correlation between MFC and sequencing MRD results was observed (r^2=0.87); as well as between ASO-PCR of Ig genes and sequencing (r^2=0.89). The Time to Tumor Progression (TTP) and Overall Survival (OS) were significantly longer in the MRD negative group compared with the MRD positive group by sequencing (TTP, median 80 *vs*. 31 months, p<0.0001; and OS, median not reached *vs*. 81 months p=0.02). When restricting the analysis to the 62 patients that were in conventional CR (negative immunofixation), 36 of 62 patients were positive by sequencing at MRD levels at 10^{-5} and higher and 26 were MRD negative. There was a significantly improved TTP in the MRD negative group compared with the MRD positive group (median 131 *vs*. 35 months, p=0.0009). Patients were grouped into 3 categories according to their MRD levels by sequencing: i) ≥10^{-3} (n=43), ii) 10^{-3} to 10^{-5} (n=37) and iii)<10^{-5} (n=30). The median TTP were: 27 months, 48 months, and 80 months, respectively (*P* from 0.003 to 0.0001) (Figure 1). This sensitivity analysis was extended to assess OS across the 3 categories of MRD levels. Similar to the TTP analysis, MRD levels of<10^{-5} were associated with significantly longer OS compared to patients with high MRD level (defined as >10^{-3}) (median not reached *vs*. 55 months, *P*=0.002). Similar results were found when comparing patients with MRD levels of 10^{-3} to 10^{-5} to patients with high MRD level (>10^{-3}) (median not reach *vs*. 55 months, *P*=0.02).
Figure 1. Time to Progression of the series stratified according to different minimal residual disease (MRD) levels (>10^{-3} *vs*. 10^{-3} to 10^{-5} *vs*.<10^{-5}) as determined by deep sequencing.

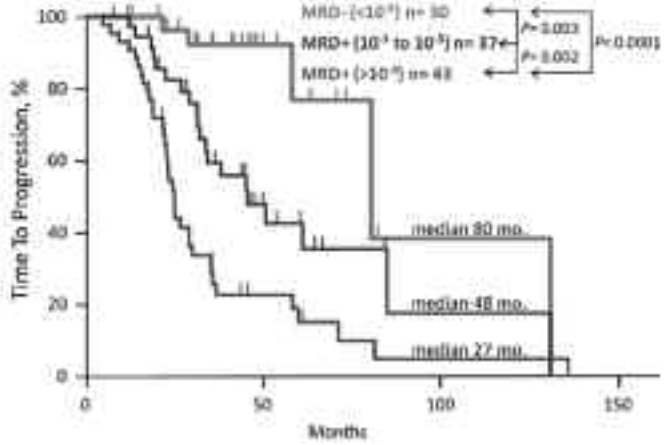

Figure 1.

Summary and Conclusion: The sequencing applicability for MRD studies in MM is 91%, which is essentially equivalent with that of MFC (96% Rawstron AC et al, JCO 2013). There is a high correlation between MRD levels by sequencing, ASO-PCR and MFC. These results demonstrate that MRD assessment by sequencing is highly prognostic even in patients who are in CR. Thus, MRD assessment by sequencing is a useful method for patient risk stratification and can be used to determine molecular CR in MM.

S1286

PROTEASOME INHIBITOR TREATMENT RESPONSE CAN BE PREDICTED BY GENE EXPRESSION PROFILING IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a heterogeneous disease in terms of genetic background, survival and treatment response for which more and more 'novel agents' become available. However, there is a large diversity between subgroups of patients in treatment response and survival. This signifies an ever increasing need for predictive markers for MM that may allow identification of such subgroups at time of diagnosis. Gene Expression Profiling (GEP) studies have resulted in several markers, such as the EMC92/SKY92 high risk signature, virtual karyotyping (t(4;14), t(11;14), etc.), and the GEP clusters (MS, MF, etc.).
Aims: To identify predictive GEP based markers capable of distinguishing patients that benefit from proteasome inhibitor based treatment.
Methods: The data from the HOVON-65/GMMG-HD4 phase 3 trial was used, in which bortezomib, doxorubicin, and dexamethasone (PAD), followed by high dose melphalan (HDM)/autologous stem cell transplantation (ASCT) and bortezomib maintenance was compared with vincristine, doxorubicin, and dexamethasone (VAD) followed by HDM/ASCT and thalidomide maintenance. GEP was performed (Affymetrix U133 Plus 2.0 GeneChip; algorithms: MMprofiler assay) for 329 MM patients. A subset of this dataset was previously used as a training set for the EMC92/SKY92 signature (290 patients), for the signatures of the 12 clusters (320 patients), as well as for the signatures for chromosomal aberrations (virtual karyotyping of t(4;14), t(11;14), t(14;16)/t(14;20), add1q, add9q. del13, and del17, number of patients depending on the FISH). The predictive power for Overall Survival (OS) of those markers in relation to the PAD/VAD treatment arms was assessed. For each marker, a Cox Proportional Hazards model was fit on the patients that are positive for that marker, with samples split into PAD/VAD as covariate. A Hazard Ratio (HR) larger than 1 indicates that PAD patients had longer OS than those treated with VAD (uncorrected p values also reported).
Results: The predictive power of the EMC92/SKY92 signature, markers for the various chromosomal aberrations and GEP clusters was assessed by comparing VAD with PAD. Five markers, EMC92/SKY92, t(14;16)/t(14;20), add1q21, MF and CD2 cluster, were found to have longer OS when treated with PAD (HR ranging from 2.2 to 12.9, p<0.05, Figure 1A). t(4;14) shows improved survival when treated with PAD (HR=1.8), but is not significant (p=0.15). These markers capture different biological mechanisms, and therefore different sets of patients. For example, the EMC92/SKY92 (23.1% of patients) and cluster MF (6.4% of patients) together identify 26.1% of patients with an HR of 2.7 (Figure 1A and B). These results indicate that GEP markers may provide a good predictive marker for proteasome inhibition therapy.

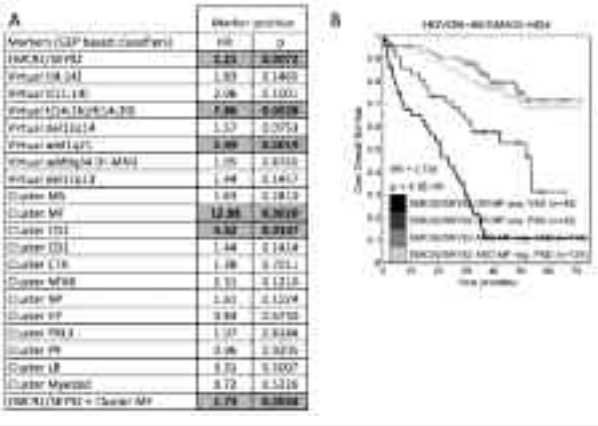

Figure 1. A) Predictive hazards ratios of all MMprofiler markers and one marker combination (VAD *versus* PAD treatments arms). Bold text indicates p<0.05. B) Kaplan-Meier curve for the combination of EMC92/SKY92 and MF cluster split out for the two treatment arms. The Hazard Ratio compares the treatment arms within the "EMC92/SKY92 positive OR MF cluster positive" group.

Summary and Conclusion: Five GEP markers have been found that can predict longer OS in subsets of MM patients when treated with proteasome inhibitors (PAD/Bortezomib), suggesting that they may serve as a predictive marker. The predictive power of these markers must be validated in the EMN-02/HOVON-95 clinical trial which will include a bortezomib based induction regimen, and consolidation regimen.

S1287

PROGNOSTIC HETEROGENEITY AMONG HIGH RISK MULTIPLE MYELOMA PATIENTS (T(4-14) AND DEL17P) ACCORDING TO ADDITIONAL CYTOGENETIC ABNORMALITIES, THE IFM EXPERIENCE

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Background: Molecular heterogeneity is a hallmark of multiple myeloma (MM) disease. Recent works using genome sequencing reveal a new dimension on the knowledge and complexity of MM physiopathology. However there's not clear impact on patient management especially the evaluation of individual risk remains a challenge. Usually t(4-14) and del17p are considered as bad prognosis cytogenetics abnormalities (CA) and hyperdiploidy as good prognostic. However situation is increasingly more complex and prognostic of patient should be more tailored using whole acquired cytogenetic characteristics.
Aims: To illustrate this we analyzed by SNP array for 242 high risk patients (t(4-14) and/or de(l17p)) from the IFM data base to evaluate the prognostic impact of additionally CA detected.
Methods: We analyzed 242 high risk MM patients (147 with del17p, 110 with t(4-14) and 25 with both). All patients gave their informed consent. SNP array were performed on affymetrix platform (Santa Clara CA). Data were analysed in univariate and multivariate analysis in a cox model adjusted on age, treatment, beta2microglobulin (b2m) and del(13). There was in the t(4-14) group: 147 patients, median age 59.4 years, 129 (82.2%) treated with autologous stem cell transplantation (ASCT), 144 (92.3%) relapses and 103 deaths (65.6%). In the del17p group there was 110 patients, median age 60 years, 83.7% treated with ASCT, 90.8% have relapsed and 83 (76.1%) deaths. Repartition of CA in both groups will be provided during EHA meeting. Main additionnally analysed CA were hyperdiploidy, structural chromosomal changes, del(13), del (1p12), del(1p32), 1q gain, 6p gain, del(6q), del(8p), del(12p), del(14q), monosomy14, dl16q, del(22), nullisomy Y.
Results: t(4-14) group: Median follow up was 4.6 years, progression free survival (PFS) was 1.4 years and overall survival (OS) was 3.5 years. In the multivariate analyses, PFS was shorter in patients with del(6q) and low b2m (HR=3.14), but longer in patients with monosomy 14 (HR=0.49). For OS, the significant factors were molecular karyotype complexity, del(13) (HR=3.25), del(1p32) (HR=6.31), and del(12p) (HR=1.74). Hyperdiploidy or individual trisomies did not impact neither the PFS, nor the OS. Thus, in this large series of patients with t(4;14), we were not able to identify "good-risk" parameters. In contrast, several chromosomal changes are associated with a worst prognosis. Del17p group: Median follow up was 5.2 years, PFS 1.3 years and OS 2.7 years. For OS, univariate analyses identified the following most significant parameters: del(13) (HR=2.11), del(1p32 (HR=1.95), del(8p) (HR=1.62), del(16q) (HR=1.79), nullisomy Y (HR=2.09), and at least one trisomy (HR=0.57. In the multivariate analyses, PFS was shorter in patients with del(6q) (HR=2.14), and longer for patients with monosomy 14 (HR=0.42). For OS, no chromosomal factor significantly impact survival (despite some trends) in the multivariate analyses certainly due to the small size of the cohort.

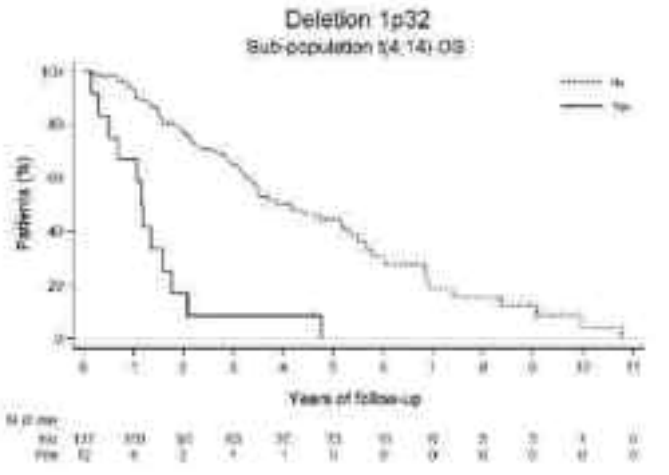

Figure 1.

Summary and Conclusion: this study in a large series of high-risk patients, analyzed with the most modern genomic technique, does not confirm the Mayo Clinic data which suggested that trisomies/hyperdiploidy may overcome the poor prognosis of t(4;14) and del(17p). In contrast, we did identify CA that worsen the prognosis of t(4;14) like del1p32, del12p, molecular karyotype complexity, or del(13). However as some abnormalities are not frequent those data have to be confirmed in a larger cohort of patients.

S1288

PET/CT IS A USEFUL TOOL FOR BOTH REFINING THE DEFINITION OF CR IN MM AND DETECTING OTHERWISE UNREVEALED PROGRESSION DURING THE FOLLOW-UP OF THE DISEASE: A SINGLE CENTRE EXPERIENCE ON 282 PATIENTS(PTS)

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Background: PET/CT is a valuable tool at the onset of MM for predicting outcomes in transplant (ASCT) candidates pts. However, the prognostic role of PET/CT after treatment and/or during the follow up of the disease, as well as in the non ASCT setting, still remains less defined.
Aims: To address these issues, we retrospectively analyzed 282 symptomatic MM pts, with a median age of 58 yo, (range 22-83), who were treated in a single institution between 2002 and 2012, and were followed for a median of 66 months. Treatment included ASCT in 70%, novel agents in 77% and was bortezomib-based in 37% of the cases.
Methods: All the pts were studied with PET/CT at baseline, then every 12-18 months during follow-up, and at the time of each subsequent relapse; for 189 of them PET/CT at baseline and 3 months after the end of first line treatment was available. The number of focal lesions (FLs), their associated standardized uptake value (SUVmax) and presence of extra-medullary disease (EMD) were recorded.
Results: 42% of the pts at diagnosis had >3 FLs and in 50% of them SUVmax was >4.2; EMD was present in 5% of the cases. On multivariate analysis, these 3 variables adversely affected PFS and OS, independently of the treatment received (including or not ASCT, bortezomib- or non-bortezomib-based). On multivariate analysis, ISS stage 3, presence of >3 FLs at PET/CT and failure to achieve CR during or after first line treatment were the leading factors independently associated with shorter PFS and OS. These 3 variables enabled the definition of a scoring system, based on the number of risk factors simultaneously present (score O: none of the 3 adverse factors, 31% of the pts; score 1: only one out of the 3, 37%, score 2+: 2 or 3 factors, whatever of them, 32%), that predicted for PFS and OS. After treatment, PET/CT negativity (PET-CR) was observed in 70% of the pts, while conventionally-defined CR was achieved in 53% of them. Attainment of PET-CR favorably influenced PFS and OS, both in uni and multivariate analyses. Notably, 29% of the pts who achieved CR according to conventional criteria still had positive PET/CT scans: their median PFS was 50 mos as compared with 90 mos for those pts who also achieved PET-CR (P=0.01) (fig. 1). OS was significantly inferior, as well, for pts not achieving PET-CR, with 6-year estimate of 65% in comparison to 90% for PET negative pts (P=0.0035) (fig. 1). On multivariate analysis, PET-CR was an independent factor predicting for prolonged PFS (P=0.004) and OS (P=0.02) within the conventionally-defined CR group. 63% of the pts experienced relapse or progression, after a median of 56 mos from the end of first-line treatment. In 37% of them, progression was only serological, both serological and skeletal

in 48%, only skeletal in 15% and in 12% of these latter patients it was exclusively detected by systematic PET/CT during the follow-up (no pain or pathological fractures). A logistic regression analysis of baseline and post treatment features revealed that persistence of SUVmax >4.2 after the end of first line treatment was independently associated with exclusive PET/CT progression.

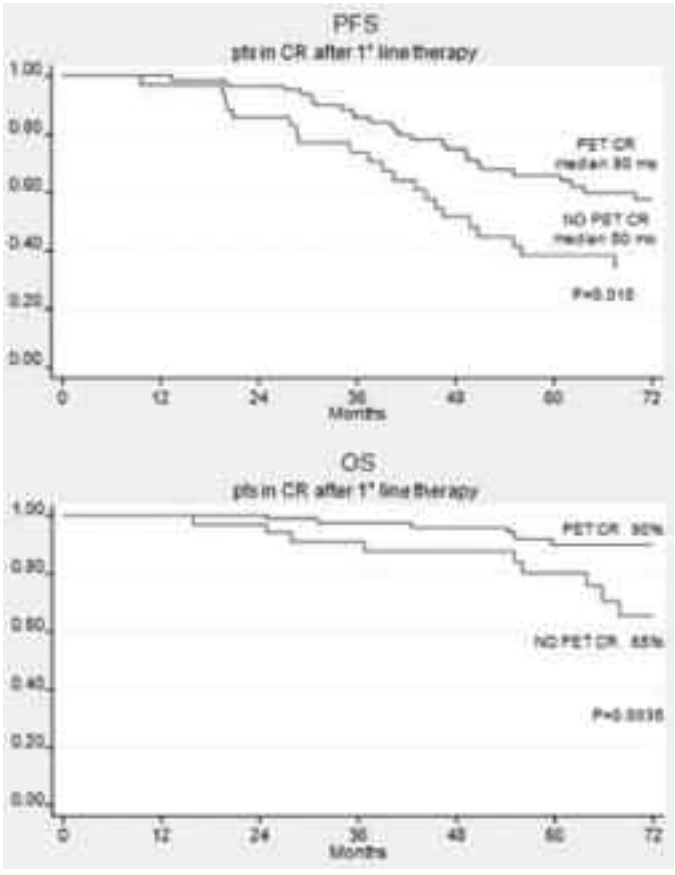

Figure 1.

Summary and Conclusion: In conclusion, PET/CT was confirmed as a reliable predictor of outcome in newly diagnosed MM pts. Importantly, PET/CT contributed to a more careful and deep evaluation of CR, going beyond the conventionally defined level. Finally, in pts with a persistent high glucose metabolism after first line treatment, PET/CT can be recommended during the follow-up, in order to point out possible progression, not otherwise identifiable.

S1289

REVISED-INTERNATIONAL STAGING SYSTEM (R-ISS): A NEW AND SIMPLE PROGNOSTIC ASSESSMENT FOR MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a heterogeneous disease and the thought that there are different diseases with variable outcome led to a definition of prognostic tools. The International Staging System (ISS) is a simple and widely used staging model, but it does not take into account the biology of MM (such as the presence of chromosomal abnormalities) and other important prognostic markers such serum LDH levels.

Aims: To evaluate the combined role of three established prognostic factors (ISS, chromosomal abnormalities and serum LDH levels) in newly diagnosed Multiple Myeloma (NDMM) patients.

Methods: Data from NDMM patients enrolled in nine international multicenter trials were pooled together and retrospectively analyzed. All patients received new drugs up-front in association with standard chemotherapy or in pre-transplant induction and post-transplant maintenance strategies. Patients evaluable for ISS stage, interphase Fluorescence *in situ* Hybridization (iFISH) and serum LDH levels were included in the analysis. The K-adaptive partitioning was used to identify the ISS/iFISH/LDH groups. Multivariate analyses of OS and PFS were performed.

Results: Median age was 63 years; 2159 (60%) patients were younger than 65 years. 1791 (50%) patients received IMIDs, 1370 (38%) proteasome inhibitors and 1606 (45%) underwent autologous stem cell transplantation (ASCT). A total of 2359 patients were evaluable for ISS, iFISH and LDH: 570 (24%) patients presented at baseline with ISS stage III, 612 (26%) had High-Risk (HR) iFISH profile [presence of del(17p) and/or t(4;14) and/or t(14;16)] and 285 (12%) had LDH values higher than the normal limit (LDH high). The recursive partitioning procedure gave an optimal number of 3 ISS/iFISH/LDH groups, defined as revised ISS [R-ISS] stages: R-ISS stage 1 [n=636 (27%)] included patients with ISS I, no HR-iFISH and low LDH; R-ISS stage III [n=246 (11%)] patients with ISS III and HR-iFISH or LDH high; R-ISS stage II included all the other patients [n=1477 (62%)]. After a median follow-up of 4 years, 5-year OS was 81% in the R-ISS I, 62% in the R-ISS II and 39% in the R-ISS III groups. The 5-year PFS was 56% in the R-ISS II, 37% in the R-ISS II and 20% in the R-ISS III groups (median PFS was 65, 41 and 25 months, respectively). Multivariate analyses of OS and PFS confirmed the strong prognostic role of R-ISS. The mortality risk was clearly increased for R-ISS II *vs* I (HR 3.02), as well as for R-ISS III *vs* I (HR 7.44). Similarly, the risk of progression was higher for R-ISS II *vs* I (HR 2.07), as well as for R-ISS Stage III *vs* I (HR 4.04).

Summary and Conclusion: The R-ISS considerably improved the risk assessment in comparison with the individual ISS, iFISH and LDH evaluations. The R-ISS is a new risk model, that includes simple and widely used prognostic markers, it is easily applicable in clinical practice and identifies three different MM entities with clearly different outcomes.

Hodgkin lymphoma - Clinical

S1290

IMPACT OF BLEOMYCIN AND DACARBAZINE WITHIN THE ABVD REGIMEN IN THE TREATMENT OF EARLY-STAGE FAVORABLE HODGKIN LYMPHOMA: FINAL RESULTS OF THE GHSG HD13 TRIAL

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Background: Combined modality treatment consisting of two cycles of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) followed by involved-field radiotherapy (IFRT) is regarded as standard of care for early-stage favorable Hodgkin Lymphoma (HL).
Aims: To analyze the impact of bleomycin and dacarbazine within the ABVD regimen.
Methods: The GHSG HD13 study compared two cycles of ABVD with a dacarbazine-deleted variant (ABV), a bleomycin-deleted variant (AVD), and a variant in which both, dacarbazine and bleomycin were deleted (AV). In each treatment arm, chemotherapy was followed by IFRT of 30 Gy. Primary objective was to demonstrate non-inferiority of the three experimental variants compared to ABVD regarding the primary endpoint freedom from treatment failure (FFTF) by excluding a difference of 6% after 5 years, corresponding to a non-inferiority margin of 1.72 for the Hazard Ratio, via a 95% confidence interval (CI). Recruitment started in 01/2003. In our continuously performed safety analyses, higher event rates were observed with AV and ABV. Thus, these two arms were closed early in 09/2005 and 02/2006, respectively. Randomization between ABVD and AVD continued until 09/2009 with a total of 1710 patients. Two hundred eight patients were excluded from the final analysis due to revision of HL diagnosis, loss to follow-up before start of treatment, revision of staging, or violation of other inclusion criteria. Of 1502 qualified patients analyzed for therapy adherence, toxicity, and efficacy, 566 were randomized into the standard arm and 198, 571, and 167 patients were randomized to receive experimental chemotherapy with ABV, AVD, or AV, respectively.
Results: Patient characteristics were well balanced between the four treatment arms: median age was 39 years, 67% had stage II disease, and there were more male patients included (60%). The most frequent histologic subtypes were mixed cellularity and nodular sclerosis (40% and 37%, respectively). The rate of acute toxicities ranged between 26.3% with AVD and 32.7% with ABVD; leukopenia (14.4%), hair loss (10.9%), and nausea/vomiting (5.8%) were most frequently observed. Interestingly, pulmonary toxicity was observed in four patients only, including one patient receiving AV. Only moderate reduction in acute toxicities was observed when omitting Bleomycin (leukopenia) and Dacarbazine (nausea/vomiting). FFTF at five years was 93.1%, 81.4%, 89.2%, and 77.1% after treatment with ABVD, ABV, AVD, and AV, respectively. Inferiority of the early closed treatment arms without dacarbazine was confirmed in this final analysis with five-year-differences in FFTF of 11.5% (95% - CI 4.7% to 18.3%) with ABV and 15.2% (95% - CI 7.4% to 23.0%) with AV compared to ABVD, respectively. In addition, non-inferiority of AVD compared to ABVD could not be confirmed, with a five-year-difference in FFTF of 3.9% (95% - CI 0.1% to 7.7%). The respective Hazard Ratio was 1.5 with a 95% - CI ranging from 1.0 to 2.3, including the pre-specified non-inferiority margin. Overall survival was excellent and did not differ between treatment arms, with five-year-estimates of 97.6%, 94.1%, 97.6%, and 98.1% after treatment with ABVD, ABV, AVD, and AV, respectively.
Summary and Conclusion: Dacarbazine cannot be deleted from the ABVD regimen without a significant loss of efficacy. With respect to the predefined non-inferiority margin of 6% after 5 years, also Bleomycin cannot be safely omitted. Importantly, the reduction in tumor control in the experimental arms did not translate into inferior overall survival.

S1291

FINAL ANALYSIS OF A RANDOMIZED PHASE II STUDY WITH PREDNISONE, VINBLASTINE, DOXORUBICIN, AND GEMCITABINE IN PATIENTS WITH EARLY UNFAVORABLE HODGKIN LYMPHOMA -PVAG-14 PILOT-

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Background: Optimal treatment of early unfavorable Hodgkin lymphoma (HL) is still a matter of debate, since improved tumor control in terms of longer progression free survival (PFS) has to be weighed against possible adverse effects of the most effective treatment. With PVAG-14, the German Hodgkin Study Group (GHSG) designed a new chemotherapy regimen that included gemcitabine which is an effective drug in relapsed or refractory HL patients.
Aims: The aims of this phase-II trial were to assess toxicity and activity of PVAG-14 (benchmarks:<50% hematological grade III/IV toxicities; >50% complete responders). Secondary objectives were feasibility, efficacy and safety.
Methods: Patients with first diagnosis of early unfavorable HL according to GHSG criteria were randomized to receive eight cycles of prednisone, vinblastine, doxorubicin and gemcitabine with two different doses of doxorubicin (25 or 35 mg/m2) followed by 30 Gy involved-field radiotherapy. Between November 2008 and May 2011, 41 patients were included and the trial was prematurely closed due to poor recruitment and the initiation of HD17 trial. The patients' age at initial diagnosis ranged from 18 to 57 years (median 38 years), and 49% were male. Most of the patients were in stage IIA (76%) and had a WHO-Index of 0 (76%). Large mediastinal mass (LMM) was present in 20% of the patients.
Results: Most patients (40/41) received eight cycles of PVAG-14 with a median dose intensity of 97%, and 27 of these patients were treated with G-CSF in each cycle according to protocol. One patient switched to ABVD after three courses of PVAG-14. Grade III/IV leukopenia occurred in 4/41 patients (<10%) including one patient with grade IV leukopenia. No treatment-associated anemia or thrombocytopenia was documented. The upper 95%>confidence limit of this toxicity rate is estimated at 23%, which is markedly lower than anticipated in the trial protocol. Three patients had grade III infections (one in cycle 5, two in cycle 8), no grade IV infection was observed. All in all, 37% of the patients experienced a grade III/IV toxicity (haematological and non-haematological). Only one patient received a red blood cell transfusion, platelet transfusion was not necessary in any patient. All patients except one (98%) achieved a complete remission (CR or CRu) as final treatment result. The lower 95%>confidence limit of the complete response rate is 87% and excludes the benchmark for inefficacy defined in the protocol. One patient had progressive disease at 5 months, another patient relapsed 15 months after the end of treatment. With a median observation time of 27 months, the 2-year PFS was 94% [95%>CI: 86%>100%] and the overall survival 100%. Due to the low number of patients a comparison of the different doxorubicin doses was not possible.
Summary and Conclusion: Both endpoints for efficacy and toxicity as predefined in the protocol were met despite the premature closure of the trial. Although fewer patients than expected were included in this trial, the results are compelling. PVAG-14 is an efficient and well tolerated regimen for patients with early unfavorable HL. The observed toxicity is remarkably low. Based on these results, PVAG-14 might challenge present treatments of choice in this group of patients. A randomized trial is thus warranted.

S1292

TARGETED BEACOPP VARIANTS IN PATIENTS WITH NEWLY DIAGNOSED ADVANCED STAGE CLASSICAL HODGKIN LYMPHOMA: INTERIM RESULTS OF A RANDOMIZED PHASE II STUDY

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Background: $BEACOPP_{escalated}$ (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) has substantially

improved the outcome for patients with advanced stage Hodgkin Lymphoma (HL). However, there still is concern about the toxicities of this intensified chemotherapy regimen. Brentuximab vedotin (BV) is an anti-CD30 directed antibody-drug conjugate that has shown very promising single-agent activity and good tolerability in HL.

Aims: We introduced BV into the BEACOPP regimen in order to improve its toxicity profile of $BEACOPP_{escalated}$ while maintaining its efficacy.

Methods: Two modified BEACOPP regimens were developed. In a more conservative variant (BrECAPP: BV, etoposide, cyclophosphamide, doxorubicin, procarbazine, prednisone), vincristine was replaced by BV and bleomycin omitted. In the more experimental variant (BrECADD: BV, etoposide, cyclophosphamide, doxorubicin, dacarbazine, dexamethasone) we additionally replaced procarbazine by dacarbazine to reduce gonadal toxicity, reduced the dose of etoposide to decrease risk of second acute myeloid leukemia while slightly increasing the dose of doxorubicin to maintain efficacy, and introduced dexamethasone (day 1-4) substituting for prednisone (day 1-14) to avoid immunosuppressive steroid treatment during neutropenia. Both regimens are administered q21d for 6 cycles. This is an ongoing randomized phase II study with the combined primary endpoint being the PET-based complete response rate after chemotherapy and the complete remission rate at final restaging including early follow-up. Here we report on the first 48 patients (planned n=100).

Results: The study started in October 2012. 75 patients have been enrolled by December 2013 and are included in this analysis. Median age is 30 years (range 18-60 years), 57% are male, and 80% have Ann-Arbor stage III or IV disease. 46 patients have been staged after 6 cycles of chemotherapy, 24 in the BrECAPP and 22 in the BrECADD arm. Four of these patients have not reached a complete response (9%, 95% CI: 2%>21%). 41 patients (91%, 95% CI: 83%>99%) achieved a CR or PET negative PR. 65 patients have received at least two cycles of chemotherapy and are evaluable for safety analyses. Hematological toxicity grade 3 or 4 of the targeted BEACOPP variants was documented in 60 of 65 patients (93%, corresponding number for 4-6 cycles of $BEACOPP_{escalated}$ in the GHSG HD15 study: 91.7%). Grade 3 or 4 organ toxicity occurred in 4/31 patients treated with BrECAPP (13%, GHSG HD15 study: 14.1%), and in 0/34 patients after treatment with BrECADD. 13/46 patients who had completed treatment showed grade 1 or 2 sensory neurotoxicity (28%). No severe neurotoxicity was reported.

Summary and Conclusion: This is the largest study of BV in combination with chemotherapy in the first line treatment of HL reported so far. Both anti-CD30 targeted BEACOPP variants are well feasible without compromising the efficacy associated with $BEACOPP_{escalated}$. Enrollment will be finished by March 2014 (at end of February 97 of 100 patients have been enrolled) and updated results will be presented.

S1293

BRENTUXIMAB VEDOTIN AS SINGLE AGENT IN REFRACTORY OR RELAPSED CD30-POSITIVE HODGKIN LYMPHOMA: THE FRENCH NAME PATIENT PROGRAM EXPERIENCE IN 241 PATIENTS

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Background: Brentuximab vedotin (BV), comprising an anti-CD30 antibody conjugated to the potent anti-microtubule agent, monomethyl auristatin E, was reported effective and safe in several refractory or relapsed CD30-positive Hodgkin's Lymphoma (HL) cohorts including 12 to 102 patients (*Younes A, JCO 2012*).

Aims: Here is presented the French experience of BV used as single agent in refractory or relapsed CD30+ HL patients enrolled in the name patient program (NPP).

Methods: 241 patients (median age at 34 years [range 17-79]) treated between January 2011 and January 2014 were retrospectively analysed using specific files. All patients had histological documented CD30+ HL and 59% have an Ann Arbor stage III-IV disease at initial diagnosis. All patients received first line chemotherapy (CT)(ABVD: 83% or BEACOPP: 12%) and 74% were primary refractory (no response to CT or response duration<12 months). After a median of 3 lines of CT [range 1-13], BV was administrated by intravenous infusion at 1.8 mg/kg every 3 weeks. BV response assessment was determined according to Cheson 1999 criteria for 22% of patients and to Cheson revised criteria for 78%. The primary endpoint was the best response after BV treatment. Response at the end of BV treatment, duration of response, disease-free survival (DFS), progression-free survival (PFS), overall survival (OS) and toxicity profile were secondary endpoints.

Results: At initiation of BV, 77% of patients had a stage III-IV disease and performance status was less than 2 in 81% of patients. 8% of them had a preexistant peripheral neuropathy. The median time since diagnosis was 31 months [range 3-335] and median time since last line of treatment 2 months [range 0.1-132] with 47% of patients progressive to the last CT. 61% of patients had received at least one transplant (including 36% who received two transplants), allogeneic transplantation was performed in 16% of patients and 45% relapsed less than 6 months after transplant. Patients received a median of 6 cycles of BV [range 1-16]; 17% of them needed at least one dose adaptation and 7% had a CT with BV. At the analysis date, 222 patients (92%) discontinued BV treatment, mostly because of progression (54%), but also to perform a transplant (25%) and 5% for adverse event. Among the 241 patients, 70 received a consolidation treatment after BV: radiotherapy in 11 cases, autologous transplant in 29 cases, allogeneic regimen in 27 cases. The primary endpoint, best response was observed after a median of 4 cycles in 223 patients: CR/uCR in 32% of patients, PR in 26% (ORR 58%, 43% according to Cheson 1999 and 68% according to Cheson 2007). At the end of treatment, CR/uCR rate was 23%, and PR rate was 9% (ORR 32%). Median duration of response was 8 months [95% CI 6-14] in the 140 patients in CR/uCR/PR; median DFS of 19 months [95% CI 13-NR]. With a median follow-up of 16 months, median PFS was 7 months [95% CI 6-8] and median OS was not reached (estimated 1 yr-OS at 76%, 2 yr-OS at 58%). Among the 75 patients who died, most common causes of death were lymphoma progression (68%), concurrent illness (9%), toxicity of additional treatment (5%). No deaths have been linked to BV toxicity. The most common treatment-related adverse events were peripheral sensory neuropathy (26% grade 1-2 ; 2% grade 3-4), anemia (39%), thrombocytopenia (27%), neutropenia (23%, with 7% of grade 3-4 infections) and diarrhea (14%).

Summary and Conclusion: This largest retrospective analysis supports the previously reported efficacy of BV in heavily pretreated CD30+ HL patients with manageable toxicity. Due to a short duration of response, the use of autologous or allogeneic transplantation should be considered quickly in responder patients as a consolidation approach to cure the disease.

S1294

BENDAMUSTINE-CONTAINING REGIMEN (BEGEV) EFFICIENTLY MOBILIZES CD34+ HEMATOPOIETIC CELLS IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA

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Background: Bendamustine has demonstrated clinical activity as single agent in a variety of lymphoproliferative disorders, including Hodgkin lymphoma (HL). Despite the wide use of this compound alone or in combination, limited data are so far available to support its capacity to mobilize peripheral blood stem cells (PBSC).

Aims: A phase II open-label prospective study is currently ongoing to evaluate the efficacy of the BeGEV (Bendamustine, Gemcitabine and Vinorelbine) regimen as induction chemotherapy before autologous stem cell transplant (ASCT). One of the study objectives is to investigate the stem cell mobilization activity of this Bendamustine-containing regimen.

Methods: Between August 2011 and January 2014, 40 consecutive patients with relapsed/refractory HL were enrolled in this phase II open-label prospective study with BeGEV followed by ASCT. The treatment schedule was: Bendamustine (90 mg/sqm, days 2-3), Gemcitabine (800 mg/sqm, day 1 and 4) and Vinorelbine (25 mg/sqm, day 1) plus G-CSF 10 µg/Kg beginning on day 7 and continued daily until collection of the target CD34+ cell yield (3 x 10^6 CD34+ cells/kg body weight). PBSC collection was planned after cycle 1 or cycle 3 in case of bone marrow involvement. Readout of PBSC harvesting included the absolute number of collected CD34+ cells/Kg, the number of leukapheresis (LK) per cycle, the value of pre-leukapheresis circulating CD34+ cells/µL, white blood cell (WBC) counts and the day of first collection. Adverse events were also recorded. All patients provided written informed consent at the time of study inclusion.

Results: Of the 40 patients enrolled, 37 underwent PBSC mobilization and harvesting and are evaluable. Two patients stopped treatment due to disease progression, while the other patient is currently receiving the first cycle of therapy. Successful PBSC mobilization was observed in 36 out of 37 evaluable patients who achieved the target yield of 3 x 10^6 CD34+/Kg. The median yield of CD34+ cells/Kg was 8.6 x 10^6 CD34+/Kg (range, 3.6- 56) after a median of 1 procedure (range, 1-2). Median values of pre-collection CD34+ cells/µL and WBC count/µL were 82/µL (range, 22-339) and 24,380/µL (range, 5,400-87,000), respectively. PBSC collection was performed after a median of 12 days (range, 9-15). Twenty-one patients underwent LK at cycle 1, 10 after cycle

2 (due to logistic reasons in 9 patients and Cytomegalovirus reactivation in 1 case), 5 after cycle 3 and one patient collected CD34+ cells after 4th cycle. Grade 1/2 hematologic and non-hematologic side effects were limited and no toxic death occurred. One patient developed grade 1 hypotension during LK, but she was able to complete the procedure. To date, 25 patients (67%) have been autografted, achieving neutrophils and platelets recovery on day 10 (range 9-21) day 12 (range 9-26), respectively.

Summary and Conclusion: This is the first prospective study evaluating the mobilizing activity of a Bendamustine-containing regimen in relapsed/refractory HL patients undergoing ASCT. Our results demonstrate that Bendamustine has neither stem cell toxicity nor detrimental effects on stem cell mobilization, and that BeGEV regimen has a high PBSC mobilizing activity.

Myelodysplastic syndromes - Clinical

S1295

THE ADDITION OF LENALIDOMIDE TO AZACITIDINE IN HIGHER RISK MDS IS DELIVERABLE WITH HIGHER RESPONSE RATES; FIRST ANALYSIS OF THE ALLG MDS4 RANDOMISED PHASE II STUDY

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Background: Azacitidine (AZA) improves OS compared to conventional care regimens in high risk MDS; lower risk MDS obtain clinically relevant responses. Virtually all patients (pts) however ultimately progress with poor prognosis. AZA with lenalidomide (LEN) is a rational combination to investigate for these pts, given the differing mechanisms and established single agent activities. Early phase trials have developed dose and schedule with acceptable toxicity. We present a first analysis of secondary endpoints of the ALLG MDS4 – a randomised phase II study comparing AZA +/- LEN in higher risk MDS and low blast AML.

Aims: 1. assess response rates of AZA and AZA + LEN 2. describe toxicity and deliverability of this combination.

Methods: Thirty centres participated; eligible pts provided informed consent and had low blast, non-proliferative AML, MDS (and RCUD and RARS with at least one clinically significant cytopenia) or non-proliferative CMML, with no prior demethylating agent or IMiD. Pts were stratified according to IPSS, site, diagnosis, and randomised 1:1. Treatment for all pts was AZA 75mg/m^2/d sc 5-2-2 schedule until progression or intolerance; those randomised to combination therapy began LEN at cycle3, 10mg oral D1-21 of each 28d cycle for total 10 cycles with AZA reduced to 5d schedule. Primary endpoint of the study (not analysed to date) is rate of clinical benefit (alive with absence PD) at 12mths. Close-out date for this analysis is Dec31 2013. Responses reported according to IWG criteria and ITT (only 1 pt received no drug).

Table 1.

	AZA (n=77)	AZA+LEN (n=76)
Male	65%	74%
Median age (yrs)	69.1 (42.5-85.9)	71.4 (44.1-87.2)
Median from diagnosis	0.5 (0.1-13.1) yrs	0.4 (0.0-9.4) yrs
ECOG (%)		
0	53%	50%
1-2	47%	50%
Diagnosis		
RARS	8%	4%
RCMD	29%	34%
RAEB-1	14%	13%
RAEB-2	21%	20%
MDS isolated 5q-	3%	1%
AML	10%	14%
CMML	14%	12%
Other	1%	1%
IPSS-R		
Very good	0%	2%
Good	38%	34%
Intermediate	25%	27%
Poor	17%	9%
Very poor	21%	29%
Carrying 5q-	11%	17%
Number cytopenias		
0-1	42%	39%
2-3	58%	61%
Best response		
CR	22%	25%
PR	0	3%
mCR	12%	13%
HI*	18% n=14 patients HI-E n=9 HI-P n=5 HI-N n=5	25% n=19 patients HI-E n=10 HI-P n=12 HI-N n=1
SD	29%	21%
PD	4%	5%
Not evaluable/missing	16%	8%

*Note patients could have HI response in more than one lineage

Results: Between March 2011 and March 2013 160 pts were randomized; for those continuing on study, only pts ≥12 months from study commencement are included (n=153). Median follow up is 11.7mths (0.7-26.7), median number

cycles aza=11 (AZA) v 10 (AZA+LEN); median cycles LEN in combination arm=8. Number AZA cycles dose reduced 2.5% AZA v 2.4% AZA+LEN. A mean of 3.2% per patient LEN cycles were dosed<10mg. See table for baseline data and best responses. ORR (CR to HI) 52% (AZA) v 66% (AZA+LEN) (p=0.08). Median time to first response 2.8mths (AZA) v 2.7mths (AZA+LEN) and to best response 5.5mths (AZA) v 4.7mths (AZA+LEN) (p=0.13). Median PFS 14.4mths (AZA) v 16.4mths (AZA+LEN). Overall rate Gr3+ nonhaem AEs 61% (AZA) v 68% (AZA+LEN); Gr3+ infections 43% (AZA) v 45% (AZA+LEN) including febrile neutropenia 20% both arms. Only other Gr3+ AE >5% pts was raised GGT in AZA+LEN 15%, to be further assessed. Emerging Gr3+ haematologic toxicity: new Hb<80g/L in 41% both AZA and AZA+LEN, neutrophils<1x10^9/L 43% AZA v 49% AZA+LEN, platelets<50x10^9/L in 37% AZA v 40% AZA+LEN. Most haematologic toxicity was seen in the first 2-4 cycles.
Summary and Conclusion: The regimen of concurrent AZA+LEN in pts with higher risk MDS/low blast AML/CMML is deliverable with numerically higher response rates and a trend for shorter time to best response than AZA alone. Toxicity is not excessive, with similar rates of emerging haematologic toxicity and infections. We await main analysis for assessment of primary endpoint of clinical benefit at 12mths treatment and OS.

S1296

ACE-536 INCREASES HEMOGLOBIN LEVELS IN PATIENTS WITH LOW OR INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROMES (MDS): PRELIMINARY RESULTS FROM A PHASE 2 STUDY

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Background: ACE-536 is a recombinant fusion protein containing modified activin receptor type IIB, being developed for the treatment of anemias due to ineffective erythropoiesis, such as myelodysplastic syndromes (MDS). Patients with MDS often have elevated levels of erythropoietin (EPO) and may be non-responsive/refractory to erythropoiesis-stimulating agents (ESAs). ACE-536 binds to ligands in the TGF-ß superfamily and promotes late-stage erythroid differentiation via a mechanism distinct from ESAs. In a healthy volunteer study, ACE-536 was well-tolerated and increased hemoglobin (Hgb) levels (Attie K *et al.*, Am J Hematol 2014). RAP-536 (murine ortholog of ACE-536) increased Hgb levels and decreased bone marrow erythroid hyperplasia in a mouse model of MDS (Suragani R *et al.*, Blood 2012;120:3796).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study to evaluate the effects of ACE-536 on anemia in patients with transfusion-dependent (TD) or non-transfusion dependent (NTD) low or int-1 risk MDS. Study outcomes include erythroid response (either Hgb increase in NTD patients or reduced transfusion burden in TD patients), safety, tolerability, PK, and PD biomarkers.
Methods: Inclusion criteria included low or int-1 risk MDS, age ≥ 18 yr, with anemia defined as either Hgb<10.0 g/dL (NTD, defined as<4 units RBCs/8 wks prior to baseline) or ≥4 units RBCs/8 weeks prior to baseline (TD), EPO >500 U/L or non-responsive/refractory to ESAs, no prior azacitidine or decitabine, and no current treatment with ESA, G-CSF, GM-CSF, or lenalidomide. ACE-536 was administered by subcutaneous (SC) injection once every 3 weeks in sequential cohorts (n=3-6) at dose levels ranging from 0.125 to 1.33 mg/kg for up to 5 doses with a 3-month follow-up. Further possible dose escalation and an expansion cohort (n=30) are planned, contingent on periodic safety data review.
Results: Preliminary data were available for 21 patients (12F/9M, 6 NTD/15 TD) enrolled as of 13Feb2014. Median age was 71 yr (range: 27-88 yr). 48% had prior EPO therapy and 19% had prior lenalidomide. Mean (SD) baseline Hgb for NTD patients (n=6) was 9.0 (0.4) g/dL. Mean (SD) units RBCs transfused in the 8 weeks prior to baseline for TD patients (n=15) was 6.2 (2.5) units. Preliminary efficacy data were available for the 15 patients (5 NTD/10 TD) treated in the first 4 cohorts (0.125, 0.25, 0.5, or 0.75 mg/kg). The 5 NTD patients demonstrated dose-dependent increases in Hgb on treatment, with maximum Hgb increase ranging from 0.8 to 3.3 g/dL. The 3 NTD pts in the 0.75 mg/kg group were either ESA refractory or non-responders and had maximum Hgb increases of 1.6, 1.9, and 3.3 g/dL. One NTD patient in this group had a Hgb increase ≥1.5 g/dL sustained for ~15 weeks. Four of the 10 TD patients had a ≥50% reduction in units transfused during an 8-week interval on treatment compared to the 8 weeks prior to treatment, including 1 pt, previously ESA and lenalidomide non-responsive, who was transfusion-free while on study (~22 weeks). Transient increases in reticulocytes and/or neutrophils were observed in some patients. ACE-536 was generally well tolerated. No related serious AEs have been reported to date. No patients discontinued treatment early due to a related AE.
Summary and Conclusion: Based on preliminary data in low or int-1 MDS patients with high baseline EPO levels or lack of response to ESA treatment, ACE-536 administered SC every 3 weeks increased Hgb levels in NTD patients and decreased transfusion requirement in some TD patients, with a favorable safety profile. These data support further evaluation of ACE-536 in patients with MDS.

S1297

MUTATIONAL ANALYSIS AND LONG TERM OUTCOME IN ADVANCED CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) TREATED BY DECITABINE: AN UPDATE OF THE GFM-CMML-2007 PHASE II TRIAL

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Background: Treatment of advanced CMML remains a challenge with no standard approach except HSCT for a minority of patients. Recently, we and others reported that the hypomethylating agents decitabine (DAC) (Braun et al, Blood 2011) and azacitidine (AZA) (Costa et al, Cancer 2011; Adès et al, Leuk Res, 2013) yield responses ranging from 35 to 45% in this setting.
Aims: Here we analyzed long term outcome in our previously published prospective phase II trial of DAC of advanced CMML (Braun et al, Blood 2011) to identify prognostic factors.
Methods: Between Nov 2008 and June 2009, 39 CMML patients (according to WHO) were included in this clinical trial if they had the following poor prognostic factors, based on a previous prognostic analysis (Wattel et al, Blood 1996): WBC<13G/l and IPSS 1.5; or WBC 13G/l, and two of the following criteria: marrow blasts 5%, Hb<10g/dl, plts<100G/l, abnormal cytogenetics, splenomegaly (SMG) >5cm below costal margin, (SMG>5cm), extra medullary disease (EMD),. Patients received DAC 20mg/m2/d IV for 5 days every 28 days for at least 3 cycles. Response criteria were based on IWG 2006 for patients with WBC<13G/L and also included evolution of WBC, SMG and EMD for patients with WBC ≥ 13 G/L. Data were analyzed 48 months after the last inclusion. For mutational analysis, DNA could be extracted for 37 patients from total BM nucleated cells or PB monocytes, and the following genes studied: ASXL1, CBL, FLT3 mutation and ITD, JAK2, NRAS and K RAS, RUNX1 and TET2. Statistical analysis was performed on Stata SE 10.1 (StataCorp, College Station, TX, USA).
Results: Median age was 71 years M/F: 30/9. 17 patients had CMML 1 and 22 had CMML 2. Nine patients had WBC<13G/l and 30 WBC ≥13G/l. Abnormal karyotype was found in 18 (46.2%) patients, including +8 and -7 in 7 and 1 case, respectively. 15 patients (38.6%) had SMG >5cm and 8 (20.5%) EMD. 58% patients were mutated for ASXL1, 50% for TET2, 31% for RAS (6 NRAS/5 KRAS), 28% for RUNX1, 14% for CBL, 6% for FLT3-ITD, 3% for FLT3 mutation and 3% for JAK2. 58% patients had at least 2 mutations. Overall Response Rate (ORR) was 38.6% with 4 (10.3%) CR, 8 (20.5%) marrow CR and 3 (7.7%) Stable Disease (SD). Median overall survival (OS) was 18 months. WBC, monocytes, Hb level, marrow blast %, SMG or EMD were not significantly prognostic of ORR, response duration or OS. 7/11 (63.6%) RAS mutated patients responded *vs* 8/27 (29.6.%) RAS germline patients (p=0.07). In univariate analysis, RAS mutation, known to have in CMML an unfavorable outcome (at least for NRAS) was associated with better OS (28 *vs* 17 months; p=0.05) and longer response duration (17.8 *vs* 9.2 months, p=0.056) compared to RAS germline. The ORR was 38.1% and 29.4%, and median OS was 18.2 months and 18.4 months in patients with ASXL1 mutations (an overall poor prognostic factor in CMML) and ASXL1 germline patients, respectively (p=0.28 and p=0.36, respectively). 6 patients had prolonged response to DAC of 18, 18, 39, 39, 48 and 58 months respectively. All those 6 patients had ASXL1 mutation, and 4 had concomitant RAS mutation. Another patient was allografted after 6 cycles in CR and was still alive (56+ months).
Summary and Conclusion: In this study of 39 advanced CMML treated with DAC with long term follow up, no conventional prognostic factor of response or survival to DAC emerged, while RAS mutations were associated with a better outcome and ASXL1 had no prognostic value. This possibly suggested a positive effect of DAC in CMML cases with RAS and/or ASXL1 mutation (ie with poor prognostic features), which however requires confirmation in larger series.

S1298

ISOLATED TOTAL MONOSOMY 7 IS ASSOCIATED WITH BETTER PROGNOSIS IN A LARGE COHORT OF MDS PATIENTS COMPARED WITH OTHER ABNORMALITIES OF CHROMOSOME 7- A SINGLE INSTITUTE EXPERIENCE

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Background: Karyotype is a strong independent prognostic factor in myeloid

neoplasms and abnormalities of chromosome 7 are the second commonest abnormality in MDS. The differential impact of monosomy 7, del7q (partial loss) and -7 (total loss) on prognosis has been demonstrated and hence incorporated into separate groups in revised IPSS.
Aims: To evaluate the clinical features, treatment and outcome of a large cohort of myelodysplastic syndromes (MDS) and related myeloid neoplasms in patients with cytogenetic aberration involving chromosome 7.
Methods: We retrospectively analysed 168 MDS patients who presented at diagnosis (147) or acquired during follow-up (21) any abnormality of chromosome 7. Patients were diagnosed between 1995 and 2013 at our institution.
Results: Median follow-up was 17 months (mo). Median age at diagnosis was 59 (range 16-91) years; 60% males and 60% more than 60 years. Denovo MDS were 118 (11 RA, 2 RARS, 56 RCMD, 23 RAEB1, 26 RAEB2), 12 had MDS/MPN, 38 AML including 15 secondary to MDS. Overall, 27 MDS/AML were therapy-related. According to IPSS, 15 % were at low, 48% intermediate 1, 19% intermediate 2 and 19% high risk. According to karyotype 4 subgroups were identified: -7 as a single abnormality (39%), -7 associated with other chromosomal aberrations (47%), del(7q) plus other chromosomal aberrations (5%) and patients with add(7) or translocations between 7 and other chromosomes [t(1:7),t(7:17),t(7:21),t(4:7)] (9%). Eighty-six (51%) patients had complex karyotype and 57 (34%) monosomal karyotype. Fifty-seven patients were treated in first line with intensive chemotherapy (IC), 55 with azacytidine (AZA), 32 with other therapies (including lenalidomide, low dose cytarabine) and 23 with best supportive care (BSC). Fifty-four patients underwent allogeneic haematopoietic stem cell transplantation (HSCT); 31 after IC, 15 after AZA and 8 as upfront. Median overall survival (OS) was 19 mo (range 1 to 166) and was significantly affected by karyotype: in isolated -7 group was 27 mo, in -7 plus other abnormalities group 16 mo, in del(7q) group 15 mo and in add(7)/t(7;?) group 8 mo (p<0.016). Moreover, as expected, both complex karyotype (p<0.001) and monosomal karyotype (p<0.002) were poor prognostic factors (median OS 11 and 15 mo, respectively). Patients treated with AZA as front line therapy had a better OS (27 mo) compared to patients who received IC (17 mo), other therapies (19 mo) or BSC (7mo) (p<0.041). As expected, patient who underwent HSCT had a longer survival (25 mo *vs* 18 mo, p 0.022), regardless the pre-HSCT treatment. This could be due of the positive selection of patients who survived and responded to induction therapy. In the MDS subgroup, in addition to the above described prognostic factors, IPSS, WPSS and IPSS-R too were significant outcome predictors (p 0.001). Median progression free survival was 12 mo and was affected by complex karyotype (7 mo *vs* 16 mo, p<0.001), type of 7 abnormality (-7 alone 18 mo, -7 plus other abnormalities 10 mo, del (7) 2mo and add (7)/t (7 ;?) 13 mo, p<0.001), IPSS (p<0.017) but not by therapy. Cumulative incidence of AML was 33% and 40% at 24 and 60 months, respectively.
Summary and Conclusion: In our study isolated -7 had a positive impact on OS compared to other abnormalities of 7, including del7q. This finding is discordant to recently published data, possibly due to fewer patients with isolated del7q, less untreated patients and the younger age of our cohort. AZA seemed to be good treatment option for these patients, even if the better outcome was associated with HSCT, an option for a selected group of patients.

S1299

IDENTIFICATION OF BIOMARKERS WHICH COULD PREDICT THE HEMATOLOGICAL RESPONSE OF NON DEL(5Q) LOW-RISK MDS PATIENTS TREATED BY LENALIDOMIDE ; THE GFM EXPERIENCE

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Background: Lenalidomide (Len) is a successful therapy used to treat anemia of patients with MDS del(5q) in half of the cases. Treatment by Len also induces an erythroid response in ~25% of non del(5q) low-risk or int-1 MDS patients. Len targets the protein Cereblon, a receptor for the substrates of the E3 ubiquitin ligase Cul4A-DDB1-Roc1, including Ikaros (IKZF1) and Aiolos (IKZF3) encoded by the *CRBN* gene.
Aims: Predictive biomarkers of the erythroid response to Len are needed to avoid inappropriate exposition to the risk of severe neutropenia or thrombocytopenia.
Methods: This was investigated in a cohort of 132 non del(5q) MDS patients (IPSS low and int-1), non-responders to a previous treatment by erythropoiesis-stimulating agent (ESA), enrolled in the Groupe Francophone des Myelodysplasies GFM-LenEpo 08 clinical trial (NCT01718379). Patients were randomized to Len 10mg/day 21 days/28 (L-arm) or Len 10 mg/d 21 d/28 plus Epoetin beta (60,000 units/w) (LE-arm) and evaluated after 4 cycles. Ninety-nine/132 patients were enrolled in the biological study including 41 responders and 58 non responders. We have previously reported a significantly HI-E according to IWG2006 in LE-arm (52%) *vs*. L-arm (31%) (p=0.031) (1). Extensive genotyping study of 26 genes (*ASXL1, CBL, DNMT3A, ETV6, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, PHF6, PTPN11, RIT1, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1, ZRSR2*) was conducted by a NGS approach (AmpliSeq, Life technologies). Mutations were considered as clonal when the VAF was >50%, and subclonal when 5
Results: The most frequently mutated genes were SF3B1 (73%), TET2 (45%?), ASXL1 (20%) and DNMT3A (20%), none of them influencing the response to Len or Len+Epo. Analysis of erythropoiesis based on the ratio of BFU-E/CFU-GM and the GEP signatures delineated two groups of responders patients: (i) a first group with impaired erythropoiesis, as already reported (2) and a second group with still effective erythropoiesis before treatment as assessed by normal or subnormal BFU-E number and the expression of erythroid genes. Using *Gene Set Enrichment Analysis (GSEA), the* comparison of GEP in 24 paired samples obtained before and after 4 cycles of treatment linked the response in L-arm or LE-arm to a signature of 32 up-regulated genes exclusively involved in the immune response. A supervised GSEA analysis of GEP before treatment identified a predictive signature of 36 up-regulated genes mainly involved in translation, cellular division and DNA repair. The basal expression level of 2/36 genes of this signature, further quantified by qPCR in a larger set of patients, was predictive of the response in L-arm or LE-arm (p<0.001) with a sensitivity >65% and a specificity >92%. The efficacy of Len or Len+Epo was independent of the basal expression level of CRBN, IKZF1 and IKZF3. However, a A>G polymorphism in the 5'UTR region of *CRBN* gene (rs1672753) was significantly associated with HI-E in the whole cohort (41.5% in responders *vs*. 22.4% in non-responders; p=0.048).
Summary and Conclusion: In conclusion, we have identified three biomarkers predictive of the erythroid response to Len or Len+Epo. 1. A Toma *et al.* Oral Presentation, ASCO Annual Meeting 2013 (#7002), 2. Ebert BL *et al.* PLoS Med. 2008 Feb;5(2):e35.

NHL & HL - Biology: Novel genetics and signaling

S1300

THE BRAF-MEK-ERK PATHWAY IN HAIRY CELL LEUKEMIA: A COMPREHENSIVE DISSECTION IN PRIMARY LEUKEMIC CELLS OF ITS BIOLOGICAL AND THERAPEUTIC RELEVANCE

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Background: Hairy cell leukemia (HCL) is a distinct, chronic B-cell neoplasm with a unique morphology, immunophenotype and molecular signature among other B-cell leukemias/lymphomas. Recently, we identified BRAF-V600E as the mutation underlying HCL and distinguishing it from HCL-like neoplasms (*e.g.*, HCL-variant and splenic marginal zone lymphoma) (Tiacci et al, NEJM 2011;364:2305). Because in solid tumors the BRAF-V600E activating kinase mutation leads to constitutive phosphorylation of MEK and, in turn, ERK, the BRAF-MEK-ERK pathway appears an ideal candidate to shed light on the unique biology of HCL and an ideal therapeutic target to be attacked by BRAF or MEK inhibitors, which recently proved effective in clinical trials of BRAF-V600E+ melanoma patients. However, a thorough dissection of the biological effects of the BRAF-MEK-ERK pathway in HCL is so far lacking, as are mechanistic studies on the effects of these drugs in HCL.

Aims: To study *in vitro* the effects of BRAF and MEK inhibitors on the specific biochemical, molecular, morphological and anti-apoptotic features of HCL, using patients' hairy cells (as the putative "HCL" cell lines described in the literature do not carry the BRAF-V600E mutation and are unlikely to be of true HCL origin - Tiacci et al, Blood 2012;119:5332).

Methods: Primary leukemic cells, purified (≥90%) from 23 HCL patient and 13 HCL-like patients, were exposed *in vitro* to active BRAF inhibitors (Vemurafenib, Dabrafenib or PLX4720) or the MEK inhibitor Trametinib for 15 minutes to 96 hours at various concentrations (up to 1 μM), and were then monitored for: *i)* activation status of MEK and ERK by Western blotting; *ii)* downstream transcriptional changes by genome-wide expression profiling (in 6 HCL patients); *iii*) surface morphology changes by confocal microscopy after phalloidin/Annexin-V staining to highlight the F-actin-rich hairy projections in still living cells (in 8 HCL and 5 HCL-like patients); *iv)* viability (by MTT or WST metabolic assays) and apoptosis (by AnnexinV/PI staining) in 14 HCL and 5 HCL-like patients.

Results: In all HCL patients, treatment with each BRAF inhibitor resulted in a consistent, sustained and dose-dependent dephosphorylation of MEK and ERK at all time points and at all drug concentrations tested, as compared to vehicle-treated cells and to inhibitor-treated HCL-like cells. Also Trametinib produced a strong downregulation of phospho-ERKs levels in HCL cells. Notably, gene expression profiling after 48h and 72h of BRAF-inhibition by Vemurafenib showed not only a silencing of the RAF-MEK-ERK pathway transcriptional output previously described in solid tumors, but also a loss of the HCL-specific gene expression signature previously identified by us (Basso et al, JEM 2004;199:59), as well as the downregulation of the HCL immunophenotypical markers CD25, TRAP and cyclin-D1. These *in vitro* mRNA data were validated at the protein level and *in vivo* by documenting loss of surface CD25 expression and of nuclear cyclin-D1 expression in the leukemic cells of two pluri-relapsed HCL patients being treated with Vemurafenib in our HCL-PG01 phase-2 clinical trial (EudraCT 2011-005487-13). Also interesting was the silencing of some genes important for generating cell membrane projections (*e.g.*, ACTB and LST1) and the induction of pro-apoptotic genes (*e.g.*, BCL2L11/BIM and CDKN1C/p57-Kip2). Indeed, the biochemical and transcriptional events triggered by BRAF inhibition were followed by a consistent and statistically significant ($p<0.05$): *i)* loss of the hairy projections in still viable (AnnexinV-negative) leukemic cells (see Figure); *ii)* reduction of metabolic viability (up to 51.7% relative to the drug vehicle); and *iii)* decrease of living, non-apoptotic (ANXA5/PI-negative) cells (up to 84.4% relative reduction), all of this occurring specifically in leukemic cells of the vast majority of HCL patients as opposed to none of the HCL-like patients.

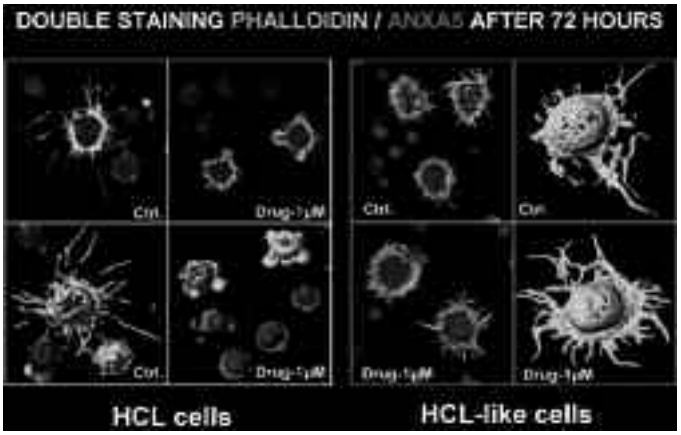

Figure 1.

Summary and Conclusion: These results represent a comprehensive biological and pre-clinical dissection of: ***i)*** the pronounced role of the BRAF-V600E mutation in driving the specific transcriptional signature, morphology and immunophenotype of HCL among other mature B-cell neoplasms; ***ii)*** the significant anti-leukemic activity obtained through inhibition of the BRAF-MEK-ERK pathway in HCL.

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S1301

A NOVEL ACTIVATING SOMATIC MUTATION OF CXCR4 PLAYS A CRUCIAL ROLE IN MODULATING WALDENSTROM MACROGLOBULINEMIA BIOLOGY

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Background: Whole-genome sequencing has enhanced our understanding of the molecular mechanisms that may contribute to WM pathogenesis. Specifically, *L265P/MYD88* has been described as a prevalent somatic mutation in WM patients; and shown to increase tumor cells growth. However, *L265P/MYD88* did not predict progression or resistance to therapy, indicating that other genetic alterations may be critical for tumor progression and dissemination to distant organs. CXCR4 represents the main regulators of tumor B-cell homing to the BM. We therefore evaluated the mutational status of CXCR4 in 429 patients with B-cell lymphoproliferative disorders; and define its functional role in WM *in vivo*.

Aims: 1) To screen the CXCR4 mutational status in 429 patients with B-cell lymphoproliferative disorders. 2) To define the biological role of the C1013G/CXCR4 variant in WM, *in vivo*. 3) To evaluate the anti-tumor activity of the novel anti-CXCR4 monoclonal antibody, BMS-936564, against both mutated and wild-type WM cells, *in vivo*.

Methods: Allele-specific(AS) PCR was performed on bone marrow (BM)-derived tumor cells of patients with WM (n: 142); IgM-MGUS (n: 40); as well as in patients with DLBCL (n: 75), splenic marginal zone lymphoma (SMZL; n: 14), B-CLL (n: 37), hairy cell leukemia (HCL; n: 35), multiple myeloma (MM; n: 36), IgA/IgG MGUS (n: 22), lymphoplasmacytic lymphoma without WM criteria (n: 13), and amyloidosis (n: 6). healthy subjects have been also studied (n: 32). CXCR4-loss and -gain of function studies were performed on WM cells stably expressing either shRNA-CXCR4, CXCR4-ORF-GFP-tagged or scramble-RFP-tagged (lentiviral infection). Lentivirus-based infection was used to generate the *C1013G/CXCR4* mutant protein in WM cells. CXCR4-overexpressing or *C1013G/CXCR4*-mutated cells and the corresponding controls were injected i.v. into SCID/Bg mice and tumor dissemination was evaluated *ex vivo* by IHC (hCD20; h-CXCR4). *C1013G/CXCR4*-mutated cells were characterized at mRNA levels (U133 plus2) using GSEA. A novel human anti-CXCR4 mAb (BMS-936564) was tested *in vitro* (cell proliferation, MTT, adhesion, migration to primary WM BM mesenchymal stromal cells) and *in vivo*. Tumor growth was evaluated by IHC *ex vivo* (hCD20; hCXCR4) and by immunofluorescence.

Results: The somatic C1013G/CXCR4 variant was detected in 28% of the 142 WM cases evaluated. The mutation was also present at IgM-MGUS stage (20%); while it was present in a minority of patients with DLBCL (1%) and SMZL (7%); and absent in all MM, IgA/IgG MGUS, B-CLL, HCL patients and it was not detected in healthy subjects. Mice injected with C1013G/CXCR4-cells

presented with a significant dissemination of tumor cells, demonstrating involvement of liver, bone marrow, lymph nodes, kidney and lung. IHC showed the presence of CXCR4+ and CD20+ cells in all the tissues examined; and quantification of CXCR4 and CD20 positivity was higher in C1013G/CXCR4-cells-, compared to parental(p)-WM cell-injected mice (P<.05). C1013G/CXCR4-cells were further characterized *in vitro*, showing increased adhesion and cell proliferation in the presence of primary WM BM-MSCs. These findings were also confirmed using CXCR4-overexpressing cells. GSEA demonstrated that genes related to invasiveness, cell proliferation, anti-apoptosis, and oncogenesis were enriched in C1013G/CXCR4-cells compared to parental-WM cells. These findings let us hypothesize that C1013G/CXCR4 may act as an activating mutation in WM cells. Indeed, CXCR4 over-expressing cells were injected into mice, showing similar phenotype to the one observed upon C1013G/CXCR4-WM cell-injected-mice. Finally, the novel anti-CXCR4 antibody (BMS-936564) exerted anti-WM activity both *in vitro* and *in vivo*, with anti-tumor effects observed also against the mutated variant, with inhibition of pro-survival pathways (p-ERK; p-AKT); induction of pro-apototic proteins (cleaved-PARP and -caspase-9); up-regulation of p-GSK3beta, p-beta catenin and subsequent beta catenin degradation.

Summary and Conclusion: *C1013G/CXCR4* acts as an activating mutation in WM; and it is targetable by using BMS-936564 thus providing the basis for translating these observations into clinical trials for WM patients.

S1302

NORDIC MCL2-3 TRIALS: MIRNA-18B OVEREXPRESSION IDENTIFIES A MANTLE CELL LYMPHOMA SUBGROUP WITH POOR SURVIVAL AND IMPROVES MIPI-B PREDICTION OF PROGNOSIS

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Background: Mantle cell lymphoma (MCL) is an aggressive NHL subtype with a variable clinical course. Based on long-term follow-up 57% of younger MCL patients (≤65 years) relapse during a 10-year period. Risk-stratification is therefore of great importance in order to identify patients who are eligible for novel or alternative treatment regimens. The current prognosticator (the MIPI-B) does however not identify specific biological pathways associated with aggressive subtypes of MCL. Over the last five years, several studies have shown that miRNAs delineate aberrant molecular pathways and predict survival in MCL patients. However, there is little coherence between the clinical studies, and at this point, miRNA expression in MCL has not yet been examined (or validated) in large, prospective, uniformly treated patient cohorts.
Aims: We assessed whether MCL miRNA expression could identify patients with poor survival and improve current prognostication of MCL patients.
Methods: Diagnostic MCL samples from 172 patients in the Nordic MCL2 and MCL3 clinical trials were retrieved. All patients had confirmed CyclinD1 overexpression and received almost identical high dose immunochemotherapy followed by ASCT. The only difference between the two regimens was that MCL3 patients with unconfirmed complete remission (CRu) or partial remission (PR) received ^{90}Y-Ibritumomab-Tiuxetan (Zevalin), which did not have any impact on survival or adverse events. The median follow-up was 6.4 years for the MCL2 cohort and 3.7 years for the MCL3 cohort. Genome-wide miRNA microarray profiling covering 1846 miRNAs was performed in 74 patients of the screening cohort (MCL2). Differentially expressed miRNAs (adjusted p-value<0.05) were re-analyzed by qRT-PCR. Prognostic miRNAs were validated by qRT-PCR in 94 patients of the validation cohort (MCL3). The main endpoint was cause-specific survival.
Results: In the screening cohort 17 miRNAs were differentially expressed by microarray analysis in patients who died from MCL. Follow-up qRT-PCR analysis of the 17 miRNAs in the validation cohort showed that three (miR-18b, miR-92a, miR378d) were differentially expressed in patients who died from MCL. Based on the screening cohort, feature selection and leave-one-out cross-validation, miR-18b was identified to hold the highest prognostic value. Multivariate analysis confirmed miR-18b as an independent prognostic variable. We generated a new MIPI-B-miR prognostic score, by combining expression-levels of miR-18b with MIPI-B data, with a significantly higher predictive value than the current gold standard MIPI-B with regards to cause-specific survival (p=0.015), overall survival (p=0.006), and progression-free survival (p<0.001).

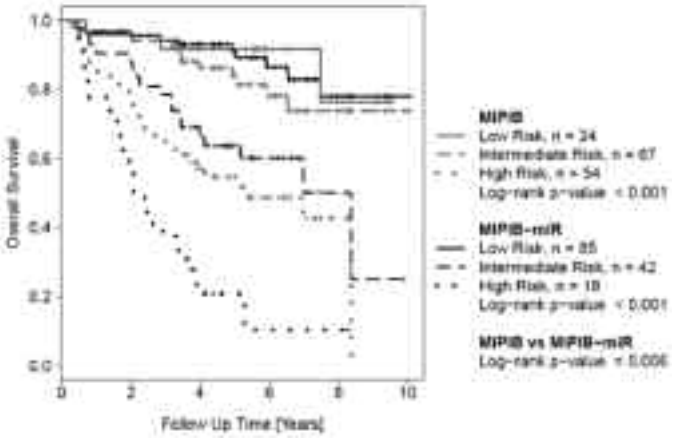

Figure 1.

Summary and Conclusion: Overexpression of miR-18b identifies patients with poor prognosis in two large prospective MCL cohorts and adds prognostic information to the MIPI-B score.

S1303

DISTINCT PATTERNS OF RHOA MUTATIONS IN ADULT T-CELL LEUKEMIA/LMPHOMA AND OTHER PERIPHERAL T-CELL LYMPHOMAS

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Background: Adult T-cell leukemia/lymphoma (ATL) is a distinct form of peripheral T-cell lymphoma (PTCL), which is etiologically associated with human T-lymphotropic virus type I (HTLV-1) infection during early infancy. Although the presence of a long latency period before the onset of ATL in late adult life suggests a critical role of accumulating genetic events in HTLV-1-infected T-cells, little is known about their gene targets. Recently, frequent somatic mutations in *RHOA*, as well as *TET2, DNMT3A*, and *IDH2* genes, were reported in angioimmunoblastic T cell lymphoma (AITL) and other PTCL characterized by follicular helper T-cell phenotypes. Most of the *RHOA* mutations invariably cause an identical amino acid change (Gly17Val), which was shown to inhibit wild-type RHOA function in a dominant-negative manner.
Aims: We aimed to investigate the mutational status of *RHOA*, as well as *TET2*, *IDH2*, *DNMT3A*, and *SF3B1* in ATL cases, which were compared with that in other PTCL, in terms of the type of mutations and their functional impact on RHOA functions.
Methods: We performed targeted deep sequencing in 205 ATL cases with different subtypes, and compared them with AITL (N=72) and PTCL-not otherwise specified (NOS) cases (N=87). Also, we analyzed the function of different RHOA mutants using luciferase assay and actin filament staining.
Results: In total, we identified 42 *RHOA* mutations in 38 (18.5%) ATL cases. Unexpectedly, however, showing a stalk contrast to AITL and other PTCL-NOS, in which *RHOA* mutations almost exclusively involved Gly17 within the GTP binding domain, *RHOA* mutations were widely distributed across all the GTP-binding domains in ATL cases. Among them, Cys16 located in exon 2 was most frequently mutated (N=11; 5.4%), while only four (1.9%) cases harbored Gly17Val mutation. These results suggest that the *RHOA* mutations act as a driver of ATL lymphomagenesis, but in a different manner from AITL and PTCL-NOS. We found no differences of *RHOA* mutational status among ATL subtypes, and no significant prognostic impact of *RHOA* mutations on survival. In AITL/PTCL-NOS, all *RHOA* mutated cases were accompanied by *TET2* mutations and often had *DNMT3A* and *IDH2* mutations, whereas no significant correlation between *RHOA* and *TET2* mutations was observed in our ATL cohort, although TET2 was also commonly mutated in 21 ATL cases (10.2%). *IDH2*, *DNMT3A*, and *SF3B1* were very rare in ATL cases. *RHOA* encodes a ras-related GTP-binding protein that functions as a molecular switch in a variety of biological processes. Modeling of three-dimensional structures suggested these mutations could affect the GTP-binding ability of RHOA protein. Indeed,

luciferase assay showed, unlike dominant-negative Gly17Val RHOA mutant, the Cys16Arg RHOA mutant enhanced transcriptional activity, suggesting gain-of-function mechanisms of this mutant. In addition, Gly17Val RHOA attenuated actin stress fiber formation in NIH3T3 cells, whereas Cys16Arg RHOA did not. These results suggest ATL-specific RHOA mutants act in an opposite manner to those characteristic of AITL and PTCL-NOS.
Summary and Conclusion: Our finding highlights a unique role of *RHOA* mutations in the development of peripheral T-cell neoplasms; as is the case with other PTCL, including AITL and other PTCL-NOS, *RHOA* mutations were also common in ATL. Nevertheless, the distribution and functional impacts of these *RHOA* mutations in ATL were distinct from the canonical *RHOA* Gly17Val mutation found in AITL and other PTCL-NOS, suggesting their distinct role in the pathogenesis of different PTCL. Further investigation should be warranted to clarify the exact role of RHOA in both normal and malignant T-cells.

S1304

DISCRIMINATION OF MUTATIONS ARISING IN PRE-MALIGNANT CELLS AND THOSE IN LYMPHOMA CELLS IN ANGIOIMMUNOBLASITC T-CELL LYMPHOMA

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is a distinct subtype of peripheral T-cell lymphoma, characterized by generalized lymphadenopathy and autoimmune-like manifestations. Regarding genetic lesions of AITL, frequent mutations in *TET2*, *IDH2* and *DNMT3A* have been identified, although all these mutations were also found in myeloid malignancies. Recently, we and others identified disease specific G17V *RHOA* mutations in AITL and AITL-related cancer.
Aims: We performed this study to further identify specific gene mutational profiles and origins in AITL and AITL-related cancer.
Methods: Targeted sequencing was analyzed for 76 genes in 79 PTCL samples. Mutational origin was analyzed by cell sorting and laser microdissection.
Results: Targeted sequencing identified 168 mutations in 33 genes, including those in *TET2* (54/79 [68.4%]), *RHOA* (39/79 [49.4] %), *DNMT3A* (21/79[26.6%]), and *IDH2* (14/79[17.7%]). Allele frequencies of *TET2* mutations were higher than those in *RHOA* or *IDH2*, while comparable to those of *DNMT3A* mutations, suggesting that *TET2/DNMT3A* mutations should precede *RHOA/IDH2* mutations. Allele frequencies of mutations in most of newly identified gene mutations were smaller than those of *TET2*, suggesting that many of these mutations should occur later than *TET2* mutations. Cell sorting and laser microdissection, followed by amplicon sequencing, revealed that *TET2/DNMT3A* mutations were found in tumor and non-tumor cells, while *RHOA* mutations and *IDH2* mutations were found only in the tumor cells. Newly identified mutations were classified into 2 types: those identified both in tumors and non-tumors, and those confined to tumors.
Summary and Conclusion: These data provide insight into more profound understanding of the genomic structure and multi-lineage involvement in development of AITL.

Stem cell transplantation - Clinical

S1305

LONG-TERM RESULTS OF 174 PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS TREATED WITH HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Background: About 40% of patients (pts) with amyloid light chain amyloidosis (AL) achieve a complete remission (CR) of their underlying monoclonal plasma cell disorder following high-dose melphalan (HDM) and autologous stem cell transplantation, these pts have an excellent survival (Cibeira, *Blood* 2011).
Aims: We report our single center experience of HDM in AL since 1998.
Methods: 174 pts were transplanted between 1998 and 2013. Inclusion criteria were age<70 years, NYHA stage<III, creatinine clearance >30 ml/min (or on dialysis) and WHO performance status<3. Median age was 56 years. Median number of involved organs was 2. Ten patients had previous cardiac transplantation. In those patients, stem cells were mobilized after G-CSF administration; in almost all other patients after chemotherapy. Furthermore, 95 patients received chemotherapy prior HDM. Patients were treated with melphalan 200 mg/m2 in median. Fifty-nine of 136 evaluable patients were Cardiac Mayo stage 1, 53 patients stage 2 and 24 patients stage 3, respectively
Results: The estimated median follow-up after transplantation is 75 months. Median overall survival (OS) is 136 months. Four patients (2%) died due to cardiac and infectious complication of HDM. Hematological remission (HR) was achieved in 80% (38% CR) and organ response in 40% of evaluable patients, respectively. For pts in PR or CR after HDM median OS is not reached
Summary and Conclusion: For HDM, identification of eligible patients is crucial. We observed a very low TRM of 2% in our center. The long-term survival is excellent. In our opinion, HDM and ASCT is the treatment of choice in younger patients who fit strict inclusion criteria.

S1306

PREDICTING UNSATISFACTORY PERIPHERAL BLOOD STEM CELL COLLECTIONS IN MYELOMA PATIENTS RECEIVING NEW DRUGS AS INDUCTION AND CYCLOPHOSPHAMIDE + G-CSF AS MOBILIZING REGIMEN: A GIMEMA MYELOMA-WP STUDY

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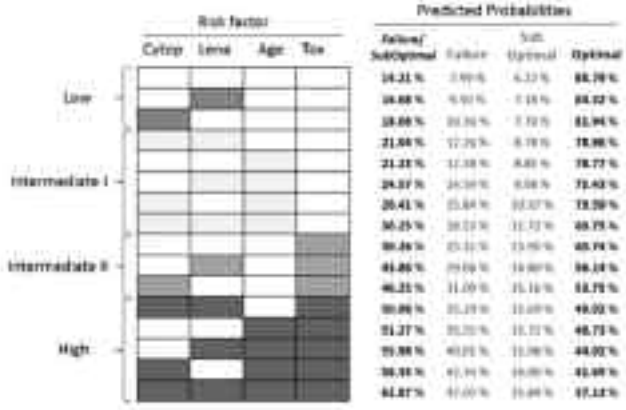
Background: Patients with multiple myeloma (MM) may fail to collect adequate amounts of CD34+ peripheral blood stem cells (PBSC) for single or multiple autologous transplantation. The percentage of “poor mobilizers”, however, differs across studies, depending on definitions, criteria to evaluate collections, age, disease phase and characteristics, treatments applied, objectives to reach, and practices for mobilization and apheresis.
Aims: We aimed to evaluate, define and forecast unsatisfactory PBSC collections in newly diagnosed MM patients receiving novel agents as induction therapy and a homogeneous mobilization procedure.
Methods: We retrospectively analyzed the role of 4 parameters potentially considered able to negatively affect releasing of PBSC and to impair their collection in MM: age (>60 years); initial use of lenalidomide; cytopenia at diagnosis (Hb<10 g/dl, neutrophil count<1.000/µl, platelet count<100.000/µl); grade 3-4 hematological toxicity during induction therapy. Overall, 1.348 patients enrolled in 5 consecutive clinical trials conducted by GIMEMA/MM Italian Network were evaluated. In all cases, the mobilizing regimen was cyclophosphamide (3-4 g/sqm) + G-CSF (10 mcg/kg). An absolute number of PBSC >20/µl was the threshold to start apheresis. According to different studies, induction regimens were TD (316 patients), VTD (258 patients), RD (396 patients) and PAD (86 patients). 292 patients received a modified VAD-regimen, without novel agents. PBSC total amounts<2 x 10^6/kg and<5 x 10^6/kg

after a single mobilization procedure were considered "failures" or "sub-optimal" results, respectively. Risk factors were analyzed by univariate and multivariate logistic regression models; the final results were internally validated using the bootstrap method. ROC curves were constructed to assess a model discriminatory power for the predictive probability, Predicted probabilities of outcomes (optimal *vs* suboptimal *vs* failure), were used to generate a risk heatmap.
Results: 946 patients (70.1%) showed at least one "negative" parameter: 560 patients (41.5%) were older than 60 years, 332 (24.6%) had baseline cytopenia, 356 (26.4%) were treated with lenalidomide and 88 (6.5%) developed grade 3-4 hematological toxicity under induction therapy. Overall, 630 patients (46.7%) had only one parameter, while 319 showed a combination of 2 (252, 18.6%), 3 (54, 4 %) or 4 (10, 0.7%) parameters, respectively. After a single mobilization (median number of apheresis: 2; range: 0-4), 280 patients (20.7 %) collected an insufficient number of PBSC (167 failures, 12.3%, and 113 sub-optimal, 8.3%). All parameters negatively influenced PBSC collection at univariate analysis, but only hematological toxicity during induction and age >60 maintained a detrimental effect at multivariate analysis (p<0.0001). The figure reports the risk heatmap obtained when the 4 parameters were pooled and weighted according to their relevance as single or combined variables. According to the cumulative probability of unsuccessful collections (failures + suboptimal), 4 risk areas were identified: low (range 14-18%), intermediate-1 (21-30%), intermediate-2 (39-46%), and high (50-63 %). In particular, the risk of failure was 9% for low-risk and 40% for high-risk patients.

Table 1.



Summary and Conclusion: In this series, about 20% of newly diagnosed MM patients showed unsatisfactory PBSC collections. Our simple model, based on a large number of patients treated frontline with novel agents and receiving the most popular mobilizing approach currently employed in Europe, provides a reliable measure for the early identification of a "poor mobilizer" phenotype, applicable in individual patients.

S1307

UPDATED EUROPEAN EXPERIENCE ON AUTOLOGOUS STEM CELL TRANSPLANTATION FOR WALDENSTROM'S MACROGLOBULINEMIA/ LYMPHOPLASMACYTIC LYMPHOMA PATIENTS. A RETROSPECTIVE STUDY OF THE EBMT LYMPHOMA WORKING PARTY

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Background: Waldenstrom's Macroglobulinemia (WM) is a rare distinct clinicopathological entity. Although it is classified as an indolent disease, its treatment remains a major challenge The role and timing of autologous stem cell transplantation (ASCT) in the management of WM patients has not been well established.
Aims: The aim of this retrospective study was to analyse the outcome of ASCT for WM.
Methods: Between 1995 and 2011, 615 patients with WM (70% were male) were reported to the European Society for Blood and Marrow Transplantation registry. The median age at ASCT was 54 years and median time from diagnosis to ASCT was 19 months (291 patients–47% - underwent ASCT within<18 months from diagnosis). Disease status at ASCT was first partial (PR1), very good partial (VGPR1) and complete remission (CR1) in 325 patients (53%). 176 (29%) patients were autografted in second, third or later CR, PR or sensitive relapse, whereas 47 (8%) patients had primary refractory or progressive disease at ASCT. Conditioning regimens were chemotherapy based in 595 patients (97%) with the majority of them receiving BEAM chemotherapy. The stem cell source was from peripheral blood in 598 patients (97%).
Results: Within a median follow up of 29.5 months, 216 patients had disease relapse or progression. The relapse rate (RR) at 5 years was 47% and it was significantly lower (p=0.001) in patients undergoing transplantation in first response (CR1, VGPR1, PR1). Disease-free survival (DFS) was 46% at 5 years and this was significantly better for patients receiving the transplant in less than 18 months from diagnosis (p=0.004), and for those transplanted in PR1, VGPR1 or CR1. The non-relapse mortality (NRM) was 2.6 % and 7.1% at 1 and 5 years respectively. The median overall survival (OS) was 112 months. Disease progression was the cause of death in 84 patients. Nine patients died of secondary malignancy. The estimated OS was 65% at 5 years and was significantly superior (p=0.033) for patients in PR1, VGPR1 and CR1 at the time of ASCT,than those transplanted in other disease status.
Summary and Conclusion: ASCT is a feasible and effective treatment in patients with WM who are transplant eligible. The outcomes are significantly better when the autologous transplantation is offered early in the course of the disease in good responders. However, the appropiate place of ASCT in the treatment algorithm of WM in the era of B cell receptor signalling inhibitors and other targeted drugs needs to be defined by additional studies.

S1308

SEQUENTIAL INTENSIfiED CONDITIONING FOLLOWED BY TAPERING OF PROPHYLACTIC IMMUNOSUPRESSANTS AND DONOR LYMPHOCYTE INFUSIONS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR REFRACTORY LEUKEMIA

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is perceived as the only curative option for refractory acute leukemia. However, the relapse rate exceeds 50% in these patients undergoing allo-HSCT with standard myeloablative regimen. To improve outcomes of allo-HSCT for refractory leukemia, we previously introduced a strategy of sequential intensified conditioning and early rapid tapering of prophylactic immunosupressants for graft-*versus*-host disease (GVHD). The results indicated this strategy could improve outcomes of refractory leukemia, with 5-year overall survival (OS) and disease-free survival (DFS) of 44.6% ± 8.1% and 38.2% ± 7.7%. However, the 3-year cumulative incidence of leukemia relapse reached to 33.3%. To reduce the relapse rate but without increasing regimen-related toxicities (RRT), we increased the dose of etoposide (VP-16) in conditioning and infused donor lymphocytes (DLI).
Aims: To assess the feasibility and efficacy of this modified strategy in patients with refractory acute leukemia.
Methods: A total of 123 patients with refractory acute leukemia undergoing allo-HSCT from January 2009 to December 2012 were enrolled. Ninety-four patients received related (73 sibling and 21 family donors), 29 unrelated donor transplants; 73 were HLA locus matched, 50 mismatched. Modified sequential intensified conditioning regimen was: fludarabine (30 mg/m^2/day, -10 to -6 days) + cytarabine (2.0 g/m^2/day, -10 to -6 days) plus TBI (total body irradiation, 4.5 Gy/day, -5, -4 days) + cyclophosphamide (60 mg/kg/ day, -3, -2 days) + VP-16 (15 mg/kg/day, -3, -2 days). Cyclosporine A (CsA) was withdrawn rapidly in a stepwise fashion (total dose reduced by 20%/week) if patients who did not experience acute GVHD (aGVHD) by day +30 post-transplantation. Donor lymphocytes (1.0×10^8/kg, once a month, 4 doses totally) would be infused in patients without II° or more than II° aGVHD by day + 60 post-transplantation.
Results: All patients achieved hematopoietic engraftment, except for two who died of infections and one who died of RRT during conditioning. All 120 evaluable patients achieved complete remission (CR) at the time of neutrophil engraftment and achieved complete donor chimerism by day +30 post-transplantation. The incidence of total RRT was 100%, and III-IV RRT was 22.0%. Within the first 100 days post-transplantation, 67 patients developed 95 episodes of infections. Twenty-one had bacterial infections, 7 had invasive fungal infections, 9 had viral infections except cytomegalovirus (CMV) and Epstein-Barr virus (EBV) viremia, 24 had mixed infections and 6 had infections of unknown etiology. Moreover, 48 patients had CMV viremia, 3 developed CMV pneumonia; 41 had EBV viremia, 13 developed EBV-associated diseases. Of the 120 evaluable patients, aGVHD occurred in 31 cases by day +30. Of the 89 patients who did not develop GVHD by day +30, 28 developed aGVHD after CsA withdrawal. Of the 66 patients who received DLI by day +60, 20 developed aGVHD, and 43 developed chronic GVHD (cGVHD, including 13 migrating

from aGVHD). cGVHD occurred in 65 of 102 patients who survived more than 100 days, including 43 after DLI. Twenty-three patients experienced leukemia relapse (hematologic in 16, genetic in 4, central nervous system in 2 and extramedullary in 1) at a median time of 165 (range, 28 to 479) days post-transplantation. The 3-year cumulative incidence of relapse was 25.3 ± 4.8%. Of the 23 patients who relapsed, 6 abandoned treatment and 17 received treatment, including 9 with chemotherapy and DLI, 6 only with chemotherapy, 1 only with DLI, and 1 with chemotherapy and radiotherapy. Only two of the 17 cases achieved CR after treatment, and the others all died of disease progress. With a median follow up of 316 (range, 7 to 1589) days post-transplantation, 75 patients survived and 48 died. Causes of death included leukemia relapse (n=21), infections (n=12), GVHD (n=8), EBV-associated diseases (n=5), cerebral hemorrhage (n=1) and secondary dyshematopoiesis (n=1). The 3-year OS and DFS was 58.8% ± 4.7% and 57.0% ± 4.8%.

Summary and Conclusion: For patients with refractory leukemia undergoing allo-HSCT, the modified strategy of sequential intensified conditioning followed by tapering of prophylactic immunosupressants and DLI not only improves OS and DFS, but also reduces leukemia relapse.

S1309

EFFECT OF IMMUNOMODULATION FOR PERIPHERAL T-CELL LYMPHOMA IN RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT: A SFGM-TC STUDY ON 64 PATIENTS

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Background: T cell lymphomas (TCL) are heterogeneous diseases, lightly sensitive to chemotherapy, with an adverse outcome and a 5-years survival rate of about 30%. Allogeneic hematopoietic stem cell transplant (allo-SCT) can lead to durable remission, even for patients with a poor response to chemotherapy. Several observations, generally reported on small samples of patients, argue for a Graft *Versus* Lymphoma (GVL) effect in TCL. However allo-SCT is not consensually recommended, at least in first line therapy.

Aims: The aim of the current study is to highlight GVL effect in TCL. To do so, we analysed the outcome of patients with relapse after allo-SCT, to evaluate the benefits of immunomodulation on disease control (donor lymphocyte infusions (DLI) and immunosuppressive therapy tapering).

Methods: Between 1988 and 2012, sixty-four patients in relapse for a TCL after allogeneic SCT were identified in 21 SFGM cooperative centers (Société Française de Greffe de Moelle). Exhaustive data were completed in 51 patients. The median age at transplant was 44 years old, 24% were cutaneous TCL (3 Sezary syndrome and 9 mycosis fongoides), whereas 76% were not (16 T nos, 11 anaplasic TCL, 5 angio-immunoblastic TCL, 4 NK/T lymphomas, 2 HTLV1 TCL and 1 EIATL). At transplant, 35% were in complete remission (CR), 49% in partial remission (PR) and 16% had progressive disease (PD). Regiment was myeloablative for 19 patients and non-myeloablative for 32. At relapse, patients were treated with either non immunologic-based strategy (chemotherapy, radiotherapy, PUVA therapy) or immunomodulation (DLI and/or discontinuation of immunosuppressive therapy), or both. We compared their outcomes according to the treatment they received at relapse.

Results: Relapse occurred at a median time of 2.7 months after transplant (from 0.5 to 18.6 months) and 14 patients had a localized cutaneous relapse (7 MF, 3 anaplasic TCL, 2 T nos, 1 AITCL and 1 NK/T lymphoma). Among the 13 patients who received DLI (DLI alone: 5, DLI + radio/chemotherapy: 8), 9 obtained a response (7CR, 2 PR) and 6 are still alive, with a median follow up of 3 years. For the 38 who did not receive DLI, immunosuppressive therapy was tapered or stopped for 23 and led to a prolonged remission, for 2 of them, along with extensive chronic GVHD. In the non-DLI group, 22/38 received a radio/chemotherapy treatment with a response rate of 50% and a 1-year survival rate of 25%. Among the 8/38 patients who experienced a response in this group, five developed a chronic GVHD. Median overall survival was 144 days in the non-DLI-group and 557 days in the DLI-group (p=0.01). In univariate analysis, time from transplant to relapse (p=0.015), chronic GVHD after relapse (p=0.036) and receiving DLI (p=0.016) were associated to a better overall survival (OS). In multivariate analysis, DLI was the only remaining factor associated with OS (p=0.005). Disease status at transplant, regiment's intensity, patient's age and relapse localization (cutaneous or disseminated, p=0.08) were not found to be associated with OS.

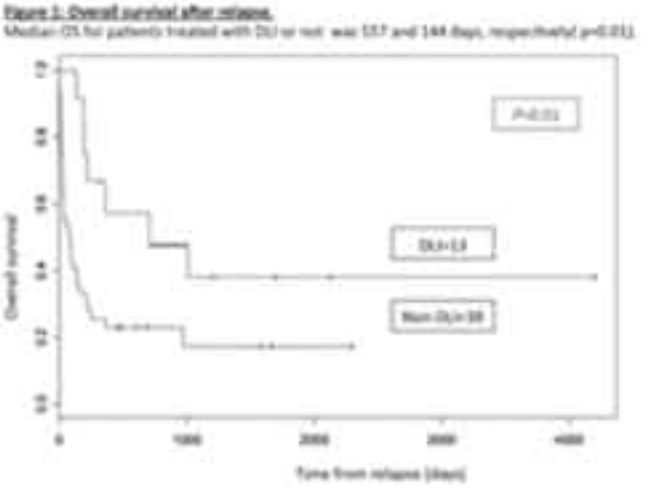

Figure 1.

Summary and Conclusion: The benefit of DLI in case of relapsing T-cell lymphomas after transplant is suggested in this study and represent an argument in favour of the existence of GVL effect in these diseases. These findings could lead to the set up of an active immunomodulation strategy after transplant for patients with high-risk disease, even if the short delay from transplant to relapse, currently observed, can limit this therapeutic option.

Acute lymphoblastic leukemia - Clinical 2

S1310

CONCURRENT DELETIONS OF IKZF1 AND PAX5, CDKN2A, CDKN2B OR PAR1 (IKZF1-PLUS) CONFER A VERY POOR PROGNOSIS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Recent technical advances in the field of genomic analyses have stimulated a large number of discovery studies on etiological and clinical endpoints in acute lymphoblastic leukemia (ALL). Recently, *GATA3* was described as a new susceptibility locus for pediatric ALL (Migliorini *et al.* Blood 2013;122:3298-3307). The variant GATA3 allele (rs3824662) was found to be specifically associated with B-other ALL (B-lineage ALL lacking recurrent chromosomal translocations and hyperdiploidy) and to confer a dismal outcome.
Aims: Analysis of somatic genetic aberrations describing B-other ALLs associated with rs3824662 to characterize the leukemia genetics underlying the prognostic germline *GATA3* ALL risk-allele.
Methods: Clinical epidemiological analysis of 500 patients treated on trial AIEOP-BFM 2000 having information on rs3824662 genotype and leukemia genetic characteristics including MLPA (SALSA kit P335-A3; MRC-Holland) and *ERG* deletion. Validation of results by employing an independent cohort of 599 patients from trial AIEOP-BFM 2000. Treatment outcome was analyzed excluding *BCR/ABL1*-positive patients.
Results: *GATA3* rs3824662 genotype was negatively associated with hyperdiploidy and positively associated with prednisone poor-response, higher loads of minimal residual disease (MRD), and deletions of *IKZF1*, *PAX5*, *CDKN2A* and *CDKN2B*. Out of the 476 patients from the discovery cohort, 88 (18.5%) bore an *IKZF1* deletion, 98 (20.6%) a *PAX5* deletion, 61 (12.8%) a heterozygous and 69 (14.5%) a homozygous deletion of *CDKN2A*, 61 (12.8%) a heterozygous and 60 (12.6%) a homozygous deletion of *CDKN2B*. Similar numbers were observed in the validation cohort. When single marker analyses were conducted, *IKZF1* deletion was the strongest determinator of outcome. In *CDKN2A*- and *CDKN2B*-deleted patients, the prognostic impact was restricted to those with homozygous deletions. When *IKZF1* deletions were analyzed in the discovery and validation cohorts in combination with *PAX5*, *CDKN2A* and *CDKN2B* deletions, patients displaying an additional deletion to that of *IKZF1* had the worst event-free survival (EFS) and highest cumulative incidence of relapse (CIR). We also analyzed *IKZF1* deletion in association with *PAR1* deletions leading to *P2RY8-CRLF2* fusion, which we previously described as prognostically relevant in our patient population (Cario *et al.* Blood 2010;115:5293-5397) and detected similar combinatorial effects as described above. Consequently, we defined a group by presence of *IKZF1* deletion and at least one additional deletion in *PAX5*, *CDKN2A*, *CDKN2B* or *PAR1*. This group comprising 6% of B-lineage ALL patients was termed *IKZF1plus* and had a very poor clinical outcome: 5y-EFS 55%±0.07 compared to 85%±0.01 in *IKZF1plus*-negatives (p<0.0001); 5y-CIR 42%±0.07 compared to 11%±0.01 (p<0.0001). In multivariate analyses including MRD, slow early response, prednisone response, ETV6/RUNX1 status, and WBC (≥100.000/µl), *IKZF1plus* displayed the highest hazard ratio for relapse (3.29; 95% CI 2.01-5.38; p<0.001).
Summary and Conclusion: The previously described effect on clinical outcome of GATA3 SNP rs3824662 is due to its association with distinct prognostic leukemic lesions. Combinatorial analysis of deletions in *IKZF1*, *PAX5*, *CDKN2A*, *CDKN2B* and *PAR1* allowed description of a very poor prognostic subgroup of ALL – termed *IKZF1plus* – with significantly worse outcome compared to the use of *IKZF1* deletion or others as a sole marker. The definition of *IKZF1plus* is likely to aid in the practical implementation of newly detected markers for risk stratification in childhood ALL. Grant support: TRANSCALL (EU FP7 - TRANSCAN, FKZ01KT1312), GACR-P302/12/G101, UNCE204012.

S1311

MINIMAL RESIDUAL DISEASE STRATIFICATION BY NEXT GENERATION SEQUENCING PROFILING OF IMMUNOGLOBULIN GENE REARRANGEMENTS AS AN ALTERNATIVE TO CLASSICAL QPCR-BASED TECHNIQUE IN CHILDHOOD ALL

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Background: MRD negativity at both day 33 and 78 is required for stratification into standard risk (SR) group in pediatric BFM protocols. Quantitative PCR (qPCR) for immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements had been standardized within the EuroMRD consortium. Next generation sequencing (NGS) of Ig/TCR repertoire is a rapidly expanding method that enables monitoring of the malignant clone, but can also show the picture of normal lymphoid cells during the treatment. Moreover, the NGS would avoid qPCR optimization for every patient, which should markedly reduce costs and handling time.
Aims: To establish the detection of multiple Ig heavy chain (IGH) rearrangements using the Ion Torrent PGM/Ion Proton platforms and to compare the NGS results with qPCR at the crucial stratification time points used in childhood BFM trials.
Methods: The sequencing libraries were created from 450 ng of bone marrow DNA mixed with 50 ng of polyclonal DNA using multiplex PCR with IGH FR3 BIOMED-2 primers. The sequencing adapters together with different multiplex identifiers allowing for parallel sequencing of different samples were introduced and sequencing was performed using the Ion Torrent 318 and Ion Proton PI chips. Bioinformatic algorithm was developed to define numbers of reads with V-D-J marker sequences selected according to their clonality in the diagnostic sample among total reads of expected size.
Results: In total 56 samples (28 x day 33, 28x day 78) from 28 patients treated according to Interim BFM 2007 protocol were sequenced with median coverage of 428,648 reads. Thirty-six samples (64%) were concordantly negative by NGS and qPCR. Five samples (9%) were negative by NGS and positive by qPCR and four samples (7%) were negative by PCR and positive by NGS (Fig.1). This would cause a shift from IR to SR in 4 patients, from SR to IR in 2 patients and from slow early response (SER) group to IR in one patient. In the 11 double-positive samples the quantitative values correlated, but the MRD levels were higher in NGS than in PCR in all but one sample (median 7x, range 1-124x). This was likely caused by the fact that NGS quantitates leukemic IGH rearrangements within the B cells only while qPCR is performed in a bulk of all mononuclear cells. Importantly, the quantitative differences would not change the stratification into treatment risk groups in any patient. After correction for percentage of $CD19^{pos}$ cells assessed by flow cytometry (FC) at respective time points the correlation with qPCR improved (R^2=0.77).

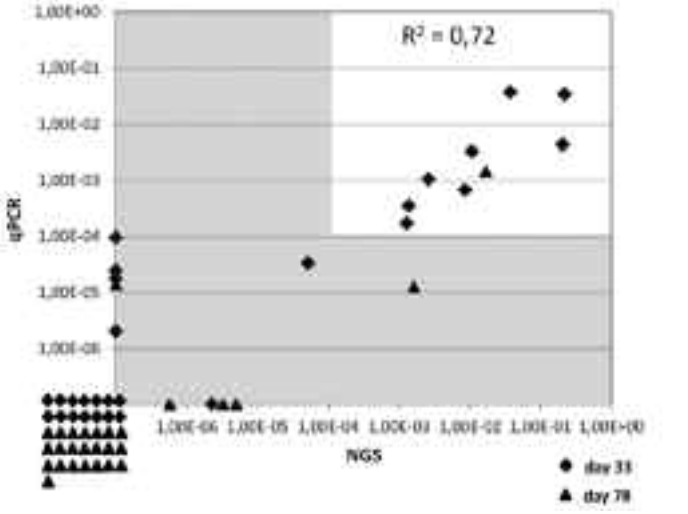

Figure 1.

Summary and Conclusion: In the NGS design we used, the sensitivity of NGS was comparable to qPCR, showing the most differences in the "grey zone" consisting of weakly positive samples. This caused a shift between risk groups in 7/28 (25%) patients, mostly from IR to SR. More investigated samples are needed to find out whether the differences in the risk group assignment would have clinical consequences. The NGS has a great potential for replacing antigen receptor-based qPCR in clinical decisions for ALL. At present, the main

drawback of Ig/TCR-exploring NGS methods is lack of standardization both in the experimental setting and in data analysis. Therefore, recently a European network, the EuroClonality NGS Consortium, has been formed to standardize the whole workflow of analytics, pre-analytics and bioinformatics not only for MRD quantification but also for clonality assessment in lymphoid neoplasms and for repertoire analysis. Supported by IGA NT14343, NT/12397 and GACR P302/12/G101.

S1312

HIGH-THROUGHPUT SEQUENCING TO DETECT MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Trials indicate that for Acute Lymphoblastic Leukemia patients, minimal residual disease (MRD) is a better predictor of relapse risk than pre-treatment risk factors. Several methods are used clinically to detect MRD including flow cytometry and qPCR. However, flow detects MRD in the range of 1:10,000 cells while the more sensitive qPCR assays need to be personalized for each patient. Amplifying and sequencing, at high-throughput, rearranged TCR and Ig CDR3 chains has the potential to be a universal assay with high-sensitivity.
Aims: Here we test the utility of detecting MRD using high-throughput sequencing (HTS) of the variable regions of T-cell receptor Beta (TCRB) and Immunoglobulin Heavy chain (IGH).
Methods: Pre-treatment and 29 days post-treatment samples were collected at University of Washington's Hematopathology Laboratory from 43 patients diagnosed with T-ALL and 99 patients diagnosed with B-ALL enrolled in the Children's Oncology Group AALL0434 and AALL0932 trials, respectively. As part of the trials, multi parametric flow cytometry (mpFC) was used to monitor MRD. Adaptive Biotechnologies developed and offers a method that amplifies rearranged TCR and BCR CDR3 sequences and uses high-throughput sequencing (HTS) to sequence tens of thousands of chains simultaneously, named immunoSEQ. Adaptive used the immunoSEQ assay to amplify and sequence the TCRB or IGH repertoire, dependent on diagnosis, from unselected de-identified residual material. MRD detected using HTS immunoSEQ assay was not used for clinical decisions.
Results: Pre-treatment samples were used to identify and define clonal TCRB or IGH CDR3 sequences. Twenty-nine days following treatment, samples were evaluated for MRD by surveying the TCRB or IGH repertoire for the presence and frequency of the previously identified clonal sequence(s). These data were compared to mpFC data that was used to identify abnormal T or B lymphoblasts for both pre and post samples. For patients diagnosed with T-ALL; using HTS, 31 of the 43 samples had a clonal TCRB and by mpFC, five of the 43 cases had a pre-treatment immunophenotype compatible with early thymic precursor (ETP) T-ALL and nine had an ETP-like immunophenotype that we designate as, "near-ETP". All five of the ETP phenotype samples and six of the nine samples with a nETP phenotype lacked a TCRB clone by HTS. In the 31 post-samples for which there was a pre-treatment TCRB clone, HTS detected MRD in 22 of the 31 samples while mpFC detected MRD in 12 of the 31 samples. For patients diagnosed with B-ALL using HTS detected a clonal IGH rearrangement in 91 of the 98 pre-treatment samples. In the 91 post-samples for which there was a pre-treatment IGH clone, HTS detected MRD in 51 of the 91 samples while mpFC detected MRD in 23 of the 91 cases. For both patient groups, HTS detected MRD in every case for which mpFC detected MRD. However, HTS detected MRD in an additional 10 samples for the T-ALL study and 28 cases in the B-ALL study.
Summary and Conclusion: HTS is a viable technology to detect clonality and track MRD in T-ALL and could allow lower detection thresholds for MRD.

S1313

IMMUNOPHENOTYPIC STUDIES IN INFANT ACUTE LEUKEMIA

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Background: Acute leukemia (AL) in children less than 1 year old is the relatively rare disease with specific biological features and poor outcome. It is also characterized by high incidence of *MLL* gene rearrangements. Immunophenotype of infants' leukemia varies due to presence or absence of *MLL* gene rearrangements.
Aims: Description of immunophenotype in infant acute lymphoblastic and acute myeloid leukemia (ALL and AML respectively).
Methods: Totally 540 cases of pediatric AL were studied. 113 patients (77 with ALL, 31 with AML and 5 with MPAL) aged from 5 days to 11 months were included in the study group. Their data was compared to 427 cases of acute leukemia in older children. Tumor cells immunophenotyping was performed by 6-8-color flow cytometry. Detection of various types of *MLL*-gene rearrangements was done by fluorescence in-situ hybridization, reverse-transcriptase polymerase chain reaction (PCR) and long-distance inverse PCR.
Results: ALL was found less frequently in infants than in older children (68.1% and 86.9% respectively, p<0.001) while percentage of acute myeloid leukemia cases was higher in infants (27.4% and 11.5% respectively, p<0.001). Significant immunophenotypic differences were observed in patients with and without *MLL* gene rearrangements in both ALL and AML. Number of ALL cases in those tumor cells expressed CD10, CD20, CD45, CD133, CD15, CD65 NG2 significantly varied between *MLL*-positive and *MLL*-negative groups (p<0.001, p<0.001, p=0.002, p<0.001, p=0.004, p=0.019 and p<0.001 respectively). NG2-positivity represented the highest overall correct prediction (OCP) rate for presence of *MLL*-rearrangements (90.6%). Diagnostic accuracy of CD20-negativity and CD45-positivity was lower (81.2% and 81.9% respectively) while OCP for CD10-negativity (76.4%), CD133-positivity (76.5%) CD15-positivity (67.7%) and CD65-positivity (53.7) was not sufficient enough. Nevertheless CD10-positive BCP-ALL with *MLL*-rearrangements differed from CD10(+) cases in *MLL*-germline group. CD10 homogeneous expression was noted frequently in *MLL*-germline cases *MLL*-rearranged than in ones (p=0.001). Although there were found no significant differences in CD22-positive patients' number, CD22(+)-cells percentage was significantly lower in *MLL*-positive cases (median 89.9%, range 25.2-99.7% and median 99.9%, range 96.0-99.9% respectively, p=0.003). Thus CD20-negativity, CD10-negativity/low expression, high CD45, CD15, CD65, CD133 and NG2 expression, decreased CD22-expression are immunophenotypic signatures of *MLL*-rearranged infant ALL, although NG2 has the highest diagnostic efficacy. Interestingly CD10-negativity and positivity for CD34, CD15 and CD65 could be able to distinguish *MLL-AF4*-positive cases from patients carrying other types of *MLL*-rearrangements. Number of AML cases in those tumor cells expressed CD99, CD133, CD15, CD65, CD4, CD56, CD61, NG2 varied between *MLL*-positive and *MLL*-negative groups ((p=0.019, p=0.012, p=0.002, p=0.008, p=0.013, p=0.012 and p<0.001 respectively). Thus CD61-negativity, high CD99, CD15, CD133 and NG2 expression were immunophenotypic signatures of *MLL*-rearranged infant AML, although NG2 and CD65 had the highest diagnostic efficacy (89.7% and 83.3% respectively).
Summary and Conclusion: Thus immunophenotype of AL in children less than 1 year old differs significantly from patients of older age groups. Infants' ALL and AML immunophenotype varies greatly due to the presence of *MLL* gene rearrangements. Complex diagnostic immunophenotyping of infants' AL allows predicting presence of *MLL* rearrangements and NG2 is the most applicable single marker.

S1314

EFFECTS OF THE BITE® ANTIBODY BLINATUMOMAB ON MOLECULAR RESPONSE IN A PHASE 2 OPEN-LABEL, MULTICENTER CONFIRMATORY STUDY IN RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (R/R ALL)

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Background: Molecular response, determined by evaluation of minimal residual disease (MRD), is a strong predictor of outcomes in de novo ALL but limited data are available for adult r/r ALL. Blinatumomab, an investigational bispecific T-cell engager (BiTE®) antibody, directs T cells to $CD19^+$ B cells, resulting in serial lysis. In an exploratory study, 80% of patients (pts) with MRD-positive ALL were MRD-negative within 1 cycle of blinatumomab. The study described herein has evaluated efficacy and toxicity of blinatumomab in adult r/r ALL (results from the primary analysis are presented separately).
Aims: The objective of this analysis was to evaluate the efficacy of single-agent blinatumomab in terms of molecular response in adult pts with r/r ALL.
Methods: Eligible pts (≥18 yrs old) had Ph-negative r/r ALL, including primary refractory disease after standard induction, 1st salvage in early 1st relapse

(within 12 months), relapse within 12 months of allogeneic stem cell transplantation (SCT), refractory or later relapses. Pts received blinatumomab by continuous IV infusion (4 wks on/2 wks off), for up to 5 cycles at a dose of 28 µg/d (cycle 1 only: 9 µg/d on days 1-7, 28 µg/d on days 8-28). The primary endpoint was complete remission (CR) or CR with partial hematologic recovery (CRh*: platelets >50,000/µL, neutrophils >500/µL) within the first 2 treatment cycles (assessed on day 29 of each cycle). MRD in bone marrow was assessed using allele-specific real-time quantitative PCR for clonally rearranged Ig and/or TCR genes, with a sensitivity of at least 10^{-4} (central laboratory). MRD response (fewer than 10^{-4} detectable blasts) and complete MRD response (no detectable blasts) within the first 2 treatment cycles were exploratory endpoints.
Results: 189 pts from 37 centers in Europe and the US were treated; median (range) age was 39 (18–79) yrs. 64 pts (34%) had prior SCT. 81 pts had a CR (n=63) or CRh* (n=18) during the first 2 treatment cycles. 17 pts had a morphologically blast-free or aplastic bone marrow. 73 of 81 pts (90%) with CR/CRh* and 10 of 17 pts (59%) with blast-free bone marrow had evaluable MRD results. Of those, 60 pts (82%) with CR/CRh* had an MRD response; 51 (70%) had a complete MRD response. MRD and complete MRD responses occurred in pts with CR and CRh*. 5 of the 10 pts (50%) with blast-free marrow and CR/CRh* had an MRD response (**Table**). Among pts who achieved CR/CRh*, the rate of MRD response was 81% in pts without and 85% in pts with prior SCT. Across the study population, the most frequent grade ≥3 adverse events regardless of causality included febrile neutropenia (25%), neutropenia (16%), and anemia (14%).
Summary and Conclusion: This is the largest adult patient population with r/r ALL to date with PCR-based MRD assessment using a central laboratory. The method was feasible in the setting of an international multicenter trial. High MRD response rates (82%) were observed in pts with CR or CRh*, and a proportion of pts with blast-free bone marrow showed MRD response. Analyses of MRD in subtypes of relapses, the role of MRD assessment in different types of cytologic response, and the impact of MRD response on outcome are ongoing.

Table 1.

Response	Pts with best hematologic response and MRD data n	Pts with MRD response n (%)[a,b]	Pts with MRD complete response n (%)[a,b]
CR/CRh*	73	60 (82)	51 (70)
CR	58	50 (86)	43 (74)
CRh*	15	10 (67)	8 (53)
Blast-free hypoplastic or aplastic bone marrow	10	5 (50)	2 (20)

[a]During first 2 cycles; [b]Percentage based on number of pts with MRD data

Chronic myeloid leukemia - Biology

S1315

RECURRENT KIT D816V MUTATION IN ATYPICAL CHRONIC MYELOID LEUKEMIA

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Background: Atypical Chronic Myeloid Leukemia (aCML) is a heterogeneous disorder belonging to the group of myelodysplastic/myeloproliferative syndromes. Since 2013, no recurrent somatic mutation was specifically associated with aCML. By applying Next Generation Sequencing (NGS) technologies on 8 aCML cases, we demonstrated the presence of recurrent somatic mutations in the *SETBP1* gene. These mutations were identified in approximately 30% of aCML cases, which suggested the presence of other yet unknown pro-oncogenic events in the remaining 70%
Aims: To gain insight into the molecular lesions responsible for the occurrence of the *SETBP1*-negative aCML cases, we applied whole-exome and transcriptome sequencing to a total of 16 matched samples taken at onset of the disease
Methods: Whole-exome and transcriptome sequencing data were generated using an Illumina Genome Analyzer IIx following standard library-prep protocols. Alignment to the reference GRCh37/hg19 genome was performed using BWA. Alignment data were processed using Samtools. Single nucleotide and small indels detection was performed using in-house software. Copy number analyses from whole-exome data were generated using CEQer and gene fusions transcriptome data were screened using FusionAnalyser
Results: The application of NGS techniques to a large cohort of aCML cases led to the identification of a somatic, non-synonymous single-nucleotide mutation (chr4:g.55599321A>T) in the *KIT* gene in 1/16 (6%) cases. At protein level this mutation translated into the D816V variant. KIT D816V has been already found in several clonal disorders, among them in systemic mastocitosis, gastrointestinal stromal tumors and acute myeloid leukemia and it is responsible for the constitutive activation of the pro-oncogenic tyrosine kinase activity of the KIT protein. To assess whether the mutation identified by NGS was recurrent, we performed targeted Sanger sequencing on a larger cohort of aCML patients, comprising a total of 52 cases. This analysis revealed the presence of *KIT* mutations in additional 3 patients, indicating that they are recurrent in aCML. Notably, all the *KIT* mutations identified so far translate to the D816V variant at protein level, suggesting that the activation of the KIT tyrosine kinase signaling plays an important role in this subset of aCML patients. It is known from the literature that KIT D816V is highly sensitive to the tyrosine kinase inhibitor dasatinib. To test if dasatinib was able to impair cell growth of the leukemic clone in KIT+ aCML cases, we performed *ex vivo* tritiated thymidine proliferation assays on bone marrow cells from one of the KIT D816V aCML patients in presence of either dasatinib, imatinib or vehicle alone: the proliferation assay showed that dasatinib was able to inhibit the proliferation of the leukemic clone with an IC50 of 1nM, while neither imatinib nor vehicle alone were able to significantly impair cell growth. In line with these data, western blot with an Anti-Phospho-KIT antibody on KIT+ lysates after treatment with increasing concentration of dasatinib showed that the drug was highly effective in inhibiting KIT autophosphorylation.

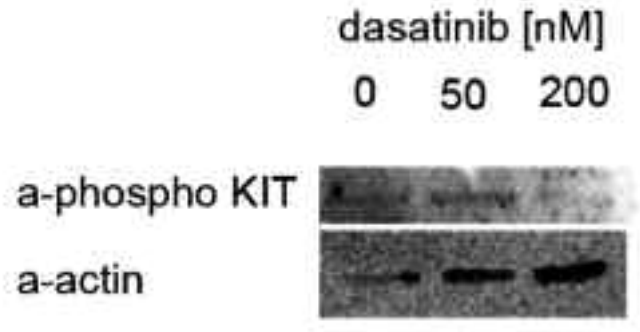

Figure 1.

Summary and Conclusion: These data indicate that KIT D816V is a pro-oncogenic lesion recurrently present in aCML, albeit with low frequency (4/68, 6%) and that aCML cells bearing this mutation are highly sensitive to dasatinib, *in vitro*. Given the very poor prognosis of this disorder, these findings suggest a new, highly efficient targeted treatment for a subset of aCML patients.

S1316

TRANSCRIPT LEVELS OF THE HEDGEHOG PATHWAY MEMBERS PTCH1 AND SMO ARE PREDICTIVE OF IMATINIB FAILURE IN PRE-TREATMENT CHRONIC MYELOID LEUKAEMIA

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Background: Chronic myeloid leukaemia (CML) is characterised by the *BCR-ABL1* fusion gene and resultant dysregulated tyrosine kinase oncoprotein. First-line treatment is the tyrosine kinase inhibitor (TKI), imatinib (IM), to which 60-70% of patients will have an adequate response. 2nd generation TKIs are available, which exhibit greater potency, but their use upfront is controversial because of their adverse event profiles and greater cost. Biomarkers for IM response are therefore needed to optimise TKI usage upfront; the current best indicator of response is *BCR-ABL1* level by RT-qPCR at 3 months from start of treatment. *PTCH1* and *SMO* are genes encoding protein members of the Hedgehog signalling pathway. Previous work showed that gene-expression levels of *PTCH1* are predictive of IM response in CML and since signalling via SMO is inhibited by non-Hedgehog ligated PTCH1, it was reasoned that transcript levels of *SMO* would also be predictive. It was also hypothesised that since 2nd generation TKIs, such as dasatinib (DAS), are more potent BCR-ABL1 inhibitors, and their failure is characterised more by intolerance than progression, gene-expression biomarkers would be therefore less predictive in DAS recipients.
Aims: To evaluate PTCH1 and SMO as biomarkers for response to TKI therapy in CML
Methods: 438 pre-treatment cDNA samples from patients enrolled on the SPIRIT2 clinical trial (randomised to IM 400mg or DAS 100mg) were utilised, with appropriate consent. Custom TaqMan probes and primers for *PTCH1* and *SMO* were used in multiplex RT-qPCR reactions with 3 control genes (*GUSB*, *B2M* and *18S*) and run in fast-mode on a Step-One-Plus analyser. The geometric mean of the control genes was used to generate a relative expression metric using the delta-Cq method. The data were analysed separately for the IM and DAS, with TKI failure free survival (TFFS) as the primary endpoint; patients failing treatment because of intolerance were excluded from analysis. Patients were grouped into good and poor responders by attainment of<9.84% *BCR-ABL1* (international scale, IS) at 3 months and receiver operating characteristic (ROC) used to determine optimal expression cut-off for high and low categorisation. Outcome data from each expression group were then assessed by Kaplan-Meier (K-M) analyses to determine if gene expression, either alone or in combination, was predictive for TFFS.
Results: 354 patients were assessable (180 IM and 174 DAS); 145 IM and 190 DAS were<9.84% IS by 3mnth RT-qPCR (good); 46 IM and 14 DAS were >=9.84% (poor). The median delta-Cq for the IM good and poor groups were: *PTCH1,* 1.088 and 0.0062, respectively (P=0.006); SMO, 0.0997 and 0.1622 (P=0.22); *PTCH1/SMO* ratio, 9.642 and 2.786 (P=0.008). For the DAS good and poor groups delta-Cq: *PTCH1*, 0.9350 and 0.4460, respectively (P=0.038); *SMO*, 0.0972 and 0.1804 (P=0.15); PTCH1/SMO, 7.109 and 2.749 (P=0.093). The ROC cut-offs for IM were:<0.51 for *PTCH1*, >0.16 *SMO*; and<2.81 *PTCH1/SMO*. For DAS, the ROC cut-offs were:<0.47 *PTCH1*, >0.11 *SMO* and<3.31 *PTCH1/SMO*. K-M analysis of the IM patients showed that high *PTCH1* expression (P=0.029), low SMO expression (P=0.013) and high *PTCH1/SMO* ratio (P<0.0001; Figure 1A) were predictive of TFFS with hazard ratios of 2.002, 0.4522 and 3.328, respectively. The K-M for DAS showed that neither the single genes nor the ratio were predictive (all P>0.05; Figure 1B).

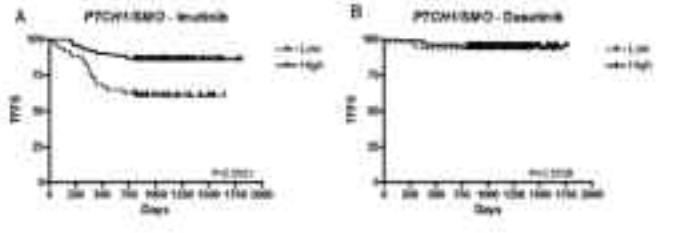

Figure 1. Kaplan-Meier analysis showing TKI Failure Free Survival (TFFS) of patients on A: imatinib and B: dasanitib, with high and low PTCH1/SMO expression ratio.

Summary and Conclusion: High *PTCH1* and low *SMO* expression levels are both predictive for response to IM and this predictive power seems to be enhanced by using the two in combination (higher hazard ratio and lower P-value). There was no predictive power for patients on DAS, suggesting that the greater potency of this TKI was able to overcome the inherent genetic disposition to IM failure. There is a need for inexpensive and robust assays to assist in the implementation of stratified medicine in CML. In this study, we demonstrate a simple multiplex RT-qPCR assay that can be used to identify those patients who may be expected to experience a sub-optimal response to IM and who therefore may benefit from a more potent 2nd generation TKI upfront.

S1317

PRO-ATHEROGENIC AND ANTI-ANGIOGENIC EFFECTS OF NILOTINIB ON ENDOTHELIAL CELLS: A POTENTIAL MECHANISM TO EXPLAIN VASCULOPATHIES IN CML PATIENTS TREATED WITH NILOTINIB

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Background: The BCR/ABL1 inhibitor nilotinib is increasingly used to treat patients with chronic myeloid leukemia (CML). However, nilotinib has been associated with metabolic changes, including an increase in the fasting glucose level. In addition, vascular adverse events, including peripheral arterial occlusive disease (AOD), have been reported in nilotinib-treated patients.
Aims: We reviewed and updated AOD events in our CML patients receiving nilotinib (n=34) and initiated *in vitro* and *in vivo* studies to evaluate potential targets and mechanisms.
Results: After a median observation time (MOT) of 24 months, the frequency of AOD (26.5%) and severe AOD requiring surgical intervention and/or prolonged hospitalization (17.6%) was higher in nilotinib-treated patients compared to risk factor-, observation time-, and age-matched controls (34 imatinib-treated patients with CML, 34 with myelodysplastic syndromes, 34 with JAK2-mutated MPN and 34 with lymphoid neoplasms;<5% AOD, p<0.05). After a MOT of 36 months, the frequency of AOD amounted to 36.1% and the frequency of severe AOD was 19.4%. We next examined the *in vitro* effects of nilotinib on cultured human umbilical vein endothelial cells (HUVEC), human coronary artery-derived endothelial cells (HCAEC), and the human microvascular endothelial cell line HMEC-1. Nilotinib was found to inhibit the proliferation of endothelial cells in a dose-dependent manner, with pharmacologically relevant IC_{50} values obtained in HUVEC (1.0 µM), HCAEC (100 nM), and HMEC-1 (1.0 µM), whereas imatinib showed little if any effect. Moreover, nilotinib was found to inhibit the migration of HUVEC in a wound-scratch-assay as well as angiogenesis in a tube-formation assay (relative capillary tubes: VEGF+control: 1.8±0.1, VEGF+nilotinib (100 nM): 1.3±0.1, VEGF+imatinib (100 nM): 1.7±0.05; n=3, p<0.01 for VEGF alone *vs* VEGF+nilotinib). In a mouse model of hindlimb ischemia, nilotinib (75 mg/kg/day p.o. for 28 days) was found to slow blood flow-recovery after induction of ischemia whereas imatinib (100 mg/kg/day p.o. for 28 days) showed no comparable effect (laser Doppler perfusion imaging ratio ischemic/control leg: control mice: 0.81±0.03, imatinib-treated mice: 0.79±0.04, nilotinib-treated mice: 0.68±0.04; n=13/group; p<0.05 for nilotinib *vs* control and for nilotinib *vs* imatinib). The decreased blood perfusion was accompanied by an increased rate of limb necrosis (necrosis score: control: 1.15±0.08, imatinib: 1.17±0.05, nilotinib: 1.54±0.18; p<0.05 for nilotinib *vs* control and nilotinib *vs* imatinib). Moreover, microvessel density was significantly lower in the affected hind limb in nilotinib-treated mice compared to imatinib-treated mice (p<0.05). In addition, we found that nilotinib, but not imatinib, promotes the expression of pro-atherogenic cytoadhesion molecules on HUVEC, including ICAM-1, VCAM-1 and E-Selectin. As assessed by proteomics profiling and phospho-array analysis, several angiogenesis-related antigens, including TEK, ABL2, JAK1, and BRAF were identified as targets of nilotinib, whereas imatinib did not bind to these proteins in endothelial cells. Neither nilotinib nor imatinib showed *in vitro* or *in vivo* effects on platelet adhesion or platelet aggregation.
Summary and Conclusion: In conclusion, nilotinib exerts multiple effects on vascular endothelial cells, presumably through multiple mechanisms and targets. We hypothesize that these effects may contribute to nilotinib-induced vasculopathy in CML.

S1318

IDENTIFICATION OF RECURRENT GENETIC ALTERATIONS ASSOCIATED WITH BLAST CRISIS TRANSFORMATION IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic Myeloid Leukemia (CML) is caused by the appearance of the BCR/ABL fusion gene, as a result of a reciprocal translocation between chromosome 9 and 22 t(9;22)(q34;q11). The hybrid gene translates into an oncogenic fusion protein which is characterized by the constitutive activation of its tyrosine kinase activity, being responsible for malignant transformation. CML evolves from a druggable chronic phase (CP) to an aggressive advanced phase (blast phase-BP). Despite the aggressiveness of BP and the poor overall survival of BP patients, little is known about the molecular mechanisms responsible for the progression of the disease.

Aims: To gain insight into the molecular lesions responsible for BP, we present here the results obtained from the whole-exome and RNA-seq analysis of paired CP/BP CML samples from patients that underwent progression to blast crisis after standard therapy. The access to matched CP/BP samples renders this data highly valuable.

Methods: We performed whole-exome sequencing and RNA-seq analysis using high-throughput technologies (Illumina Genome Analyzer IIx) from genomic DNA and total RNA of paired samples. The cross-match between BP and CP exomes was performed with dedicated in-house software. The mutations identified were confirmed through standard sequencing methodologies. Evaluation of copy number abnormalities was achieved using CEQer (Comparative-Exonic-Quantification-Analyzer) while fusions were analysed by using FusionAnalyser.

Results: We evaluated 11 paired CP (used as a control) and BP samples by comparing exome-sequences. The median time between CP and BP samples was 36 months (range 1-73 months). We found a total of 38 single nucleotide mutations occurring in BP samples that were absent in the paired CP, corresponding to an average of 3 mutations/patient (range of 0-8 mutations). Most of the mutations found (n=21) scored positively in the GeneRanker software. By using this approach we found recurrent, somatic, single nucleotide mutations in *RUNX1* and *UBE2A* in 2 out of 10 BP samples. Detailed data are presented in Table 1. CEQer analysis of 6 matched BP/CP exomes reveals the presence of 6 chromosomal alterations. In one of the patients without acquired mutations we found the presence of a Philadelphia chromosome duplication. Data obtained after CEQer analysis also indicated that 2 out of 6 patients had complete loss of chromosome 7. In patient #1 and #4 respectively, two major regulators of the cell cycle control (CDKN2A and p53) were also deleted. The analysis of RNA-seq data of 6 patients with FusionAnalyser did not show any new fusion event specific for transformation.

Table 1.

Summary and Conclusion: Our study shows the presence of a limited number of acquired mutations in the blast crisis samples when compared with matched chronic phase. Notably, we were able to find 2 recurrently mutated genes associated with blastic transformation, RUNX1 and UBE2A, with the last one never been detected in CML samples. Ongoing analysis on additional BP/CP samples and *in vitro* experiments will help to clarify the role of UBE2A mutations in CML progression.

S1319

MYC::MAX HETERODIMER ACTS ON BCR PROMOTER TO MODULATE BCR AND BCR-ABL EXPRESSION

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Background: Chronic Myeloid Leukemia (CML) is caused by the BCR/ABL fusion gene, the product of is so called Philadelphia (Ph) chromosome. If untreated CML progresses within 3 years from a mild and easy to control form, called chronic phase (CP), into an aggressive and deadly acute leukemia called blast crisis (BC). BC is marked, as any acute leukemia, by a complete differentiation block and the ensuing accumulation of blasts with frequent over-expression of BCR/ABL protein. After the oncogenic translocation, the BCR-ABL gene is under the transcriptional control of BCR promoter. In spite of all the research performed in the field, the molecular mechanisms involved in the regulation of BCR promoter are mostly unknown Therefore we need to understand what are the transcription factors or the mechanisms that regulate BCR-ABL expression.

Aims: To identify the transcription factors which are binding to the BCR promoter and modulate BCR-ABL expression.

Methods: *In silico* analysis was performed to identify putative binding sites of transcription factors on BCR promoter through Jasper database (http://jaspar.genereg.net/), and TRANSFAC Biobase (http://www.biobase-international.com/product/transcription-factor-binding-sites).Chromatin Immunoprecipitation assays were used to confirm *in silico* data. To determine the role of selected transcription factors on BCR and BCR/ABL expression, the K562 BCR/ABL positive CML cell line was transfected with specific eukaryotic expression vectors. mRNA and protein expression level of BCR and BCR-ABL were detected by real time PCR and Western blotting. BCR reporter activity was also analyzed by luciferase assay.

Results: Several putative protein binding sites (PBS) for different transcription factors have been identified along the sequence of BCR promoter by *in silico* analysis. Criteria for selecting the transcription factors (TF) for subsequent studies were: 1) TF have role in haematopoiesis or differentiation of hematopoietic progenitors; 2) TF binds to a DNase protected area of BCR promoter. Hereby, we found that **MYC and MAX proteins** putatively bind to BCR promoter at four different loci: PBS1 (- 1354 to -1341 from ATG codon), PBS2 (-1283 to -1263) PBS3 (- 813 to -801) and PBS4 (-768 to -756). By using Chromatin Immunoprecipitation we confirmed the binding of MYC and MAX on BCR promoter to all the above mentioned sites. Quantitative PCR and Western Blot showed that, when overexpressed, MYC::MAX heterodimer significantly up-regulates the BCR (MYCMAX 2.33±1.08) and BCR-ABL (MYCMAX 2.61±0.3) expression in comparison with K562 empty cells. This induction seems to be stronger when both the MYC and MAX gene are over-expressed (BCR expression in MAX: 0.75±0.2, MYC: 1.40±0.4, MYCMAX 2.33±1.08) and BCR-ABL (MAX: 1.28±0.20, MYC: 2.32±0.4, MYCMAX 2.61±0.3). By using a luciferase reporter assay we saw 3.5 fold increase in the luciferase activity when K562 MYC::MAX transfectants were cotrasfected with the full BCR promoter (-1444 base pair from the ATG starting site cloned in the pGL3 promoter comprising all the 4 putative PBS) in contrast to K562 not over-expressing MYC::MAX. This indicates that MYC::MAX overexpression induces an up-regulation of BCR promoter activity, when all the four putative MYC::MAX binding sites (PBS1, PBS2, PBS3 and PBS4) are present. In contrast when PBS3 and PBS4 were deleted, a minimal decrease of luciferase activity was observed, thus identifying these two regions as critical for BCR and BCR/ABL regulation.

Summary and Conclusion: These data demonstrate that binding of MYC::MAX heterocomplex to the BCR promoter leads to upregulation of BCR and BCR-ABL expression at both transcriptional and protein level. Since MYC is frequently over-expressed in BC, this phenomenon could be responsible for the BCR/ABL upregulation and thus ensuring differentiation block.

Acute myeloid leukemia - Biology 2

S1320

CONSTITUTIVE EXPRESSION OF IRF8 INHIBITS MN1 INDUCED AML: ROLE OF IMMUNE GENES IN DIFFERENTIATION

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Background: Acute myeloid leukemia (AML) is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation, due to sequential accumulation of multiple rare genetic or epigenetic events. Functionally, disrupted transcription factors are one of the most important causes of abrogated differentiation of hematopoietic stem cells. MN1 (a transcriptional regulator) protein is one such factor, whose overexpression serves as a prognostically important factor in AML patients. Overexpression of MN1 induces AML in mice and causes resistance to cytarabine and all-trans-retinoic acid (ATRA) induced cytotoxic/differentiation effects.
Aims: To investigate whether MN1 plays a role in myeloid differentiation through transcriptional activation or repression, we fused transcriptional activation (VP16) and repression (M33) domains with MN1 for functional analyses. In order to evaluate, whether re-expression of repressed genes (Ccl9 and Irf8) could overcome the MN1-induced leukemia in mice, we overexpressed Ccl9 and Irf8 in MN1 expressing cells and evaluated their effects *in vitro* and *in vivo*.
Results: Transcriptional activation of MN1 target genes by the MN1VP16 fusion gene induced myeloproliferative disease with long latency (median 146 days compared to 35 days for MN1 expressing cells, P<0.001), and full differentiation potential to mature neutrophils *in vivo*. Conversely, transcriptional repression of MN1 target genes by the MN1M33 fusion gene induced AML with the same latency as MN1 (median 39days, P=0.6). Gene expression profiling identified a large proportion of differentially expressed genes between leukemic MN1 and differentiation-permissive MN1VP16 cells that belonged to the immune response pathway including Irf8 and Ccl9. As MN1 is a co-factor of the transcription factors MEIS1 and RARA, we compared chromatin occupancy between these genes. Immune response genes that were upregulated in MN1VP16 cells were co-targeted by MN1 and MEIS1, but not RARA, suggesting that myeloid differentiation is blocked through transcriptional repression of shared target genes of MN1 and MEIS1. Constitutive expression of Irf8 or its target gene Ccl9 identified these genes as potent inhibitors of MN1-induced leukemia. Ectopic expression of Irf8 and Ccl9 in MN1 leukemic cells induced partial differentiation, prevented leukemic outgrowth and significantly extended survival of mice (P<0.001). Specifically, 60% of the mice transplanted with MN1+Irf8 showed no leukemic phenotype and 40% mice developed myeloproliferative disease but not AML. In addition, we utilized NOD/SCID/IL2rg^{null} mice model to preclude the role of adaptive immunity for the leukemic inhibition, wherein we observed that inhibition of leukemia by IRF8 was independent of an immune response.
Summary and Conclusion: Our data shows that MN1 prevents activation of the immune response pathway, and suggests restoration of Irf8 signalling as a novel therapeutic mechanism in AML.

S1321

CD99 IS A STEM CELL MARKER AND THERAPEUTIC TARGET IN AML/MDS

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Background: The myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are initiated and sustained by self-renewing stem cells. These cells are likely resistant to conventional therapies, and they may represent the reservoir of minimal residual disease (MRD) that leads to disease relapse.
Aims: We sought to identify aberrant cell surface proteins on MDS and AML disease-initiating cells since they may serve as biomarkers of disease status as well as serve as potential therapeutic targets.
Methods: Flow cytometry was performed on *de novo* and therapy-related MDS and AML samples to assess cell surface expression of CD99, originally identified in a prior transcriptome analysis of purified hematopoietic stem cells (HSCs) from MDS patients. AML cell lines overexpressing CD99 cDNA or CD99 shRNA were transplantation into sublethally irradiated, NSG immunodeficient mice or evaluated in transendothelial migration assays. Anti-CD99 clones H036-1.1 and 12E7 were obtained from commercial sources. For cytotoxicity assays, AML cell lines and primary cells were incubated with these antibodies for 72 hrs, with cell number quantitated by flow cytometry.
Results: Flow cytometry of MDS patient bone marrow (BM) samples (n=26) confirmed that CD99 is frequently increased on MDS HSCs (85%). Assessment of 78 paired diagnosis/relapse AML specimens revealed that CD99 is also frequently overexpressed at diagnosis (81%) and relapse (83%). Providing support for CD99 as a leukemia stem cell (LSC) marker in AML, CD99 surface expression is higher in the LSC-enriched CD34+CD38- fraction compared to bulk blasts (p=0.003), as well as in relapsed as compared to diagnostic specimens (p=0.007). In addition, CD99 high cells exhibit a CD34+CD38-CD90-CD45RA+ LMPP-like immunophenotype, previously shown to be enriched for LSC activity. To determine the function of CD99 in AML, we transduced MOLM13 AML cells with a CD99 shRNA (8.0-fold knockdown). NSG mice transplanted with these cells showed improved survival compared to vector controls (58d *vs*. 34d, p=0.02). Consistent with its role in leukocyte trafficking, AML cell lines overexpressing CD99 showed increased transendothelial migration in transwell assays, and CD99 expression was higher on AML patient PB blasts than BM blasts (p=0.03). Unexpectedly, CD99 transcript expression in the ECOG 1900 AML patient cohort (n=308) positively correlated with survival (p=0.001). We propose that CD99 may improve survival in the context of chemotherapy by promoting mobilization and thus chemosensitivity of AML cells. To determine whether CD99 may be therapeutically target, we tested the ability of monoclonal antibodies (mAb) against CD99 to induce direct cytotoxicity *in vitro*. Anti-CD99 mAbs induced significant cell death in 11 AML and two MDS-derived cell lines, as well as in primary AML blasts (n=7) and MDS HSCs/CD34+ cells (n=3), with relative sparing of primary human HSCs and endothelial cells. Pre-coating of primary human LSCs with anti-CD99 mAb prior to transplantation into NSG mice led to impaired engraftment (20% *vs*. 67%, p=0.009) and improved survival (p=0.05). Immunofluorescence demonstrated that mAb ligation promotes clustering of CD99, and this clustering appears to be critical for cytotoxicity. Anti-CD99 mAb treatment was also associated with activation of Src-family kinases, and inhibitors of Src signaling attenuate anti-CD99 mAb induced cytotoxicity.
Summary and Conclusion: Our results establish CD99 as a cell surface marker expressed in AML and MDS stem cells, as a mediator of transendothelial migration, and as a promising therapeutic target for direct targeting by mAbs. Further studies are needed to evaluate the potential of targeting CD99 in AML/MDS *in vivo* and to further characterize the mechanisms of CD99 mediated cell death.

S1322

GENOME-WIDE DNA METHYLATION PROFILING REVEALS A RARE CPG ISLAND METHYLATOR PHENOTYPE IN AML

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Background: Acute myeloid leukemia (AML) represents a clonal myeloid stem cell disorder. The combination of differentiation arrest and excessive proliferation of the immature progenitor pool results in the accumulation of non-functional progenitor cells in the bone marrow and peripheral blood where they hamper the production or functions of normal blood cells. It has become evident that besides genetic alterations, epigenetic changes play an essential role in leukemogenesis.
Aims: To increase our understanding of how DNA methylation may affect leukemic transformation in AML, and to define marker genes for improved patient outcome prediction, diagnosis and prognosis we performed a systematically genome-wide screening of DNA methylation profiles.
Methods: Initially we analyzed the DNA methylation status of about 23.000 CpG islands of 28 acute myeloid leukemia samples using the Methyl-CpG-Immunoprecipitation assay combined to CpG island microarrays (MCIp-chip). These profiles were then used to select a subset of aberrantly methylated CpG islands (380 regions covering 15.000 individual CpGs) for quantitative DNA methylation profiling in a larger cohort of 185 AML patients (50% normal karyotype) using MALDI-TOF analysis of bisulfite-treated DNA.
Results: Meta analysis clearly separated a subgroup of CpG island regions showing highly correlated DNA methylation changes that were also marked by histone H3 lysine 27 trimethylation in normal hematopoietic progenitor cells (HPC). The residual group of CpG islands not targeted by polycomb group (PcG) repressors in HPC displayed heterogeneous methylation patterns across patients that (in contrast to the PcG target group) clustered with genetic markers, including oncofusion proteins (CBFB-MYH11, AML-ETO), or certain mutations (NPM1, CEBPA, IDH1/2). A fraction of AML patients (5/185) displayed aberrant hypermethylation at almost all studied loci, representing a rare CpG island methylator phenotype (CIMP) in AML. These patients present immature

leukemia (FAB MO, M1) with various chromosomal aberrations but very few mutations (*e.g.* no IDH1/2, KRAS, DNMT3A) that might explain the CIMP phenotype. The patient group that we identified shows high resemblance with a recently reported CEBPA methylated subgroup displaying a similar strong methylation signature (Wouters et al, 2007 and Figueroa et al, 2009). DNA methylation analysis revealed that most of these CEBPA-silenced cases fall into our CIMP category. To further characterize this interesting group of hypermethylated patients from both studies we performed in-depth global DNA methylation analyses (MCIp-seq) and detected more than 3000 genes that were specifically methylated in the patients of the CIMP-cluster. Interestingly, hypermethylated gene promoters represent many transcriptional regulators that are involved in the differentiation of myeloid lineages (including CEBPA, CEBPD, IRF8, GATA2, KLF4, MITF or MAFB). In addition, these patients frequently show hypermethylation of the TET2 promoter, which could result in a loss of maintenance DNA demethylation and successive hypermethylation at CpG islands.

Summary and Conclusion: It is likely that the aberrant silencing of key lineage regulators facilitates/stabilizes the differentiation arrest in these cells, and that these patients may particularly benefit from therapies that revert DNA methylation. To test whether the DNA methylation phenotype correlates with common genetic alterations we are systematically analyzing and correlating genetic and epigenetic alterations of these patients.

S1323

REQUIREMENT FOR CDK6 IN MLL-REARRANGED ACUTE MYELOID LEUKEMIA

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Background: Chromosomal rearrangements involving the H3K4 methyltransferase MLL trigger aberrant gene expression in hematopoietic progenitors and give rise to an aggressive subtype of acute myeloid leukemia (AML). Insights into MLL fusion-mediated leukemogenesis have not yet translated into better therapies, because MLL is difficult to target directly and the identity of the genes downstream of MLL whose altered transcription mediates leukemic transformation are poorly annotated.

Aims: To search for essential signaling pathways in MLL-rearranged AML that might serve as new therapeutic targets, we performed loss-of-function RNA interference (RNAi) screens in 5 AML cell lines (NOMO-1, THP-1, OCI-AML3, HL-60, U937) using a lentiviral short hairpin RNA (shRNA) library.

Results: We observed that MLL-AF9-driven NOMO-1 and THP-1 cells are exceptionally reliant on the cell cycle regulator CDK6, but not its functional homolog CDK4. The enhanced CDK6 dependence of MLL-rearranged cells was confirmed in an expanded panel of AML cell lines (MLL-rearranged, n=6; MLL wildtype [WT], n=4) that also included cell lines harboring alternate MLL fusions (MLL-AF4 and MLL-AF6), and the RNAi-induced phenotype was countered by overexpression of an shRNA-resistant *CDK6* cDNA. Stable knockdown of MLL-AF9 in MLL-AF9-positive cell lines and overexpression of MLL-AF9 in WT MLL-expressing cell lines, normal human CD34-positive cells, or Ba/F3 murine pro-B cells led to concordant changes in CDK6 mRNA and protein levels that resembled those of HOXA9, a known MLL-AF9 target. Furthermore, chromatin immunoprecipitation-sequencing analysis demonstrated that MLL-AF9 binds to the *Cdk6* locus in mouse bone marrow (BM) cells transduced with MLL-AF9 and in MLL-AF9-driven murine AML, indicating that CDK6 is rendered essential via direct targeting by truncated MLL. Analysis of cell cycle, apoptosis, and myeloid differentiation demonstrated that the differential growth-inhibitory effect of CDK6 suppression was mainly attributable to myeloid differentiation, as MLL-rearranged cell lines upregulated CD11b expression and assumed a more mature, macrophage-like morphology upon CDK6 knockdown, effects not observed in WT MLL-expressing cells. Furthermore, the immature phenotype of MLL-rearranged cells was rescued by overexpression of an shRNA-resistant *CDK6* cDNA. Knockdown of Cdk6 also impaired the proliferation and *in vitro* clonogenic activity of primary murine BM cells stably transduced with MLL-AF9, whereas cells expressing another leukemogenic fusion gene (*MOZ-TIF2*) and Ba/F3 cells were largely unaffected. We also expressed MLL-AF9 in unfractionated BM derived from *Cdk6* knockout mice and observed that colony numbers were gradually reduced in cultures initiated with $Cdk6^{+/-}$ and $Cdk6^{-/-}$ BM compared to WT BM. Finally, depletion of Cdk6 was found to overcome the differentiation block of MLL-AF9-driven AML and prolonged survival in a murine BM transplantation model of MLL-AF9-induced leukemia. The context-dependent effects of lowering CDK6 expression were closely phenocopied in cell lines and primary human AML specimens by palbociclib (also known as PD-0332991), a small-molecule inhibitor of CDK4 and CDK6 enzymatic activity that is in clinical development as an anticancer agent.

Summary and Conclusion: These data identify CDK6 as critical effector of MLL fusions in leukemogenesis that might be targeted to overcome the differentiation block associated with MLL-rearranged AML, and underscore that cell cycle regulators may have distinct, non-canonical and non-redundant functions in different contexts.

S1324

REVERSE ENGINEERING OF HEMATOPOIETIC TRANSFORMATION: DECONSTRUCTING MIXED LINEAGE LEUKEMIA BY GENOME WIDE TECHNIQUES

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Background: For a complete understanding of the pathomechanism leading to a neoplastic disease it is necessary to elucidate the primary events that convert a normal differentiating cell into a malignant self-renewable entity. Simply cataloging the differences between the end-stage of the neoplastic development and normal tissue suffers from several pitfalls. Tissue and tumor heterogeneity, the unknown nature of the "normal" target for transformation as well as the multi-tiered nature of biochemical processes with unclear inter-dependencies makes the identification of the primary causative events very cumbersome. Using mixed lineage leukemia (MLL) as an example we are using a "reverse engineering" strategy to avoid this problems enabling us to pinpoint the earliest changes that cause a normal hematopoietic cell to deviate towards a transformed fate.

Aims: MLL is caused by the presence of MLL fusion proteins. These aberrant transcription factors are potent leukemogenic agents that are created by chromosomal translocations joining the N-terminus of the histone methyltransferase MLL to a variety of different fusion partners. As a consequence a transcriptional elongation and chromatin modification complex is recruited to an unknown number of target genes leading to ectopic expression and eventually a block in differentiation. Here we wanted to elucidate the genome-wide genetic changes that accompany transformation by a MLL fusion protein.

Methods: We used a genetically modified mouse with an inducible knock-in of a MLL fusion into the natural MLL locus. With this model we were able to generate large numbers of hematopoietic cells harboring a single transforming mutation. Inactivation of the conditional MLL fusion leads to reversion of the neoplastic phenotype enabling access to a matched malignant/normal population thus obviating the need to identify and isolate the rare primary target of initial transformation. We employed genome wide techniques of ChIP-Seq and nascent RNA-Seq to determine changes in RNA synthesis rate, chromatin modification and transcription factor occupancy during this reversal to normal.

Results: A kinetic sampling across several time points allowed a hierarchical classification of the observed changes and an exact correlation to the physiological state of the cell. In this way a comprehensive list of 109 MLL target genes could be assembled. Next to well-known genes under control of MLL fusions like the HOXA and HOXB cluster and their cooperation partners MEIS1 and PBX3 a significant number of hitherto unknown targets could be identified. Most importantly the kinetic data allowed the identification of an "early changing" group that can be seamlessly assembled into a molecular network controlling cell cycle and differentiation. Biochemical studies probing the significance of individual net-nodes for the overall transformation process are under way.

Summary and Conclusion: In summary we present a generally applicable method to deconvolute the tangled development towards a neoplastic state. This approach may potentially reveal sensitive points for therapeutical intervention.

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S1325

THE JAK-INHIBITOR RUXOLITINIB SUBSTANTIALLY AFFECTS NK CELL BIOLOGY

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Background: Ruxolitinib is a potent JAK-inhibitor, approved for the treatment of myelofibrosis (MF). It exerts strong anti-inflammatory activities, thereby improving splenomegaly and cytokine-mediated symptoms but also increases infection rates in these patients. NK cells are critical for cancer-immune surveillance and cytokine-mediated signals are central for proper NK cell activation.
Aims: We aimed to characterize in detail the effects of JAK inhibition on human NK cells by phenotypic and functional analysis.
Methods: Highly purified NK cells from human peripheral buffy coats were exposed to increasing concentrations of ruxolitinib (0.1-10 µM). Cytokine (1000U/ml IL-2, 25ng/ml IL-15)-induced NK cell proliferation was analyzed by CFSE dilution. Phenotypic and functional NK cell activation markers (NKp46, NKG2D, Granzyme B, CD16, and CD69) were analyzed by flow cytometry (including CD107a expression for degranulation). NK cell function was tested by flow-cytometry-based killing assays and quantification of IFN-γ production upon stimulation with either MHC class I-deficient K562 target cells or cytokines (IL-12, IL-18). In addition, phenotypic and functional analyses were also tested during NK receptor activation via plate-bound activating NKp46 antibodies. Signaling events were analyzed by phospho-flow technology to evaluate ruxolitinib-mediated changes of cytokine-dependent signalling cascades (pS6, pSTAT1, pSTAT3, pSTAT5, pERK, pAKT, pP38, and pZAP70). Synapse formation of ruxolitinib exposed NK-92 cells towards the target cell K562 was tested either by flow cytometry with differentially labelled cells or by confocal microscopy with additional staining of perforin and filamentous actin.
Results: Our results provide first evidence that ruxolitinib profoundly affects cytokine-induced NK cell activation. This includes a significant and dose-dependent reduction of NK cell proliferation, reduced induction of activation-associated surface markers as well as impaired killing activity against the classical NK target cell line K562. In addition, all main functional activities of NK cells are down-regulated as shown by reduced cytotoxic capacity, impaired degranulation, IFN-γ production and synapse formation without affecting cell viability. After wash-out, the inhibitory effects of ruxolitinib on NK cells are fully reversible, as shown by proper re-activation by cytokines. In contrast to cytokine-mediated NK cell activation, stimulation *via* the NK-specific receptor NKp46 is not affected by ruxolitinib. On a molecular level, phospho-flow analyses revealed that cytokine associated signaling events, such as phosphorylation of STAT5 and S6 were dose-dependently reduced by ruxolitinib in primary human NK cells.
Summary and Conclusion: Ruxolitinib strongly inhibits NK cell activation leading to impaired proliferation and functional activity. Experiments verifying these effects in patients are currently ongoing. Our findings may have important clinical implications, when considering the application of ruxolitinib as GvHD therapy as NK cells are critically involved in the GvL effect after allogeneic stem cell transplantation.

S1326

THE KI-1 ANTIGEN (CD30) IS A NOVEL MARKER AND POTENTIAL THERAPEUTIC TARGET IN ADVANCED SYSTEMIC MASTOCYTOSIS

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Background: Systemic mastocytosis (SM) is a hematologic neoplasm characterized by increased expansion and pathologic accumulation of neoplastic mast cells (MC) in various organs. In most patients, neoplastic cells exhibit the transforming *KIT* mutation D816V. The Ki-1 antigen (CD30) is an established therapeutic target in Hodgkin´s lymphoma and anaplastic large cell lymphoma. Recent data suggest that neoplastic MC in patients with aggressive SM (ASM) and mast cell leukemia (MCL) express substantial amounts of cytoplasmic CD30.
Aims: In the present study, we examined the expression of CD30 in various human MC lines (HMC-1, MCPV) and in primary neoplastic MC in patients with indolent SM (ISM, n=11) and advanced SM (ASM, n=5; MCL, n=2). In addition, we asked whether CD30 serves as a potential therapeutic target in SM.
Results: As assessed by flow cytometry, neoplastic MC were found to express cell surface CD30 in most patients with SM, namely in 10/11 patients with ISM and in 4/7 patients with ASM/MCL. The immature, RAS-transformed MCL line MCPV (KIT D816V-negative) also expressed cell surface CD30, whereas the MCL line HMC-1.1 (lacking KIT D816V) expressed only low amounts of CD30, and the HMC-1.2 subclone (KIT D816V+) was found to stain negative for CD30. We next applied the CD30-targeting drug brentuximab vedotin (provided by Millennium: The Takeda Oncology Company, Cambridge, MA, USA) which is an antibody-conjugate consisting of a chimeric anti-CD30 antibody and the microtuble inhibitor monomethyl auristatin E (MMAE). Brentuximab vedotin has recently been described to induce dose-dependent growth-arrest in human CD30+ lymphoblastic cell lines. In the present study, brentuximab vedotin was found to inhibit the proliferation of MCPV and HMC-1 cells in a dose-dependent manner, with lower IC50 values found in CD30+ MCPV and HMC-1.1 cells (5 µg/ml) than in CD30− HMC-1.2 cells (10 µg/ml). As assessed by AnnexinV/PI staining and staining for active-caspase-3, brentuximab vedotin also induced dose-dependent apoptosis in MCPV and HMC-1.1 cells, but did not induce substantial apoptosis in CD30− HMC-1.2 cells. Brentuximab vedotin also induced growth inhibition and apoptosis in the CD30+ canine-mastocytoma cell line C2. Furthermore, brentuximab vedotin was found to downregulate anti-IgE induced histamine release in basophils. We next examined the effects of brentuximab vedotin on *in vitro* survival (apoptosis) of primary neoplastic MC in patients with CD30− SM (n=3) and in patients with CD30+ SM (n=3). In these experiments, brentuximab vedotin was found to induce dose-dependent apoptosis in neoplastic MC in patients with CD30+ SM at pharmacologically relevant concentrations, whereas no drug effects were seen in patients with CD30− SM. Finally, we examined the effects of a drug combination consisting of brentuximab vedotin and the KIT D816V-targeting drug PKC412 (midostaurin). We found that both drugs synergize with each other in inhibiting the *in vitro* proliferation of CD30+ MCPV cells.
Summary and Conclusion: In conclusion, our data provide evidence that CD30 is expressed on the surface of neoplastic MC in patients with indolent and advanced SM. Our data also show that the CD30-targeting antibody brentuximab vedotin induces growth arrest and apoptosis in neoplastic MC and synergizes with midostaurin in inhibiting the growth of CD30+ neoplastic MC. Whether these effects also occur *in vivo* in patients with advanced mastocytosis, remains to be determined in clinical trials.

S1327

THE EPIGENETIC READER BRD4 SERVES AS A NOVEL MARKER AND TARGET IN AGGRESSIVE SYSTEMIC MASTOCYTOSIS AND MAST CELL LEUKEMIA

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Background: Advanced mast cell (MC) neoplasms are characterized by uncontrolled growth and rapid expansion of neoplastic MC in various organ systems and a poor prognosis. In human patients, advanced systemic mastocytosis (SM) is rare and usually presents as aggressive SM (ASM) or mast cell leukemia (MCL). Unfortunately, so far, no effective treatment concept has been established for these patients. This is due to the fact that neoplastic MC in advanced SM usually are resistant against various cytoreductive agents. Therefore, research is currently focusing on potential drug targets and the effects of novel targeted drugs on neoplastic MC. We have recently identified the bromodomain-containing protein-4 (BRD4) as a promising new drug target expressed by neoplastic cells in acute myeloid leukemia (Zuber et al, Nature 2011;478:524-528).
Aims: In the present study, we examined BRD4 as a potential therapeutic target in ASM and MCL
Methods: Immunocytochemistry (ICC) and immunohistochemistry (IHC), Flow cytometric evaluation of apoptosis, qPCR,^{3}H-thymidine uptak, Flow cytometry
Results: As assessed by immunohistochemistry, neoplastic MC expressed substantial amounts of cytoplasmic and nuclear BRD4 in ASM and MCL, whereas in indolent SM (ISM), MC expressed low amounts of BRD4 or did not express cytoplasmic BRD4. All human MCL lines tested, including HMC-1.1 (KIT D816V-), HMC-1.2 (KIT D816V+), $ROSA_{wt\ KIT}$ (KIT D816V-) and $ROSA_{KIT\ D816V}$ (KIT D816V+) also expressed cytoplasmic BRD4. In order to study the

potential biological function of BRD4, we applied a BRD4-specific shRNA. The shRNA-induced knockdown of BRD4 was found to be associated with a markedly reduced proliferation of neoplastic MC. Correspondingly, JQ1, a BRD4-targeting drug, induced dose-dependent growth inhibition in all MC lines examined, with IC50 values of about 1.0 μM. As assessed by morphology and AnnexinV/PI staining, JQ1 also produced apoptosis in neoplastic MCs (Figure). The apoptosis-inducing effect of JQ1 was also demonstrable by staining for active caspase 3. Furthermore, JQ1 was found to downregulate the expression of CD63 and CD71 in HMC-1 cells, and CD63, CD71 and FceRI in ROSA cells. Moreover, JQ1 was found to suppress proliferation of primary neoplastic MCs obtained from patients with ASM or MCL (IC_{50} 0.1-0.5 μM). We next screened for potential drug partners that would potentiate the anti-neoplastic effects of JQ1. Of all drugs tested, all-trans retinoic acid (ATRA) and PKC412 were identified as most potent drug partners of JQ1. These drugs were found to act highly synergistic with JQ1 in producing apoptosis and growth inhibition in HMC-1.1 and HMC-1.2 cells (Figure).

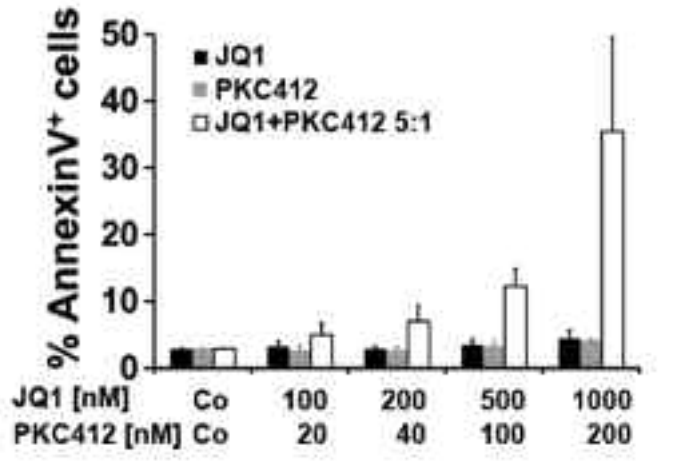

Figure 1.

Summary and Conclusion: Together, BRD4 is a novel marker and promising target in advanced SM. Whether JQ1 or other BET bromodomain inhibitors are effective *in vivo* in patients with advanced SM or canine MCT remains to be elucidated.
Legends to Figure: HMC-1.2 cells were incubated in control medium (Co) or in various concentrations of JQ1 (black bars), PKC412 (gray bars), or a combination of both agents at a ratio of 5:1 (white bars) at 37°C for 48 hours. Then, AnnexinV/propidium iodide (PI) staining was performed. The percentage of AnnexinV+ cells was determined by flow cytometry. Results represent the mean±S.D. of 3 independent experiments.

S1328

MUTATIONAL PROFILE IN ESSENTIAL THROMBOCYTEMIA (ET) « NEGATIVE » FOR JAK2 AND MPL

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Background: Until recently, in about 40% of ET, the driver mutations targeting signaling pathway responsible for thrombocytosis were unknown. Indeed, JAK2 V617F is present in about 50% of ET and primary myelofibrosis (PMF) patients, *MPL* mutations, particularly (W515), in 3%>5% ET and PMF and mutations in *SH2B3* and *CBL* in around 1%. In addition, mutations in other pathways are rare in ET and usually associated with mutations in *JAK2* or *MPL*. These additional mutations may play a role in initiation of the disease but also in disease progression.
Aims: Find out new driver mutations responsible of the thrombocytosis
Methods: 36 ET and 3 MF patients were studied who were selected on the absence of JAK2V617F and MPLW515K/L mutations in the granulocytes, except one PMF patient with a MPLW515K, whose sample was used as a positive control. Whole Exome Sequencing (WES) was performed on granulocytes (tumoral sample) and on T cells (germline control). An exon capture sequencing was performed with Agilent technique using a HiSeq 2000 in granulocytes and T cells. Identified mutations were validated by Sanger sequencing and the *CALR* mutations by Sanger sequencing and high-resolution sizing of fluorescent dye–labeled PCR products.
Results: We performed sequencing of 39 patients by WES with a mean depth of 100. A total of 119 mutations were confirmed by Sanger sequencing, with a median of 2 mutations per patients (from 0 to 15). In the ET patients, most of the mutations found concerned genes involved in signaling. We found that 21 patients presented *CALR* mutations with a majority of 52pb deletion in exon9. This type of mutation appeared as a 1bp deletion in WES analysis, even if the mutation was present at homozygous state in one patient. In contrast, the insertion of 5bp could be correctly detected. 3 patients displayed previously described MPL mutations, two in exon 10 (MPL W515R, MPL S505N), and one in exon 4 (S204P). Furthermore, 1 patient showed a homozygous nonsense mutation in *SH2B3*. In addition, we identified 2 new mutations in genes coding for PI3K pathway, both associated with *CALR* mutations. We also found mutations in epigenetic regulators such as *DNMT3A, ASXL1, TET2, IDH1* and *SETDB1* and in spliceosome or translation such as *SF3B1, SRSF1* and *PHF6*, but generally in the context of disease progression (myelofibrosis or myelodysplastic syndrome). Nevertheless, *CALR* mutation was associated either with *ASXL1* or *TET2* mutation in two isolated chronic forms of ET. Seventeen ET patients remain negative for signaling mutations; however 10 of them showed a clonal hematopoiesis in granulocytes. Seven patients had no clonal mutations and were considered as polyclonal although they have all clinico-biological criteria for a myeloproliferative neoplasm. Therefore, we hypothesize that some "polyclonal" ET may have a clonal hematopoiesis, but only in the megakaryocyte lineage. In two of these cases, we detected JAK2V617F in platelets, bringing more arguments in favor of this hypothesis.
Summary and Conclusion: This study further demonstrates that ET are very early disorders in the occurrence of malignancies with a very low numbers of mutations, and that the discovery of *CALR* mutations has drastically reduced the number of patients with unknown "driver" mutations, called triple negative. Some of them may correspond to a clonal hematopoiesis restricted to the platelet/megakaryocyte lineage.

S1329

CHARACTERIZATION OF CULTURED ENDOTHELIAL CELLS FROM PATIENTS WITH POLYCYTHEMIA VERA WITH AND WITHOUT PRIOR THROMBOSIS BY GENE EXPRESSION ANALYSIS

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Background: In polycythemia vera (PV) the major cause of morbidity and mortality are thrombotic complications with frequent splanchnic thrombosis, especially Budd-Chiari syndrome. Association of the JAK2 V617F mutation with thrombosis has been shown in different studies, suggesting an involvement of the malignant clone. In addition to activated thrombocytes and leukocytes, pathological bone marrow-derived endothelial cells (EC) have been shown to be involved.
Aims: Our aim was to investigate EC from patients with PV and to define their role in thrombosis formation.
Methods: Endothelial colony forming cells (ECFC) were cultured from peripheral blood mononuclear cells of 24 patients with PV carrying the JAK2 V617F mutation. Patients were grouped according to the prior occurrence of thromboembolic events. Immunocytochemical staining for vascular endothelial growth factor receptor 2 (VEGFR2) and von Willebrand factor (vWF) was applied to confirm endothelial cell nature. Total RNA was extracted from ECFC. Gene expression analysis of genes related to endothelial cell biology was performed by real-time RT-PCR. ECFC were analyzed for the JAK2 V617F mutation by allele-specific PCR. DNA from blood samples and corresponding ECFC was screened for additional mutations by next generation sequencing (Ion Torrent PGM™). We designed a panel including the genes ASXL1, DNMT3A, EZH2, IDH1, IDH2, JAK2, MPL, SOCS1 and TET2.
Results: The mRNA expression of 29 genes involved in endothelial cell activation and function showed a significant upregulation (>2.5 fold) compared to human umbilical vein endothelial cells (HUVEC). When compared to liver endothelial cells, 22 genes were higher expressed. The expression of the adhesion molecules VCAM-1, ICAM-1, E-Selectin and PECAM-1 was increased up to 1000□fold and also the cytokines TNFα and IL-1β that play a role in the regulation of the adhesion molecules were highly expressed. Many of the adhesion related genes were higher expressed in ECFC of patients with prior thrombosis compared to patients without thrombosis, *i.e.* ICAM-1 (3.7-fold, median) and VCAM-1 (2.2-fold) as well as the cytokines TNFα (3.8-fold) and IL-1β (3.4-fold). PECAM-1 was lower expressed (0.7fold). The JAK2 V617F mutation was present in 8/24 EC cultures. Screening for additional mutations identified mutations in the ASXL1 and SOCS1 genes that were present in blood samples and corresponding ECFC. Five mutations in ASXL1, IDH1 and MPL were detected in ECFC only implying a clonal origin of EC in these patients.
Summary and Conclusion: We found increased expression of genes involved in endothelial cell activation and function in ECFC from patients with PV indicating endothelial cell activation. In particular, adhesion molecules and their regulators were overexpressed. A specific pattern of higher expression of ICAM-1, VCAM-1, and lower PECAM-1 expression was identified in ECFC from

patients with prior thrombosis. In accordance with published results, the JAK2 V617F mutation is only present in a minority of ECFC. Additional mutations in ASXL1 and SOCS1 were identified that could drive malignant hemopoiesis and endothelial cell development from hemangioblasts. In conclusion, our results suggest a contribution of dysfunctional endothelial cells to a prothrombotic state in patients with PV.

Quality of life

S1330

A RANDOMIZED PHASE III TRIAL OF RETINOIC ACID AND ARSENIC TRIOXIDE VERSUS RETINOIC ACID AND CHEMOTHERAPY IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS: HEALTH-RELATED QUALITY OF LIFE OUTCOMES

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Background: Recently, a randomized clinical trial (RCT) compared efficacy and toxicity of ATRA plus chemotherapy *versus* ATRA plus arsenic trioxide (ATO) in patients with newly diagnosed, low-intermediate risk APL (Lo Coco *et al.*, *N Engl J Med*. 2013;369:111-121).
Aims: As potential clinical benefits of newer experimental treatments need to be weighed against possible patients' burden and given the lack of published data on the impact of ATO on patients' symptoms and wellbeing, health-related quality of life (HRQOL) assessment was included as a secondary endpoint of this RCT. We herein report HRQOL findings of this study.
Methods: The study was a prospective**,** randomized, multicenter, open-label, phase-III non-inferiority trial. The study was designed to show that ATRA-arsenic trioxide was not inferior to ATRA-chemotherapy with respect to the event-free survival rate at 2 years. HRQOL was a secondary endpoint of this trial. The EORTC QLQ-C30 was used to assess HRQOL at the end of induction and after the consolidation therapy. This validated questionnaire consists of five functioning scales: physical, role, emotional, cognitive and social; three symptom scales: fatigue, nausea/ vomiting and pain; six single item scales: dyspnoea, sleep disturbance, appetite loss, constipation, diarrhea and financial impact; and the global QOL scale. All analyses were based on the intention-to-treat principle, the groups defined according to the randomly assigned treatment. Primary analysis was performed estimating mean HRQOL scores over time, and their differences between treatment arms by a linear mixed model with an unrestricted covariance structure.
Results: Out of 156 patients analyzed in the primary clinical analysis, 150 were eligible for HRQOL evaluation at the end of induction phase and one hundred forty two at the end of consolidation phase. Overall compliance with HRQOL forms was 80.1%. After induction, 115 HRQOL forms were received out of 150 expected (compliance of 77%) while after consolidation phase 119 forms were received out of 142 expected (compliance of 84%). No statistically significant differences were found in compliance rates between treatment arms. The largest difference, favoring patients treated with ATRA-ATO was found for fatigue severity (mean score difference of -9.3, confidence interval [CI] of -17.8 and -0.7, P=.033), at the end of induction therapy. All other symptoms, except for pain, were favoring patients treated with ATRA plus ATO. Similarly, except for social functioning, all functional and global HRQOL scores favored patients treated with ATRA plus ATO. A comparison by treatment group, each of which *versus* their respective peers in the general population, revealed greater HRQOL impairments in patients treated with ATRA plus chemotherapy in several symptom and functional scales. Large impairments (*i.e.*, effect size (ES) of ≥.8) were noted in physical (ES=.85), role (ES=.83), and social functioning (ES=.84) as well as fatigue (ES=.95) only for patients treated with ATRA plus chemotherapy. HRQOL differences between treatment arms at the end of consolidation showed that, for several scales, differences between treatment arms were marginal.
Summary and Conclusion: The HRQOL benefits of ATRA plus ATO therapy over standard ATRA plus chemotherapy, are mainly relevant at the end of induction phase. Current HRQOL results, extend previous clinical findings, and can help physicians to make more informed treatment decisions on first line therapy for their newly diagnosed APL patients.

S1331

CLINICAL BENEFIT OF LENALIDOMIDE TREATMENT FOR LOW AND INTERMEDIATE-1 IPSS RISK MYELODYSPLASTIC SYNDROME WITH DEL(5Q) BEFORE TRANSFUSION DEPENDENCE

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Background: Lenalidomide is approved for the treatment of transfusion-dependent (TD) patients with anemia due to International Prognostic Scoring System (IPSS)-defined Low- or Intermediate-1- (Int-1)-risk myelodysplastic syndromes (MDS) associated with a deletion of the long arm of chromosome 5 [del(5q)]. Treatment induces erythroid and cytogenetic responses in a high proportion of patients associated with an advantage in survival. Quality of life (QoL) benefit has also been shown in clinical trials. Whether early lenalidomide treatment be of benefit in MDS with del5q transfusion-free (TF) patients with moderate anemia has not been explored.
Aims: To evaluate objective and subjective clinical outcomes in TF patients with MDS with del(5q) treated with lenalidomide.
Methods: Untreated patients (N=381) with MDS and del5q from the report by Germing, et al (Leukemia, 2012) were divided in 3 groups : TD patients (N=316), TI patients with Hb ≥ 10 g/dl (N=88) and TI patients with Hb<10 g/dl (N=96) for the purpose of comparison for survival and incidence of non-leukemic deaths (NLD). Anemic patients with Hb<10 g/dL enrolled in the phase II, multicenter, non-randomized, open-label RevMDS trial on the efficacy and safety of lenalidomide in IPSS Low- or Int-1-risk MDS associated with del(5q) (Oliva et al, 2012) were evaluated for safety, efficacy and changes in QoL during treatment, using the QOL-E instrument. Secondary endpoints included efficacy and survival. Patients received oral lenalidomide 10 mg once-daily continuous dosing for up to 12 months. Twelve patients enrolled were TF at baseline according to current MDS criteria and had a baseline Hb value<10 g/dL with sufficient QoL data to form the subset for the present report.
Results: *Results from the German cohort*: The hazard ratio (HR) of death for the patients with Hb≥10 g/dl *versus* the TD patients was 0.441 (95%>CI: 0.286-0.678, p<0.001).; the HR for the TF patients with Hb<10 g/dl *versus* the TD patients was 0.552 (95%>CI: 0.376-0.810, p=0.002). The HR of non-leukemic death for the TF patients with Hb≥10 g/dl *versus* the TD patients was 0.343 (95%>CI: 0.192-0.614, p<0.001); the HR for the TF patients with Hb<10 g/dl *versus* the TD patients was 0.424 (95%>CI: 0.253-0.709, p=0.001). On the whole, these data pointed to the identification of TI patients with lower Hb levels as an intermediate-risk category. However, there was no significant difference in survival between the two TF groups. *Results from the RevMDS trial*: TF patients experienced earlier Hb increases at 12 weeks (3.6 ± 1.6 g/dl *versus* 1.9 ± 2.1 g/dl, P=0.01) and 24 weeks (4.5±1.6 g/dl *versus* 3.1±2.2 g/dl, P=0.04) compared to TD patients. All deaths occurred only in TD patients. There was no difference in the risk of disease progression between TD and TI. Nine TF patients perceived poor QoL (score<60) at baseline in at least one dimension. Baseline Hb levels correlated with QoL-E physical (r=0.666, p=0.035) and fatigue scores (r=0.604, p=0.049). One patient refused to continue in study because she was discouraged by the drug.-related myelosuppression during the first weeks of treatment. All other patients were erythroid responders (an Hb increase of at least 1.5 g/dL). Overall, within the first 8 weeks, improvements in mean physical QoL scores (45 ± SD 25 to 58 ± SD 26, p=0.068) and fatigue (73 ± 13 to 81 ± 8, p=0.062) were observed. Changes at 12 weeks became significant for physical QoL scores (67 ± 13, p=0.005); in fact, 5 out of 6 patients with decreased baseline QoL experienced improvements in physical QoL.
Summary and Conclusion: Though advantages of early lenalidomide treatment in terms of morbidity and mortality need to be explored in a randomized study, patient reported outcomes (QoL assessment) may guide therapeutic choice. In particular, patients with moderate anemia and with poor physical QoL perceive that they require treatment before transfusion-dependence has taken over. This report highlights the utility of patient-reported outcomes for the guidance of treatment in the individual patient.

S1332

AEROBIC PHYSICAL EXERCISE FOR PATIENTS WITH HAEMTOLOGICAL MALIGNANCIES. A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Though patients with hematological malignancies have to endure long phases of therapy and immobility which is known to diminish their physical performance level, the advice to rest and avoid intensive exercises is still circulating. This recommendation is partly due to severe anemia and thrombocytopenia that many patients suffer from. The inability to perform activities of daily living restricts patients, diminishes their quality of life and may influence medical therapy.
Aims: To evaluate the efficacy and safety of aerobic physical exercise for adult patients suffering from hematological malignancies.
Methods: We searched Cochrane Central Register of Controlled Trials (CENTRAL; 01.2014) and MEDLINE (1950 to 28.01.2014) as well as conference proceedings for randomized controlled trials (RCTs). We included RCTs comparing aerobic physical exercise interventions in addition to standard care to standard care only for adult patients suffering from hematological malignancies. Moreover, we included studies that evaluated aerobic exercise in addition to strength training. We excluded studies that investigated the effect of training programs that were composed of Yoga, Tai chi chuan, or similar types of exercise. We also excluded studies solely exploring the influence of strength training. Two review authors independently screened search results, extracted data and assessed the quality of trials. Two review authors independently screened search results, extracted data and assessed the quality of trials.
Results: We included nine RCTs involving 818 patients. The majority of patients suffered from acute leukemia, lymphoma and multiple myeloma, six trials randomized patients receiving stem cell transplantation. The exercise intervention consisted mostly of walking interventions with divergent duration and intensity levels. There is no evidence for a difference in terms of mortality between patients exercising and those in the control group (RR 0.93; 95% CI 0.59 to 1.47; P=0.75). Four trials analyzed the influence of exercise intervention on quality of life (QoL). Excluding one trial with serious baseline imbalances, physical exercise improves QoL (SMD 0.26; 95% CI 0.03 to 0.49; P=0.03). This positive effect of exercise was also found in the sub-scales physical functioning (SMD 0.33; 95% CI 0.13 to 0.52; P=0.0009) and depression (SMD 0.25; 95% CI -0.00 to 0.50; P=0.05). However, there is no evidence for a difference between additional exercise and standard treatment for anxiety (SMD -0.18; 95% CI -0.64 to 0.28; P=0.45). Seven trials (N=692) evaluated fatigue. There is moderate quality evidence that exercise improves fatigue (SMD 0.24; 95% CI 0.08 to 0.40; P=0.003) (see figure 1). There is no evidence for a difference between both arms in terms of SAEs (RR 1.44; 95% CI 0.96 to 2.18; P=0.08) or AEs (RR 7.23; 95% CI 0.38 to 137.05; P=0.19).

Table 1.

Summary and Conclusion: There is no evidence for differences in mortality between both arms. Physical exercise in addition to standard care can improve quality of life, especially physical functioning and depression, and fatigue. Currently, there is inconclusive evidence regarding anxiety, serious adverse events and adverse events. Further trials with a larger sample-size and longer follow-up periods are needed to further evaluate the effects of exercise interventions for patients suffering from hematological malignancies.

S1333

PSYCHOMETRIC EVALUATION OF EORTC QUALITY-OF-LIFE CORE (QLQ-C30) AND QLQ-MULTIPLE MYELOMA (QLQ-MY20) QUESTIONNAIRES TO ASSESS PHYSICIAN PERCEPTION IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: With the increased use of novel therapeutic agents and extended life expectancy of multiple myeloma (MM) patients, it is extremely important to focus on patients' Health-Related Quality of Life (HRQoL) and physicians' awareness of their patients' well-being. In particular, comparing patient-reported outcomes (PROs) to clinician-reported outcomes (ClinROs) could help physicians better understand patients' perception of their own health status, symptom burden and overall well-being. Validated ClinROs are needed to perform comparisons between patients' and physicians' perceptions of HRQoL using appropriate measures.
Aims: To evaluate the psychometric properties of the EORTC Quality-of-Life Core (QLQ-C30) and QLQ-Multiple Myeloma (QLQ-MY20) questionnaires adapted to be completed by physicians in an observational study in relapsed/refractory MM (RRMM) patients.
Methods: A European, multicenter, observational study is being conducted in RRMM patients starting 2nd or 3rd line treatment. For this study, the EORTC questionnaires QLQ-C30 and QLQ-MY20 were adapted following a rigorous methodology to be completed by physicians in order to evaluate their patients' health status. During the study, physicians responded to both questionnaires at baseline, month 3, and month 6 or discontinuation visit. The QLQ-C30 includes 15 domains (Global Health Status/QOL, Physical Functioning, Role Functioning, Emotional Functioning, Cognitive Functioning, Social Functioning, Fatigue, Nausea and Vomiting, Pain, Dyspnea, Insomnia, Appetite Loss, Constipation, Diarrhea and Financial Difficulties); and the QLQ-MY20 includes four domains (Disease Symptoms, Side-Effects of Treatment, Body Image and Future Perspective). Construct validity was evaluated at baseline by confirming the structure using multitrait analysis and by assessing clinical validity against ECOG performance status. Internal consistency reliability was evaluated at baseline using Cronbach's alpha.
Results: As of November 2013, 32 physicians had enrolled 244 patients in the study who were included in this interim analysis. The mean age was 70, with 53% male and an average time since diagnosis of 3.2 years; 94% of patients started 2nd line treatment. At baseline, 236 (97%) EORTC questionnaires were returned by physicians for the 244 patients included. Both EORTC questionnaires were well completed at baseline: 93% of QLQ-C30 questionnaires were completed by physicians with no missing items, and 92% of QLQ-MY20 questionnaires were completed with no missing items. At baseline, physicians reported the best possible score for a high percentage of patients for the majority of HRQoL and symptoms domains, in particular for Appetite Loss (64%), Constipation (68%), Diarrhea (93%), Dyspnea (59%), Financial Difficulties (72%), Nausea and Vomiting (81%), Insomnia (54%) and Body Image (59%). The structure of multi-item QLQ-C30 and QLQ-MY20 domains was confirmed. Clinical validity of both QLQ-C30 and QLQ-MY20 was established against baseline ECOG performance status (Figure 1). Reliability was confirmed with Cronbach's alpha >0.70, except for QLQ-C30 Nausea and Vomiting (0.61).

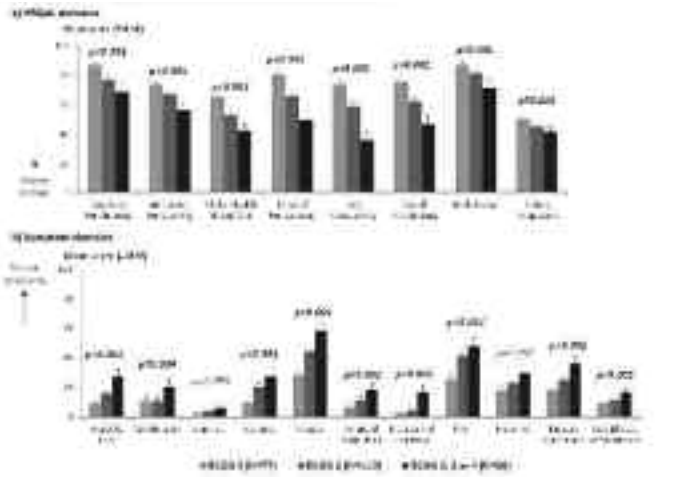

Figure 1. Clinical validity according to ECOG performance status at baseline

Summary and Conclusion: This interim analysis showed that the adapted EORTC QLQ-C30 and QLQ-MY20 questionnaires completed by physicians demonstrated satisfactory psychometric properties in terms of validity and reliability. Further investigation compared the physicians' perception to that of the patients to evaluate if physicians interpret the health of their patients differently from the patients themselves.

S1334

IMPACT OF ECOG PERFORMANCE STATUS ON OVERALL SURVIVAL AND HRQOL IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS FROM THE MM-003 TRIAL OF POMALIDOMIDE + LOW-DOSE DEXAMETHASONE (DEX) *vs* HIGH-DOSE DEX

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Background: Eastern Cooperative Oncology Group performance status (ECOG PS) is often measured in multiple myeloma (MM), but the relevance to patients of improvements in ECOG PS has not previously been shown. ECOG PS data were collected as part of the MM-003 trial comparing pomalidomide and low-dose DEX (POM + LoDEX) with high-dose DEX (HiDEX) in patients with relapsed/refractory MM (RRMM) who failed both bortezomib and lenalidomide treatment.
Aims: 1) Correlate ECOG PS with overall survival (OS) and 2) evaluate the impact of baseline ECOG PS on changes in health-related quality of life (HRQoL) in patients with RRMM from the MM-003 trial.
Methods: A Cox proportional hazards model was analyzed with OS as dependent variable (model 1) and treatment arm (POM + LoDEX *vs*. HiDEX) and ECOG PS improvement from baseline (yes *vs*. no) as covariates. ECOG PS was analyzed as a time-dependent variable. Two other variables associated with OS were added to model 1 as time-dependent variables (model 2) to adjust for progressive disease (PD) (yes *vs*. no) and subsequent POM treatment (yes *vs*. no). Upon unblinding and at PD, HiDEX patients could choose to receive POM treatment—56% of HiDEX patients subsequently received POM. Another analysis compared HRQoL results across time points for patients with ECOG PS 0, 1, or ≥ 2 at baseline, regardless of treatment. Twenty HRQoL domains from a combination of HRQoL instruments collected as part of the MM-003 trial (European Organisation for Research and Treatment of Cancer [EORTC] Quality of Life Questionnaire [QLQ] C30 and MY20 and EuroQol 5 Dimensions [EQ-5D]) were examined: Global Health Status/QoL, Physical, Emotional, Social, Role and Cognitive Functioning, Pain, Fatigue, Nausea/Vomiting, Insomnia, Constipation, Diarrhea, Dyspnea, Appetite Loss and Financial Difficulties (from EORTC QLQ-C30); Disease Symptoms, Side Effects of Treatment, Body Image and Future Perspective (from EORTC QLQ-MY20); and Utility (from EQ-5D).
Results: Both models 1 and 2 showed a significant positive impact of ECOG PS improvement on OS (model 1: HR=0.62 [95% CI, 0.44-0.86], *P*=.004; model 2: HR=0.61 [0.44-0.85], *P*=.004). POM + LoDEX treatment significantly improved OS (model 1: HR=0.75 [0.58-0.95], *P*=.019; model 2: HR=0.62 [0.48-0.79], *P*<.001). In model 2, PD increased the risk of death almost 5-fold (HR=4.97 [2.99-8.25], *P*<.001), whereas crossover lowered the risk of death (HR=0.12 [0.05-0.30], *P*<.001). Differentiating HRQoL by baseline ECOG PS showed an association between better (ie, lower) ECOG PS and better function/reduced symptom burden. Eighteen of 20 HRQoL domains demonstrated associations at baseline, all with the exception of Diarrhea and Financial Difficulties. This association continued across cycles for most HRQoL domains, with interpretation increasingly difficult in later cycles due to reduced sample sizes in the HiDEX arm.

Table 1. Individual determinants of overall survival (Cox proportional hazards model)

	Model 1			Model 2		
	Hazard Ratio	95% CI	P Value	Hazard Ratio	95% CI	P Value
Treatment	0.75	0.58-0.95	.019	0.62	0.48-0.79	<.001
ECOG improvement	0.62	0.44-0.86	.004	0.61	0.44-0.85	.004
PD		NA		4.97	2.99-8.25	<.001
Crossover		NA		0.12	0.05-0.30	<.001

Summary and Conclusion: In MM-003, improvement in ECOG PS was directly associated with improved OS and is a valuable additional endpoint. Better baseline ECOG PS status was associated with better HRQoL at baseline and over time. Similar exploratory analyses should be conducted in future studies to broadly establish the value of ECOG PS as a relevant endpoint in MM.

Myeloma and other monoclonal gammopathies - Clinical 3

S1335

IMPACT OF CONTINUOUS TREATMENT *vs* FIXED DURATION OF THERAPY IN NEWLY DIAGNOSED MYELOMA PATIENTS: PFS1, PFS2, OS ENDPOINTS

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Background: Continuous therapy significantly prolongs remission duration, but chemo-resistant relapse may reduce the duration of subsequent remissions, with negative impact on OS. In such situations, OS rather than PFS is the preferable endpoint. However, this is often deemed not feasible, thus endpoints such as PFS2 should be of importance (EMA guideline). PFS1 defines the time from randomization until the occurrence of 1st relapse. PFS2 defines the time from randomization until the occurrence of 2nd relapse, and incorporates the duration of both 1st and 2nd remissions.
Aims: To compare PFS1, PFS2 and OS in newly diagnosed multiple myeloma (NDMM) patients who received continuous treatment (CT) or fixed duration of therapy (FDT) upfront.
Methods: We included patients enrolled in 3 phase III randomized trials, comparing CT *vs*. FDT upfront (GIMEMA RV-MM-209: lenalidomide-based induction, consolidation, followed by maintenance [CT] *vs* lenalidomide-based induction, consolidation, no maintenance [FDT]; GIMEMA 0305: bortezomib-based induction followed by maintenance (CT) *vs*. bortezomib-based induction, no maintenance [FDT]; MM-015: lenalidomide-based induction, followed by maintenance [CT] *vs*. lenalidomide-based induction/melphalan-prednisone, no maintenance [FDT]). The analysis was performed on the intention-to-treat population. We evaluated PFS1 (time from randomization at diagnosis to 1st relapse), PFS2 (time from randomization at diagnosis to 2nd relapse), and OS (time from randomization at diagnosis to death) through a stratified analysis by protocol. At 1st relapse we tested 2nd PFS (time from 1st relapse to 2nd relapse) and survival from relapse (time from 1st relapse to death).
Results: In the pooled analysis, 604 patients were randomized to CT and 768 patients to FDT. The median follow-up for survivors was 48 months. CT significantly prolonged PFS1 (median 34 *vs*. 19 months, HR=0.52, 95% CI: 0.45-0.60, P<0.001), PFS2 (median 54 *vs* 38 months, HR=0.70, 95% CI: 0.61-0.82, p<0.001) and OS (median OS not reached *vs*. 60 months, HR=0.78, 95% CI: 0.65-0.93, P=0.006) in comparison with FDT. 392 patients who received CT upfront experienced 1st relapse in comparison with 626 patients who received FDT upfront. No differences in 2nd PFS and OS from relapse between patients who received CT or FDT upfront was noticed. Results were similar when the source studies were analyzed separately.
Summary and Conclusion: In NDMM patients, CT significantly improved PFS1, PFS2 and OS. Prolongation of PFS2 suggests that the clinical benefit observed during 1st remission is not cancelled by a very short 2nd remission. PFS2 should be included in all future trials to evaluate the impact of chemo-resistant relapse.

S1336

A PHASE I TRIAL OF SAR650984, A CD38 MONOCLONAL ANTIBODY, IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: SAR650984 (SAR) is a naked humanized IgG1 monoclonal antibody that binds to the CD38 receptor. SAR kills tumor cells via ADCC, CDC, direct apoptosis without secondary crosslinking and allosteric inhibition on CD38 enzymatic activity. Data on relapsed /refractory multiple myeloma (RRMM) patients (pts) in the dose escalation phase of the study are reported. (NCT01084252)
Aims: To determine the safety profile of SAR650984 in heavily pre-treated patients with relapsing and refractory multiple myeloma.
Methods: SAR was given IV weekly (QW) or every 2 weeks (Q2W). Dose levels (DL) 0.3, 1, 3, 5, 10 and 20 mg/kg Q2W and 10 mg/kg QW using the classic 3+3 design were evaluated. All patients signed an IRB approved informed consent
Results: 35 pts with RRMM were treated; median age 64 yrs (40-76); median lines of therapy were 6 (2-14), 34/35 received an IMiD® and a proteasome inhibitor (57% had carfilzomib (C) and/or pomalidomide (P). MTD was not reached at any DL. Adverse events in ≥ 10% of pts at all DL, regardless of causality, were fatigue (48.6%), nausea (34.3%), pyrexia (28.6%), anemia (28.6%), cough (25.7%), headache (25.7%), upper respiratory infection and chills (22.9%), dyspnea (20%), constipation (17.1%), diarrhea and vomiting (14.3%) and bone pain, chest discomfort, muscle spasms, thrombocytopenia and hypokalemia in 11.4% of pts. SAR related ≥ G 3 adverse events included pneumonia (n=3), with hyperglycemia, hypophosphatemia, pyrexia, apnea, fatigue, thrombocytopenia and lymphopenia in 1 pt each. Investigator assessment by EBMT response criteria (ORR ≥ PR) among 34 evaluable pts was 24% (CR n=2, PR n=6). Responses occurred at all DL ≥ 1 mg/kg. Clinical benefit response (≥ MR) was 29% with 41% SD. In the ≥10 mg/kg cohort ORR was 33% (n=6/18) and CBR was 39% (n=7/18). The time to response was 4.6 weeks and time on treatment was 9.9 weeks (2-81). 10 pts remain on treatment. The expansion cohort dose was selected based on efficacy, safety, and receptor occupancy data.

Table 1.

Response EBMT * (n=18)	All DL (n=34)	DL ≥10mg/kg
ORR	(8) 24%	(6) 33%
CBR	(10) 29%	(7) 39%
PD	(10) 29%	(4) 22%
SD	(14) 41%	(7)39%
MR	(2) 6%	(1) 5%
PR	(6) 18%	(4) 22%
CR	(2) 6%	(2) 11%

*Investigator assessment of response as of Dec 31, 2013

Summary and Conclusion: The Maximum Tolerated Dose of SAR650984 was not reached. SAR demonstrates encouraging and durable single agent efficacy in heavily pretreated RRMM pts, including those with prior (C) and (P) and warrants further evaluation.

S1337

AGE-RELATED TRENDS IN AUTOLOGOUS HAEMATOPOIETIC CELL TRANSPLANTATION FOR MULTIPLE MYELOMA IN EUROPE 1991-2010 - A STUDY BY THE EBMT CHRONIC MALIGNANCIES WORKING PARTY

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Background: Autologous haematopoietic cell transplantation (AHCT) has been linked to improved survival in multiple myeloma (MM) and is a standard of care in patients aged<65 years. The role of AHCT in older patients is unclear.
Aims: The aim of this study was to investigate age-related trends in utilisation and outcome of AHCT for MM.
Methods: We analysed 53675 patients from 31 countries and 497 centres reported to the EBMT for undergoing a first AHCT for treatment of MM in 1991-2010. Patients were grouped into four 5-year calendar periods by time of first AHCT (1991-1995, 1996-2000, 2001-2005, and 2006-2010), and into 6 age groups based on their age at the time of AHCT (<40, 40-49, 50-59, 60-64, 65-69, ≥70 years).
Results: Median age at AHCT increased from 52.8 to 59 years (p<.001). The number of patients undergoing a first AHCT increased throughout all 4 calendar periods. The highest proportional increase was observed for patients aged 65-69 and ≥70 years. These two age groups together accounted for 3% of AHCTs in 1991-1995, and for 18.8% in 2006-2010. There was a noticeable increase in patients transplanted in >PR in 2006-2010 (26.6%) compared to previous calendar periods (12.3% in 2001-2005). Utilisation of early 2nd AHCT ≤ 6 months after the 1st transplant, generally indicating a tandem AHCT approach, peaked in 2001-2005 in all age groups. While the number of tandem AHCTs decreased in 2006-2010 in all age groups, patients aged ≥70 years showed the smallest decrease and had the highest rate of tandem transplants in this calendar period. Survival rates after AHCT increased in all age groups from

1991-1995 to 2006-2010. However, we observed considerable differences between age groups in the changes of 2-year and 5-year survival. The greatest improvement in 2-year survival was observed in patients aged 65-69 years (27.6%; from 55.3% in 1991-1995 to 82.9% in 2006-2010). The improvement in 2-year survival progressively decreased with every age bracket and was smallest in patients aged<40 years (3.7%; from 82.2% in 1991-1995 to 85.9% in 2006-2010). Remarkably, a 2-year survival of >80% was observed only in patients<40 years in the first 2 calendar periods, in patients up to the age of 64 years in 2001-2005, and in all age groups in 2006-2010. 5-year survival showed similar increases from 1991-1995 to 2006-2010. However, 5-year survival for patients aged<40 and 40-49 years decreased in the most recent calendar period compared to the previous one. In patients aged<40 years, 5-year survival decreased by 11% (from 72.5% in 2001-2005 to 61.5% in 2006-2010). Over the entire observation period, factors associated with an increased risk of death after AHCT in a proportional hazards regression analysis were higher patient age, earlier calendar period, poorer remission status at AHCT, male gender, and a greater time interval between diagnosis and AHCT (p<.001 for all parameters).

Summary and Conclusion: The data demonstrate that utilisation of AHCT for MM has continued to increase in all age groups in the era of novel agents, predominantly in patients aged 65-69 and ≥70 years. Survival after AHCT has improved considerably more in older than in younger patients, resulting in a substantial narrowing of the gap in survival rates between age groups. Furthermore, our investigation reveals an emerging trend in decreasing late survival after AHCT in the youngest patient groups. AHCT is a commonly used treatment strategy with good outcome in MM patients aged ≥ 65 years.

S1338

EARLY AUTOLOGOUS STEM CELL TRANSPLANTATION IMPROVES SURVIVAL IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: Autologous stem cell transplantation (ASCT) improved outcome compared to conventional chemotherapy in newly diagnosed multiple myeloma (NDMM) patients. The role and timing of ASCT in the era of novel agents is a crucial question. PFS1 defines the time from start of therapy until the occurrence of 1st relapse. PFS2 defines the time from start of therapy until the occurrence of 2nd relapse, incorporating the duration of both 1st and 2nd remission.

Aims: To compare early ASCT *versus* ASCT at relapse in terms of PFS1, PFS2 and OS.

Methods: We analyzed pooled data from two phase III multicenter randomized trials including NDMM patients younger than 65 years (RVMM209: lenalidomide-based induction followed by ASCT *vs* chemotherapy plus lenalidomide consolidation and subsequent lenalidomide maintenance *vs* no maintenance; RVMMEMN441**:** lenalidomide-based induction followed by ASCT *vs* chemotherapy plus lenalidomide consolidation and subsequent lenalidomide-dexamethasone *vs* lenalidomide alone maintenance). In both trials, patients randomized to chemotherapy plus lenalidomide consolidation arm (CC group) who experienced progressive disease during treatment were allowed to receive ASCT at relapse. Patients randomized to ASCT consolidation arm (ASCT group) received treatment at relapse at the physician's discretion. We evaluated PFS1 (time from start of consolidation to 1st relapse), PFS2 (time from start of consolidation to 2nd relapse), and OS (time from randomization at diagnosis to death) through a stratified analysis by protocol. At 1st relapse, 2nd PFS (time from 1st relapse to 2nd relapse) and survival from relapse (time from 1st relapse to death) were evaluated. Intention to treat analysis was performed including patients who were eligible for consolidation. A subgroup analysis of PFS1, PFS2 and OS in patients who received early ASCT *vs* ASCT at relapse according to age, gender, protocol, ISS stage, cytogenetic profile and maintenance regimen was conducted.

Results: A total of 791 patients were enrolled in the two trials and 529 were eligible for consolidation: 268 in the ASCT group and 261 in the CC group. Baseline characteristic were equally distributed in the two groups. Median follow-up for survivors was 44.5 months. Early ASCT significantly improved PFS1 (3-year rate: 59% *vs* 35%, HR 0.48, CI 95% 0.37-0.62, P<0.001) and PFS2 (3-year rate: 77% *vs* 68%, HR 0.59, CI 95% 0.39-0.89, P=0.012), and marginally OS (4-year rate: 83% *vs* 72%, HR 0.64, CI 95% 0.38-1.08, P=0.096) in comparison with ASCT at relapse (Figure). 99 patients in the ASCT group experienced 1st relapsed in comparison with 159 patients in the CC group. 42% of patients in the CC group did not receive ASCT at relapse. No differences in 2nd PFS and OS from relapse between patients in the ASCT group or the CC group was noticed. The advantage of early ASCT was also observed when the two trials were analyzed separately and was confirmed in the subgroup analysis.

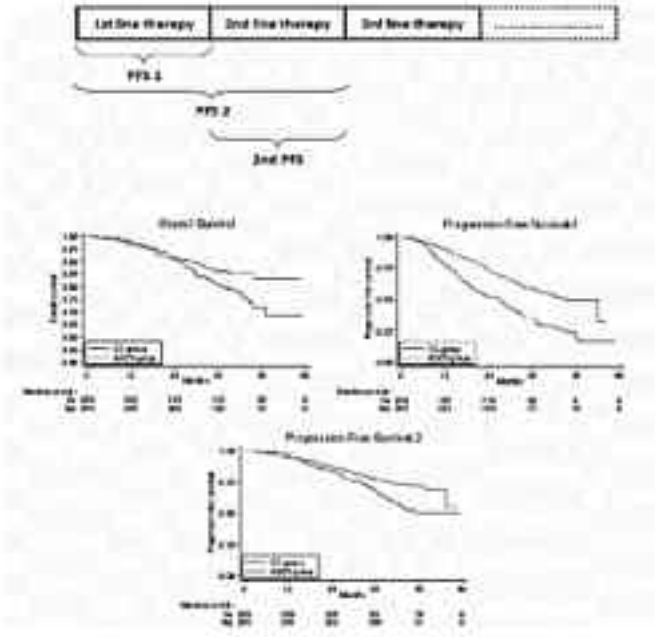

Figure 1.

Summary and Conclusion: In this pooled analysis, early ASCT improved PFS1, PFS2 and OS in NDMM patients. These results confirm the importance of ASCT as 1st line therapy even in the era of novel agents.

S1339

Abstract withdrawn

Chronic lymphocytic leukemia and related disorders - Clinical 2

S1340

DISEASE PROGRESSION ON IBRUTINIB THERAPY IS UNCOMMON AND IS ASSOCIATED WITH THE ACQUISITION OF RESISTANCE MUTATIONS: A SINGLE CENTER EXPERIENCE OF 267 PATIENTS

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Background: The Bruton's Tyrosine Kinase (BTK) inhibitor ibrutinib (I) is very effective in chronic lymphocytic leukemia (CLL), with progression free survival of 76% at 26 months (mo) for patients (pts) with relapsed disease (Byrd et al, NEJM 2013).
Aims: We evaluated pts treated with I to explore features associated with progressive disease (PD) and subsequent outcomes.
Methods: 267 pts from The Ohio State University Comprehensive Cancer Center participating in 3 Institutional Review Board approved trials of I were included; 196 pts received single agent I and 71 received I plus ofatumumab. Adjusting for type of therapy, a proportional hazards model was fit using forward selection to identify variables associated with time to discontinuation of study as measured from the date of first treatment. Similarly, Fine and Gray models of cumulative incidence were fit to identify variables associated with a particular type of failure and in the presence of competing risks. Pts who had not discontinued study were censored at date of last contact; pts who went off study for transplant or to continue treatment elsewhere (n=6) were also censored at that time. A subset of pts with PD had Ion Torrent deep sequencing (DS) performed on peripheral blood at baseline and relapse.
Results: With a median follow-up of 16.6 mo (<1 mo-42 mo), 201 pts remain on I, and 66 have discontinued due to PD (24), infection (22), toxicity (8), transplant (4), or other (8). When adjusted for treatment, number of prior therapies and complex cytogenetics were associated with discontinuation from study. While increased LDH and complex cytogenetics were associated with risk of PD, increased age and number of prior therapies were associated with higher risk of discontinuation for all other reasons. PD includes Richter's transformation (RT; n=16) or progressive CLL (n=8). RT tended to occur early, with 10 pts transforming prior to 12 mo of I. Of pts with RT, 12 developed diffuse large B cell lymphoma, 1 Hodgkin lymphoma, 1 composite B and T cell lymphoma, 1 peripheral T cell lymphoma, and 1 plasmablastic lymphoma. CLL progression tended to occur later, with 1 pt relapsing prior to 12 mo of I. 9 RT pts have died, with a median survival from date off study of 134 days. Five deaths occurred without pts receiving further therapy and within 1 month of RT diagnosis. With therapy, deaths occurred at day 68, 106, 134, and 413 post RT diagnosis. Of pts with CLL PD, median survival from date off study has not been reached; only 3 of 8 pts have died at day 25, 142, and 180 following PD and 3 pts have survived >1 year after PD. 6 pts with CLL PD received further therapy<2 mo post PD, most in ≤ 2 weeks. DS on 6 pts with CLL PD revealed BTK or PLCγ2 mutations in all. 1 pt had both BTK C481S and 3 mutations of PLCγ2, 2 had BTK C481S (1 previously reported; Chang B, ASCO 2013), 1 had BTK C481F, and 1 had PLCγ2 R665W (previously reported). An additional 2 pts who have relapsed outside of these studies both have BTK C481S.
Summary and Conclusion: This single institution experience with I confirms it to be a well tolerated and effective therapy. Patients with PD had a short time to requiring next treatment. These DS results confirm initial reports associating mutations in BTK and PLCγ2 with PD, and require further study in larger populations.

S1341

EFFICACY OF IDELALISIB IN CLL SUBPOPULATIONS HARBORING DEL(17P) AND OTHER ADVERSE PROGNOSTIC FACTORS: RESULTS FROM A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Background: Idelalisib (IDELA) is a potent and selective inhibitor of PI3Kδ, which is critical for activation, proliferation and survival of B cells and their homing and retention in lymphoid tissues. An unmet need exists for effective therapies in patients with CLL positive for del(17p) and other adverse prognostic factors.
Aims: This report describes the efficacy of IDELA in combination with rituximab (R) in patients with high-risk relapsed CLL.
Methods: Samples for del(17p), del(11q), TP53mut, IGHVmut, ZAP70 and CD38 expression, and β2-microglobulin were collected prospectively and tested using standard methods. Patients were stratified based on presence of del(17p) and/or TP53mut, and on IGHV mutational status. The endpoints evaluated in the high-risk subpopulations in the preplanned 1st interim analysis include progression-free-survival (PFS) and overall response rate (ORR). The primary study analysis was reported in NEJM 2014.
Results: IDELA+R retained robust efficacy across all high-risk subpopulations (see Table). Importantly, IDELA+R achieved 76.5% ORR and PFS HR 0.13 in the highest risk patients who were positive for both del(17p) and TP53mut, compared to 80.4% ORR and PFS HR 0.17 in those who had neither present.

Table 1.

	PFS		ORR			
	IDELA+R		IDELA+R		Placebo+R	
	HR	95%CI	n	%ORR	n	%ORR
Overall	0.16	0.09,0.28	88	80.7	88	12.5
Rai stage III or IV	0.12	0.05,0.27	52	75.0	60	11.7
Binet Stage C	0.13	0.06,0.30	47	74.5	51	13.7
Del(17p)	0.14	0.04,0.47	20	85.0	24	0.0
TP53mut	0.11	0.04,0.31	34	79.4	30	10.0
Del(17p) and/or TP53mut						
: Both	0.13	0.04,0.47	17	76.5	15	0.0
: Either one alone	0.09	0.02,0.42	20	85.0	24	12.5
: Neither	0.17	0.07,0.43	51	80.4	49	16.3
Del(11q)	0.10	0.02,0.46	28	82.1	29	6.9
IGHV unmutated	0.13	0.06,0.27	71	78.9	72	12.5
ZAP70+	0.13	0.06,0.26	77	79.2	74	12.2
CD38+	0.13	0.05,0.34	43	83.7	34	17.6
β2-microglobulin > 4mg/L	0.14	0.07,0.27	75	77.3	66	10.3

Summary and Conclusion: These results confirm the retained robust efficacy of IDELA in high-risk CLL subpopulations and support IDELA as a potentially important novel treatment for patients with CLL positive for del(17p) and other adverse prognostic factors.

S1342

IBRUTINIB INTERFERES WITH THE CELL-MEDIATED ANTI-TUMOUR ACTIVITIES OF THERAPEUTIC CD20 ANTIBODIES: IMPLICATIONS FOR COMBINATION THERAPY

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Background: The novel Bruton tyrosine kinase inhibitor ibrutinib and the PI3K-δ inhibitor idelalisib, both acting downstream of the B-cell receptor (BCR), are promising drugs for CLL and B-NHL alone or in combination with rituximab (RTX) or new generation CD20 monoclonal antibodies (mAbs). Type I CD20 mAbs act mostly through complement dependent cytotoxicity (CDC) and cell-mediated effector mechanisms, whereas Type II CD20 mAbs may induce direct cell death in addition to cell-mediated cytotoxicity, but are less effective in inducing CDC.
Aims: The aim of this study was to investigate the effect of ibrutinib and idelalisib treatment on all effector mechanisms of both Type I and Type II CD20 mAbs, using both cell lines and freshly isolated cell samples from patients with CLL.
Methods: Cell death was measured by propidium iodide (PI) or 7AAD staining based on flow cytometry, or using the alamar blue vital dye. Apoptosis was analysed by annexin V and activated caspase-3 staining. Induction of CDC in purified CLL cells or cell lines was assessed by 7AAD/CD19 staining in whole blood assays. NK cell degranulation was determined by CD107a/CD56 double staining and flow cytometry, whereas ADCC was measured by standard chromium release assays. Macrophages-mediated phagocytosis was measured by flow cytometry, using monocyte-derived macrophages and anti-CD20-opsonised CLL. PMN activation was measured as CD11b induction and phagocytosis by flow cytometry, using purified PMN or in whole blood. The type I CD20 RTX and ofatumumab (OFA) and the Type II CD20 mAb obinutuzumab (OBZ) CD20 mAbs were used throughout.

Results: Ibrutinib alone induced apoptosis of cell lines and CLL samples, but this effect was not enhanced by CD20 mAbs. Pre-treatment with ibrutinib for 1 hour did not affect complement activation or CDC of MEC-1, DOHH-2 cell lines or CLL cells. After prolongued exposure (24-72 hours) to 0.1-1 μM ibrutinib, CDC in DOHH-2 and MEC-1 cells was unaffected, although at 10 μM ibrutinib the combination of CDC and ibrunitib-mediated cytotoxicy was less than additive. This may be due to the increased expression of the complement inhibitor CD55 and decreased expression of CD20 by ibrutinib. Importantly, ibrutinib strongly inhibited all cell-mediated mechanisms induced by CD20 antibodies RTX, OFA or OBZ, either in purified systems or whole blood assays. NK cell activation and ADCC, as well as phagocytosis by macrophages or PMN were all inhibited by ibrutinib with EC_{50} of 0.3-3 μM. Analysis of CD20-mediated activation of NK cells isolated from a patient treated with oral ibrutinib suggested that repeated drug dosing inhibits NK cell activation *in vivo*. Finally we show that the PI3K-δ inhibitor idelalisib similarly inhibits CD20 mAb cell-mediated effector mechanisms, although the inhibitory effects of this drug at 10μM were weaker than those observed with ibrutinib at the same concentration (about 50% inhibition compared to over 90%).
Summary and Conclusion: The combination of ibrutinib with type II CD20 mAbs does not lead to enhanced direct cytotoxicity *in vitro*. In contrast, it inhibits all cell-mediated cytotoxic mechanisms of action of CD20 antibodies and has minor effects on CDC. Idelalisib has similar, albeit weaker, inhibitory activity on the cell-mediated effector mechanisms of CD20 mAbs. The design of combined treatment schedules with these kinase inhibitors and anti-CD20 antibodies should therefore consider the multiple negative interactions between these two classes of agents.

S1343

A PHASE 2 STUDY OF THE BTK INHIBITOR IBRUTINIB IN GENETIC RISK-STRATIFIED RELAPSED AND REFRACTORY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)/SMALL LYMPHOCYTIC LYMPHOMA (SLL)

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Background: Ibrutinib is an orally available BTK inhibitor that has demonstrated significant activity in CLL patients (pts), with an overall response rate (ORR) of 71%, an additional 20% of patients achieving partial response (PR) with lymphocytosis (420 mg approved dose) [Byrd et al, NEJM 2013]. Responses seen were independent of high-risk disease features including 17p13.1 deletion (del17p).
Aims: This phase II, single institution, non-randomized study was designed to evaluate the clinical efficacy and durable disease control of ibrutinib administered to pts with relapsed CLL/SLL of all genomic risk with patients having del17p independently evaluated.
Methods: Eligibility included pts with relapsed CLL/SLL with ≥ one prior therapy, required treatment with prior ofatumumab and allowed patients with any degree of cytopenias if attributed to bone marrow involvement by CLL. All pts had interphase cytogenetics performed prior to enrollment and were enrolled into the arm of the study that characterized their risk, del17p present or all other cytogenetic risk.
Results: 72 pts were enrolled in the study. Median age was 65 (range 37-83); median number of prior therapies was 4 (range 1-16); 27 pts (38%) had del17p and 45 (62%) pts had all other cytogenetic abnormalities. Median follow-up is 17 mos (range 10 mos-20 mos). Most frequent adverse events (AEs) considered possibly related to treatment were infection (35%), diarrhea (32%), myalgias (29%), thrombocytopenia (28%), neutropenia (26%) and bruising (22%). Grade 3-4 AEs considered possibly related to therapy occurred in 31 (43%) pts the most common including neutropenia (19%), infections (14%), and thrombocytopenia (7%). 25 pts have discontinued treatment (35%), 15 (21%) with all other cytogenetic risk and 10 (14%) with del17p. Reason for discontinuation included disease progression in 6 pts (8%); investigator or pt preference in 8 pts (11%); adverse events in 5 pts (7%) including 3 pts with pneumonia, 1 pt with progressive multifocal leukoencephalopathy (PML) and 1 pt with bilateral spontaneous subdural hematoma and death in 6 pts (8%). Of the 6 pts with disease progression, 4 had Richter's transformation (3 DLBCL and 1 HD) and 2 had progression of CLL. Of the 6 pt deaths, 3 were considered possibly related to therapy and included two pts with sepsis and one pt with a major bleed from a psoas muscle hematomahematoma. 18 of these 25 pts have died. 68 pts were evaluable for response per protocol (response assessment at 3 mos), 40 other and 28 del17p. Best ORR per investigator was 48% - all of which were PR, with an additional 24% of pts having PR with lymphocytosis. The ORR in the cohort of pts with del17p was 46% with an additional 21% having PR with lymphocytosis and ORR in the other cytogenetic risk group was 50% with an additional 25% having PR with lymphocytosis. Progression free survival (PFS) at 12 mos was 72% in the entire cohort, 68% in the del17p cohort and 79% in the other cytogenetic risk.
Summary and Conclusion: Ibrutinib therapy was well-tolerated with toxicities similar to those previously reported. One pt developed PML during cycle #12 which has not previously been reported with ibrutinib. This pt had prior rituximab with last exposure >1 yr. The ORR in pts with high-risk disease characterized by del17p was not significantly different than those pts with all other cytogenetic risk. The best ORR was lower than previously reported (48% *vs* 71%), possibly related to differences in pts at baseline, although 12 mo PFS remained high and responses may improve with time.

S1344

SF3B1 MUTATIONS AND OUTCOME IN CLL PATIENTS TREATED WITH CHLORAMBUCIL (CHL) OR OFATUMUMAB-CHL (O+CHL): RESULTS FROM THE PHASE III STUDY COMPLEMENT 1 (OMB110911)

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Background: Mutations in *SF3B1* (*SF3B1^{mut}*), a gene of the spliceosome machinery, have been found in CLL with an incidence of 10 to 20% and have been associated with male gender, CD38 positivity, unmutated *IGHV*, 11q-, absence of +12q and poor outcome. In the UK CLL4 and GCLLSG CLL8 trials *SF3B1^{mut}* was found as an independent unfavorable prognostic factor for progression free survival (PFS).
Aims: We assessed the incidence and impact of *SF3B1^{mut}* in the OMB110911 trial (1st line Chl *vs*. O+Chl) in patients considered inappropriate for fludarabine-based therapy.
Methods: Pretreatment samples were available from 376 (84.1%) patients with informed consent and representative for the full trial population (signed informed consent). We performed Illumina MiSeq amplicon based NGS for exons 14, 15, 16 and 18 of *SF3B1*. Exact variant frequency calling of the mutant allele was possible due to deep sequencing with a median depth of 2278x.

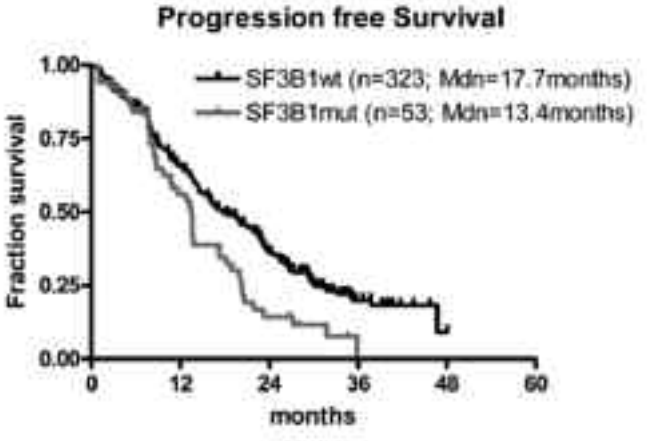

Figure 1.

Results: In total, 56 mutations in *SF3B1* were found in 53 of 376 patients (14.1%). All mutations were missense variants, and 50 of 56 mutations have been previously reported in CLL. Mean variant fraction among patients with one *SF3B1^{mut}* was 0.31 whereas it was 0.16 in 3 patients carrying two distinct *SF3B1^{mut}* mutations, indicating different subclones. *SF3B1^{mut}* was significantly associated with higher white blood cell count (WBC) at baseline (129G/L *vs*. 90G/L, p=0.01), male sex (p=0.02) and a trend to absence of +12q (p=0.06) and 17p- (p=0.09). Mutations in *NOTCH1* and *SF3B1* were mutually exclusive (p=0.01). There was no association of *SF3B1^{mut}* with age, Binet stage, B symptoms, splenomegaly, lymph node size, CIRS, ECOG, ß2-MG, *IGHV* status, deletion of 6q, 11q, and 13q. Regarding response to treatment, *SF3B1^{mut}* had no significant impact on OR or CR rate in both arms. At a median follow-up time of 29.0 months, there were 249 (66.2%) events for PFS and 63 (16.8%) for OS. *SF3B1^{mut}* was associated with shorter PFS (median 13.4 *vs*. 17.7 months, HR 1.662, p<0.01) and this impact was found in both treatment arms (O+Chl: HR=1.652 *vs*. Chl: HR=1.515). Comparison of treatments confirmed the beneficial effect of O+Chl in the subgroup with *SF3B1^{mut}* (median 17.3 *vs*. 10.8 months, HR=0.496, p=0.03). To identify factors of independent prognostic impact, we performed multivariable Cox regressions for PFS and OS including the following variables: treatment, sex, age, Binet stage, ECOG

status, CIRS, B symptoms, WBC, ß2-MG, 11q, 17p, *IGHV*, *NOTCH1 and SF3B1*. For PFS, the following independent prognostic factors were identified: O+Chl (HR 0.40, p<0.001), WBC >50Gl/l (HR 2.59, p<0.001), CIRS Score >8 (HR 1.66 p<0.01), male gender (HR 1.40 p=0.04), unmutated *IGHV* (HR 1.38 p=0.04), 17p- (HR 3.32 p<0.001) and *NOTCH1^{mut}* (HR 1.46 p=0.04), but not SF3B1 (HR=1.10 p=0.62). Interestingly, multivariable analysis with exclusion of WBC, as was frequently performed in previous studies, led to inclusion of *SF3B1^{mut}* as an independent adverse prognostic factor (HR 1.45, p=0.04). Regarding OS, WBC >50Gl/l (HR 2.60 p=0.01), ß2-MG >5mg/l (HR 2.56 p<0.01), Binet Stage C (HR 2.16 p=0.01), 17p- (HR 4.83 p=0.001) and unmutated *IGHV* (HR 1.90 p=0.05) were identified as independent prognostic factors. There was no significant impact of *SF3B1^{mut}* on OS, neither in univariate (median survival not reached, HR 1.26, p=0.5) nor in multivariate analysis, and independently of WBC inclusion.

Summary and Conclusion: In the OMB110911 trial evaluating 1st line O+Chl against Chl, *SF3B1^{mut}* was associated with high WBC, male sex, absence of *NOTCH1^{mut}* and showed a trend to absence of +12q and 17p-. *SF3B1^{mut}* was significantly associated with shorter PFS in univariate analysis but not in multivariable analysis possibly due to its association with high WBC. Subjects with *SF3B1^{mut}* benefited from the addition of Ofatumumab to Chl (O+Chl).

Aggressive Non-Hodgkin lymphoma - Clinical

S1345

PHASE 3 STUDY OF FRONTLINE RITUXIMAB, CYCLOPHOSPHAMIDE, DOXORUBICIN, AND PREDNISONE PLUS VINCRISTINE (R-CHOP) OR BORTEZOMIB (VR-CAP) IN TRANSPLANTATION-UNSUITABLE MANTLE CELL LYMPHOMA (MCL) PATIENTS

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Background: MCL is an incurable type of aggressive non-Hodgkin lymphoma. Progression-free survival (PFS) is limited with standard frontline therapy (*e.g.* R-CHOP) in newly diagnosed MCL patients (pts). Bortezomib (V) is approved for relapsed MCL in the US and >50 other countries. Incorporating V into frontline combination therapy may improve outcomes in MCL pts.

Aims: This study determined if adding V in place of vincristine in R-CHOP improved outcomes in newly diagnosed MCL pts unsuitable for bone marrow transplantation (NCT00722137).

Methods: Consenting adults with measurable stage II–IV MCL and ECOG PS 0–2 were randomized 1:1 (stratified by IPI score and disease stage) to 6–8 21-d cycles of rituximab 375 mg/m^2, cyclophosphamide 750 mg/m^2, doxorubicin 50 mg/m^2, all IV d 1, and prednisone 100 mg/m^2 PO d 1–5, plus V 1.3 mg/m^2 IV d 1, 4, 8, 11 (VR-CAP) or vincristine 1.4 mg/m^2 (max 2 mg) IV d 1 (R-CHOP). Primary endpoint was PFS by independent radiology review (IRC). Secondary endpoints were time to progression (TTP), time to next anti-lymphoma therapy (TTNT), overall survival (OS), response by modified IWRC, and safety. 486 pts were planned for 295 PFS events.

Results: Between May 2008 and Dec 2011, 244 pts were randomized to R-CHOP and 243 to VR-CAP; in all 487 pts, median age was 66 yrs, 74% were male, 74% had stage IV MCL, and 54% had an IPI score of ≥3. 97% of pts had MCL diagnosis confirmed centrally. Pts received a median of 6 cycles. After 40 mos' median follow-up, median PFS by IRC (298 PFS events) was 14.4 *vs* 24.7 mos with R-CHOP *vs* VR-CAP (Figure), and by investigator assessment (307 PFS events) was 16.1 *vs* 30.7 mos (hazard ratio [HR]=.51, *P*<.001). Median TTP by IRC was 16.1 *vs* 30.5 mos (R-CHOP *vs* VR-CAP; HR=.58, *P*<.001) and by investigator was 16.8 *vs* 35.0 mos (HR=.47, *P*<.001); median TTNT was 24.8 *vs* 44.5 mos (HR=.50, *P*<.001), and median OS was 56.3 mos *vs* not yet reached (HR=.80, *P*=.173; 4-yr OS rates: 54% *vs* 64%). By IRC, complete response (CR) + unconfirmed CR (CR+CRu) rates (bone marrow and LDH verified) were 42% *vs* 53% (R-CHOP *vs* VR-CAP; odds ratio [OR]=1.7, *P*=.007); by IRC, radiological CR+CRu rates were 71% *vs* 83% (OR=2.0, *P*=.002) and overall response rates (ORRs; CR+CRu+partial response [PR]) were 90% *vs* 92% (OR=1.4, *P*=.27). By investigator, CR+CRu rates were 28% *vs* 42% (R-CHOP *vs* VR-CAP; OR=1.9, *P*=.002) and ORRs were 92% *vs* 96% (OR=2.0, *P*=.07). Median duration of radiological response with R-CHOP *vs* VR-CAP was 18.1 *vs* 36.5 mos in pts with CR+CRu+PR by IRC and 16.6 *vs* 39.2 mos in pts with CR+CRu by IRC. For R-CHOP *vs* VR-CAP, 85% *vs* 93% of pts had grade (G) ≥3 adverse events (AEs); those in ≥10% of pts in either arm were neutropenia 67% *vs* 85%, leukopenia 29% *vs* 44%, thrombocytopenia 6% *vs* 57%, lymphopenia 9% *vs* 28%, anemia 14% *vs* 15%, and febrile neutropenia 14% *vs* 15%. Despite the difference in thrombocytopenia rates, rates of bleeding events were similar for R-CHOP *vs* VR-CAP (any G: 5% *vs* 6%; G ≥3: 3 pts *vs* 4 pts). Peripheral neuropathy rates were 29% *vs* 30% (any G) and 4% *vs* 8% (G ≥3). With R-CHOP *vs* VR-CAP, 30% *vs* 38% of pts had serious AEs, 7% *vs* 9% discontinued due to AEs, and there were 3% *vs* 2% on-treatment drug-related deaths.

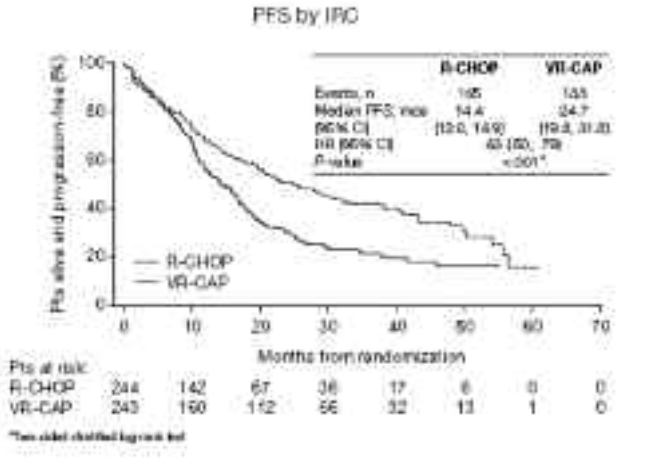

Figure 1.

Summary and Conclusion: PFS was significantly prolonged and secondary efficacy endpoints consistently improved with VR-CAP *vs* R-CHOP, with additional but manageable toxicity, in newly diagnosed MCL pts.

S1346

PROGNOSTIC MODELS FOR PRIMARY MEDIASTINAL B-CELL LYMPHOMA DERIVED FROM 18-FDG PET/CT QUANTITATIVE PARAMETERS IN THE IELSG-26 STUDY

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Background: The IELSG-26 study was designed to evaluate the role of 18-Fluorodeoxyglucose Positron-Emission Tomography/computed tomography (18FDG PET/CT) in the treatment of primary mediastinal large B-cell lymphoma (PMBCL), a distinct subtype with better outcome than other diffuse large B-cell lymphomas. However, salvage treatment for the few relapsing patients has poor results, as a consequence of rapidly-emerging chemorefractory disease. Identification of poor-prognosis patients would allow risk-stratified approaches but the international prognostic index has little value in this entity and there is a need for novel and reliable prognostic markers. We have previously shown that a Deauville score (DS) of 3 defined by a liver uptake cut-point in the post chemoimmunotherapy PET scans can accurately identify the patients who will be likely cured (Martelli et al JCO 2014, in press). We report here an analysis of the prognostic impact of quantitative PET parameters and their combination with the standard qualitative analysis.

Aims: To assess, in a prospective cohort of uniformly treated PMBCL patients, the utility of the main 18FDG PET-derived quantitative parameters alone and combined together or with visual analysis for to predict progression-free survival (PFS).

Methods: Maximum Standard Uptake Value (SUVmax), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) were measured in 100 patients baseline and after the combination chemoimmunotherapy with doxorubicin and rituximab-based regimens; 93 patients also underwent consolidation radiotherapy. Cut-off value of the MTV, TLG and SUVmax and their changes (Δ) during treatment were calculated using the ROC curve. The metabolic response after treatment was also visually evaluated with a 5-point scale (Deauville score - DS).

Results: After a median follow-up of 36 months the PFS rate was 90%. All the individual quantitative parameters considered had very high NPV (0.94-1.0) but low PPV (0.18–0.45). Most patients (86%) had an initial mediastinal mass >7.5cm and nearly all (90%) had a residual morphological lesion at the end of chemoimmunotherapy. SUV max, MTV and TLG at the end of therapy performed better in predicting PDF than the same values estimated at baseline and their changes during immunochemotherapy. The end-of-therapy TLG was the best individual PFS predictor, but the combination of baseline TLG and end of treatment DS resulted in a better PPV without a detrimental effect on the NPV. The combination of these parameters can identify the patients at risk of poor PFS (Figure 1). The univariate analysis of PFS according to the main PET parameters is summarized in the following table:

Table 1.

Parameter		Cut-off	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	Log-rank test (p-value)
Deauville Score	(best cut-point)	3	93	77	99	39	<0.0001
SUV max	baseline	22.33	60	76.6	94	27	0.037
	end-of-therapy	2.6	93	71	98	28	<0.0001
	Δ	78%	60	89	94	53	0.004
MTV	baseline	607 ml	80	80	97	31	<0.0001
	end-of-therapy	35 ml	100	78.9	100	34	<0.0001
	Δ	94%	100	49	100	18	0.0034
TLG	baseline	5814	93	77	99	35	<0.0001
	end-of-therapy	84.4	100	88.7	100	45	<0.0001
	Δ	98%	60	85	95	29	0.0012
baseline TLG + Deauville Score		-	80	92	98	53	<0.0001
end-of-therapy TLG + Deauville Score		-	93	89	99	47	<0.0001

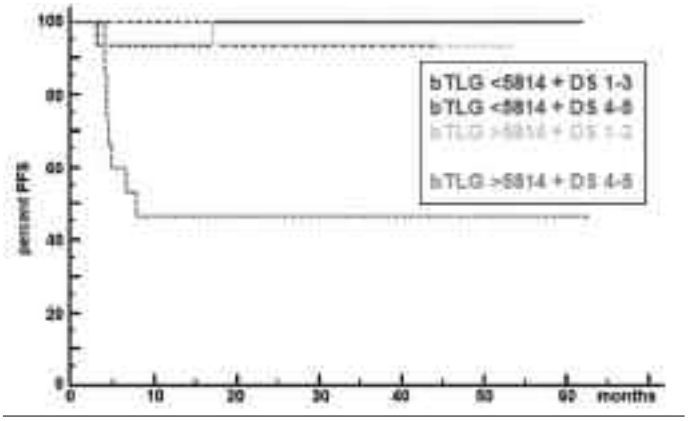

Figure 1. PFS according to baseline TLG and Deauville Score

Summary and Conclusion: Both visual analysis (Deauville qualitative scale) and PET-derived quantitative parameters can predict PFS in patients with PMBCL. In comparison with each parameter alone, combination of baseline TLG and end of treatment DS showed a high PPV and may be used to build a model that can accurately identify the poor-risk patients (Figure 1).

S1347

INCREASED RITUXIMAB (R) DOSES ELIMINATE INCREASED RISK AND IMPROVE OUTCOME OF ELDERLY MALE PATIENTS WITH AGGRESSIVE CD20+ B-CELL LYMPHOMAS: THE SEXIE-R-CHOP-14 TRIAL OF THE DSHNHL

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Background: Elderly male patients have significantly lower R serum levels, shorter exposure times and a worse outcome in RICOVER-60, RICOVER-noRTh, and Pegfilgrastim trials (Blood 2012, 119:3276; Blood 2014, 123:640-646).

Aims: To test increasing R doses, male patients received 500 instead of 375 mg/m^2 in the SEXIE-R-CHOP-14 trial which also compared six cycles of CHOP-14 in combination with eight 2-week applications with eight upfront dose-dense applications of R (days -1, 0, 3, 7, 14, 21, 28, 42) in a randomized phase-II trial. 271 patients (61-80 years) were randomized, 268 patients are evaluable. 148 patients males received 500 mg/m^2, 120 females 375 mg/m^2 R.

Results: Protocol adherence was excellent with median relative doses of R and myelosuppressive drugs >98%. During the treatment period, the increased R dose in males resulted in slightly higher trough serum levels than in females; however, R levels dropped faster in males resulting in very similar serum levels thereafter and a only a marginally longer overall R exposure time. The increased R dose in males was not associated with increased toxicities. 3-year PFS was 74% in males and 68% in females (p=0.396), 3-year OS was 80% in males and 72% in females (p=0.111). In a multivariable analysis adjusting for IPI factors, male hazard was 0.9 (p=0.817) for PFS, and 0.8 for OS (p=0.317). In a historical comparison by multivariable analysis adjusting IPI risk factors and age >70 years, of 148 elderly males who received 500 mg/m^2 in SEXIE-R-

CHOP-14 and 250 males who received 375 mg/m^2 in RICOVER-60, the increased dose of R was associated with a reduced risk for an event in PFS (HR=0.7; p=0.128) and in OS (HR=0.7; p=0.223).
Summary and Conclusion: Increasing R dose by one third from 375 mg/m^2 to 500 mg/m^2 eliminated the increased risk of elderly males. That the increased R dose significantly improves outcome not only of elderly male patients, but also of young male and female patients who have a R pharmacokinetics similar to elderly males should be confirmed in a larger randomized study of these subpopulations with aggressive CD20$^+$ B-cell lymphomas. *Supported by Roche and Deutsche Krebshilfe.*

S1348

PHASE I STUDY OF ABT-199 (GDC-0199) IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA: RESPONSES OBSERVED IN DIFFUSE LARGE B-CELL (DLBCL) AND FOLLICULAR LYMPHOMA (FL) AT HIGHER COHORT DOSES

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Background: The anti-apoptotic protein BCL-2 is highly expressed in Non-Hodgkin Lymphoma (NHL) and contributes to chemotherapy resistance. ABT-199 is a selective, orally bioavailable, small molecule BCL-2 inhibitor that is a promising agent for the treatment of patients (pts) with NHL.
Aims: Objectives of this Phase I, dose-escalation study include evaluations of safety, pharmacokinetics (PK), and preliminary efficacy in pts with relapsed/refractory (R/R) NHL.
Methods: ABT-199 was given on Week 1 Day -7 (W1D-7), followed by continuous, once-daily dosing from W1D1 until progressive disease or unacceptable toxicity. A 2 to 3 week ramp-up period with stepwise dose increases was implemented. Final cohort doses of 200 - 900 mg have been evaluated.
Results: As of December 4, 2013, 44 pts have been enrolled, 15 (35%) with mantle cell lymphoma (MCL), 11 (26%) with FL, 10 (23%) with DLBCL, 4 (9%) with Waldenström macroglobulinemia (WM), 2 (5%) with marginal zone (MZL), 1 (2%) with primary mediastinal B-cell lymphoma (PMBCL), and 1 (2%) with multiple myeloma (MM). The most common AEs (≥20% of pts) were nausea (34%), upper respiratory tract infection (27%), diarrhea (25%), and fatigue (21%). Grade (G) 3/4 AEs occurring in >3 pts were anemia (14%), neutropenia (11%), and thrombocytopenia (9%). G 3/4 thrombocytopenia was not dose-dependent or dose-limiting. Two of 10 pts in cohort 5 experienced a DLT (G3 febrile neutropenia and G4 neutropenia) at the target dose of 600 mg. G3 laboratory tumor lysis syndrome was seen after the initial dose in 1 pt with bulky MCL (elevations in phosphate and potassium only) and 1 pt with DLBCL (elevations in phosphate and uric acid only). For the 40 pts evaluable for efficacy, the overall response rate was 48%, 9/12 MCL (1 CR); 3/11 FL; 3/9 DLBCL (1 CR); 3/4 WM (1 CR); 1/2 MZL; 0/1 PMBCL; 0/1 MM. All responses in DLBCL and FL pts were observed at doses ≥600 mg; 3/8 DLBCL (38%) and 3/6 FL (50%).
Summary and Conclusion: ABT-199 monotherapy showed anti-tumor activity across the range of ABT-199 cohort doses for several NHL subtypes, most notably in MCL and WM. In DLBCL and FL, responses were observed at higher doses. Dose escalation is continuing to determine the maximum tolerated dose and recommended phase two dose.

S1349

PRELIMINARY RESULTS OF A PHASE II RANDOMIZED STUDY (ROMULUS) OF POLATUZUMAB VEDOTIN OR PINATUZUMAB VEDOTIN PLUS RITUXIMAB IN PATIENTS WITH RELAPSED/REFRACTORY NON-HODGKIN LYMPHOMA (NHL)

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Background: Polatuzumab vedotin (PoV) and pinatuzumab vedotin (PiV), antibody drug conjugates (ADC) containing the anti-mitotic MMAE targeting CD79b (PoV) and CD22 (PiV), respectively, showed clinical activity in Phase I studies of patients with relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and indolent NHL.
Aims: The current study aims to compare PoV and PiV, each in combination with rituximab (RTX) in R/R DLBCL and follicular lymphoma (FL).
Methods: Patients were randomized to receive PoV + RTX or PiV + RTX (ADC 2.4 mg/kg + RTX 375 mg/m^2) every 21 days until disease progression or unacceptable toxicity to a maximum of one year. Tumor assessments were performed every 3 months per revised IWG criteria.
Results: As of 8 November 2013, 58 patients received PoV + RTX (38 DLBCL; 20 FL), and 63 patients received PiV + RTX (42 DLBCL; 21 FL). Median prior therapies [DLBCL, 3 (1-10); FL, 2 (1-8)] were balanced between the two treatment arms for both disease subtypes. 46% of all patients were RTX refractory. The median number of treatment cycles administered in DLBCL patients was 5 for both ADC (1-15); in FL the median number of treatment cycles administered was 8.5 for PoV (3-15) and 6 for PiV (1-13). Overall safety profiles of both regimens were similar. Treatment-emergent adverse events (AE) in >25% of patients included fatigue (52%), diarrhea (42%), nausea (37%), peripheral neuropathy (PN) (32%), and constipation (26%). Grade ≥ 3 AE in >3% of patients included neutropenia (21%), diarrhea (6%), dyspnea (4%), febrile neutropenia (4%), hyperglycemia (4%) and PN (4%). Serious AE were reported in 36% of patients. Thirty-eight patients (31%) discontinued study treatment for AE after a median 5 doses (range 1-14); 16 patients discontinued study treatment for PN. Treatment delays and ADC dose reductions reported in 27% and 22%, respectively. Two of 7 deaths (sepsis, urosepsis) unrelated to NHL were attributed to PiV. Complete (CR) and partial (PR) responses, n (%) [% 95% CI]:

Table 1.

	PoV (CD79b) + RTX [95% CI]	PiV (CD22) + RTX [95% CI]
R/R DLBCL	N=37	N=37
ORR	19 (51%) [34, 68]	20 (54%) [37, 71]
CR	5 (14%) [5, 29]	7 (19%) [8, 35]
PR	14 (38%) [23, 55]	13 (35%) [20, 53]
R/R FL	N=20	N=21
ORR	12 (60%) [36, 81]	14 (67%) [43, 85]
CR	6 (30%) [12, 54]	1 (5%) [0.1, 24]
PR	6 (30%) [12, 54]	13 (62%) [38, 82]

Pharmacokinetic profiles were similar for both ADCs across DLBCL and FL with no free MMAE accumulation.
Summary and Conclusion: PoV and PiV + RTX were generally well-tolerated with similar toxicity profiles. Neutropenia, PN and diarrhea were principal toxicities. Similar efficacy was observed with both ADCs in heavily pretreated patients with DLBCL. The higher CR rate with PoV + RTX suggests greater clinical activity in R/R FL. Combination studies of RTX + PoV with chemotherapy and with ADC schedules to reduce PN are ongoing or in planning.

Acute myeloid leukemia - Clinical 2

S1350

TARGETING LEUKEMIC STEM CELLS IN AML USING THE BISPECIFIC CD33/CD3 BITE® ANTIBODY AMG 330

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Background: Antibody-based immunotherapy represents a promising strategy to target and eliminate chemoresistant leukemic cells in AML. In our previous work, we evaluated the cytotoxic effect of AMG 330 on AML cells. Using an AML long-term culture system, we could show efficient elimination of primary AML cells through AMG 330 activated and expanded autologous T-cells.
Aims: As relapse rates in AML are high and are proposed to be caused by leukemic stem cells (LSCs), the ability of AMG 330 to redirect T-cells to LSCs was evaluated by assessing CD33 expression pattern within the LSC compartment as well as by limiting dilution transplantation assays in NOD SCID gamma null (NSG) mice.
Results: CD33 expression intensity on AML cells was assessed by flow cytometry (specific fluorescence intensity, SFI) and by surface antigen quantification (specific antigen binding sites, SABC). Median SFI and corresponding SABC was significantly higher on AML bulk cells compared to $CD34^+/CD38^-$ LSCs (AML bulk: SFI: 59.1, SABC: 47280; LSC SFI: 30.7, SABC: 24560, n=24, *p<0.001*). Importantly, we could show significantly higher CD33 expression on LSCs compared to hematopoietic stem cells (HSC) (median SFI 8.1, 6480 SABC, n=7, *p=0.047*). To further investigate how many CD33 molecules were needed for efficient AMG 330 mediated lysis, $CD33^{BRIGHT}$ cells (MV4-11: SFI 31) were mixed with $CD33^{DIM}$ cells (OCI-AML3: SFI 3) and co-incubated with healthy donor (HD) T-cells (E:T ratio 1:1, 5ng/ml AMG 330 or control BiTE®). Lysis kinetics revealed a dependency on CD33 expression level (24h: OCI-AML3: 93%, MV4-11: 16%). However after 48 hours both cell lines were completely lysed suggesting that AMG 330 is active at low antigen density. Genetic variations of CD33 could also impact CD33 targeted therapy. We therefore genotyped 4 CD33 single nucleotide polymorphisms (SNPs) in 13 primary AML patient samples. Patients homozygous for the reference allele GG of rs35112940 and CC of rs12459419 showed a trend towards higher CD33 expression compared to the other genotypes. As low CD33 expression levels might be sufficient for AMG 330 mediated lysis, we evaluated unwanted on-target toxicity in a colony-forming unit (CFU) assay. No significant difference in CFUs was detected between HD bone marrow samples pretreated with AMG 330 or control BiTE® *(p=0.12)*. Limiting Dilution Transplantation (LDTA) assays were performed to test the potential of AMG 330 to effectively mediate lysis of LSCs. Patient-derived AML cells were lentivirally transduced with luciferase as a transgene, re-transplanted into NSG mice for cell amplification, enriched for transgenic cells by flow cytometry and co-cultured with HD T-cells and either AMG 330 or control BiTE® for 7 days. Residual $CD3^-$ cells were injected into NSG mice and monitored for AML outgrowth by *in-vivo* imaging and peripheral blood analysis. Control mice developed leukemia within 21 days post injection (3/3). In contrast, surviving cells from AMG 330 treated cultures did not initiate leukemia in NSG mice (engraftment in 0/6 mice; median follow up 119 days).
Summary and Conclusion: We conclude from our data that AMG 330 has the potential to eliminate LSCs, while potentially sparing primitive HSCs. Since our previous analysis of 621 AML patients demonstrated a highly variable CD33 expression pattern, we anticipate that AMG 330 mediated lysis will also differ between individual patients. Further investigations and clinical studies are needed to elucidate the impact of CD33 expression on response to AMG 330 mediated immunotherapy.

S1351

IMPACT OF THE COMPOSITION OF SALVAGE REGIMENS ON RESPONSE AND OVERALL SURVIVAL IN PRIMARY REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that nonresponse to the first induction therapy is a major unfavourable prognostic factor for outcome, and conventional chemotherapy offers almost no chance of cure for these patients. Therefore, allogeneic hematopoietic stem cell transplantation (HSCT) has been used for these patients. However, outcome even after allogeneic HSCT remains unfavourable and one major reason for this is that only a minority of these patients achieve a complete (CR) or partial (PR) remission before transplant. Thus, more effective salvage regimens in these primary refractory patients prior to transplant may improve outcome.
Aims: To evaluate the effectiveness of different salvage therapies in primary refractory patients in a cohort analysis of the German-Austrian AMLSG.
Methods: A total of n=3324 patients (age 18-60 years, n=2377; age >60 years, n=947) were accrued between 1993 and 2008 in 5 prospective treatment trials of the AMLSG. After first induction therapy with ICE, n=848 patients (26%) had primary refractory disease (age 18-60 years, 22%; age >60 years, 34%). Salvage therapies had been documented as well as further treatment including allogeneic HSCT. The following salvage regimens were used: 7+3-based (n=59), high-dose cytarabine-based (HiDAC) in the majority of patients combined with mitoxantrone (n=531), in combination with all-trans retinoic acid (ATRA) (n=357) and gemtuzumab ozogamicin (GO) (n=129) or fludarabine (FLU) within the FLA(G) regimen +/- idarubicine/mitoxantrone (n=73), as well as other intensive regimens (n=68), experimental treatment (n=26), and best supportive care (n=124). A total of 380 patients proceeded to an allogeneic HSCT, in 40 patients as a direct salvage therapy.
Results: Of 848 patients, 724 received intensive salvage therapy (85%; age 18-60 years, 96%; age >60 years, 68%). Response to salvage therapy in patients intensively treated was 37% CR and 9% PR resulting in an overall response rate of 46% (age 18-60 years, 56%; age >60 years, 22%). Multivariable regression models on the endpoint overall response in younger intensively treated adults (age 18-60 years) revealed salvage regimens including ATRA (OR, 2.23; p=0.0005), GO (OR, 3.05; p<0.0001), FLU (OR, 2.60; p=0.02) as well as direct allogeneic HSCT (OR, 5.56; p=0.0001) as favourable parameters, whereas high-risk cytogenetics (OR, 0.51; p=0.001) and log(10) white blood count (WBC) (OR 0.72, p=0.02) as unfavourable parameters. In older patients (age >60 years) again salvage regimens including FLU (OR, 3.37; p=0.003) was associated with better overall response. An extended Cox regression model (Andersen-Gill) on the endpoint overall survival in all patients including allogeneic HSCT as a time-dependent covariable revealed allogeneic HSCT (HR, 0.58; p<0.0001), salvage regimens including ATRA in combination with GO (HR, 0.61; p=0.05) and FLU (HR, 0.48, p=0.005) as favourable prognostic markers, whereas high-risk cytogenetics (HR, 2.05; p<0.0001), log(10)WBC (HR, 1.37; p<0.0001) and *FLT3*-ITD (OR, 1.40; p=0.007) were unfavourable covariables. Overall survival for patients proceeding to allogeneic HSCT after 4 years was 34% (95%>CI, 29-40%) and compared favourable to that of patients not proceeding to allogeneic HSCT with 8% (95%>CI, 2-13%, p<0.0001).
Summary and Conclusion: ATRA, GO and FLU as adjunct to intensive HiDAC-based salvage therapy improved response to salvage therapy and prolonged survival in primary refractory AML.

S1352

PHASE I/II PANOBIDARA STUDY OF PANOBINOSTAT IN COMBINATION WITH IDARUBICIN AND CYTARABINE IN PATIENTS AGED 65 YEARS OR OLDER WITH NEWLY DIAGNOSED ACUTE MYELOBLASTIC LEUKAEMIA (AML)

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Background: Elderly patients with Acute Myeloblastic Leukemia (AML) represent an unmet medical need and new strategies are required to improve CR rates and, most importantly, to prolong the TTP and OS. Panobinostat is a pan-deacetylase inhibitor, which has demonstrated *in vitro* antileukemic activity and high synergy with anthracyclines (Maiso *et al.* Leukemia 2009).
Aims: A dose-escalating phase I to define the MTD of panobinostat in this combination was followed by a phase 2 expansion phase. The initial schema included one or two induction cycles with idarubicine (8 mg/m^2 days 1-3) + cytarabine (100 mg/m^2 days 1-7) in combination with three weeks of oral panobinostat (days 8, 10, 12, 15, 17, 19, 22, 24, 26). Patients achieving CR/CRi received an identical consolidation cycle and afterwards maintenance with 40 mg oral panobinostat (3 days/week) for 3 weeks on and 1 week off. After the six first patients, panobinostat was reduced to two weeks in the cycles in combination and to every other week in the maintenance phase.
For comparison we have used an historical control of elderly patients treated in the Pethema group, using the same induction and consolidation without panobinostat, followed by an intensification with cytarabine + daunorubicine.
Results: 38 patients were included in the final schedule, 20 in the phase I and 18 in the phase II. Median age was 71 (65-83) with 32% of patients being ≥75. Median % blasts was 45 (20-93). Displastic features, adverse cytogenetics and hyperleucocytosis were present in 26%, 17% and 30% of patients respectively. Panobinostat 20 mg during induction resulted in 2/6 DLTs (G3 hyperbilirrubinaemia in both, and one of them also G3 oedema), and, accordingly the dose was reduced to 10 mg, without further DLTs. Despite the advanced age of this patient population, treatment was well tolerated in the intensive cycles with the toxicity typical of standard induction chemotherapy. Only 4 (11%) deaths were observed during induction (tumoral lysis syndrome, respiratory infection, acute pulmonary oedema and head trauma). Three more patients died in CR during consolidation due to infectious SAEs. Maintenance phase with panobinostat monotherapy was also well tolerated, being GI toxicity, haematological and asthenia the most frequent AEs (mostly G1-2). Seven out of the 19 patients in maintenance required dose reduction of panobinostat. The causes of reduction were haematological toxicity (2), GI (2); both hematological and GI (2) and a Clostridium difficile infection (1). No patient required panobinostat discontinuation. In terms of efficacy, 22 patients (58%) achieved CR plus 2 more (5%) CRi. With a median follow up of 13.5 months (range 5-29), median survival is 13 months (0-27) for the global population and has not been reached for patients achieving CR. This is superior to the 7.6 months (14.5 for patients in CR) observed in our historical control. Importantly, one of the main objectives was to test if panobinostat maintenance was able to prolong TTP in responding patients. Among the 22 patients that achieved CR, 9 have progressed to date, yielding a TTP of 17.0 months, that improves the 11.7 months of our historical control. Moreover, in at least two patients that started maintenance with positive MRD, panobinostat was able to eliminate the residual blasts.
Summary and Conclusion: This combination was shown to be safe at the MTD. Preliminary efficacy results are encouraging, particularly for the potential benefit of the maintenance phase, in prolonging TTP and subsequently OS in this poor prognostic population.

S1353

A PHASE 1 DOSE ESCALATION STUDY OF THE ORAL SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) SELINEXOR (KPT-330) IN PATIENTS (PTS) WITH RELAPSED / REFRACTORY ACUTE MYELOID LEUKEMIA (AML)

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Background: Selinexor (KPT-330) is a slowly reversible exportin 1 (XPO1) inhibitor that forces the nuclear retention and activation of >10 tumor suppressor proteins (TSP) including p53, and reduction in protein levels of the oncogenes Flt3 and Kit, and induces AML cell death while sparing normal hematopoietic cells.
Aims: Here we investigate the effect of selinexor on p53 and Flt3 in AML patient bone marrow (BM) biopsies as well as present updated results of selinexor in patients with AML.
Methods: Oral selinexor was given at 8-10 doses per 28-day cycle to patients with relapsed/refractory AML. Elevation in leukocyte XPO1 mRNA following XPO1 inhibition was used as a pharmacodynamic (PDn) marker for selinexor activity. Other PDn endpoints such as p53 and Flt3 were assessed in bone marrow (BM) before treatment and at Day 24 of Cycle 1. Pharmacokinetic (PK) analyses were performed. Appetite stimulants and anti-emetics were given as part of supportive care.
Results: Fifty-two heavily pretreated pts (median 3 prior regimens [range 1-7]; 25 M / 27 F; median age 67 yrs; ECOG PS 0/1: 13/39 received selinexor (8-10 doses / 4-week cycle) across 5 dose levels (16.8 - 55 mg/m^2). There have been no DLTs. Dosing at 70mg/m^2 is ongoing. Cycle 1 Grade 3/4 non-DLT, non-hematologic adverse events (AEs) in >1 pt included: fatigue (8%) and nausea (4%). The most common Cycle 1 Grade 1/2 AEs for 8 / 10 doses were diarrhea (42%/30%), anorexia (28%/42%), nausea (32%/46%), & fatigue (28%/29%). Prolonged administration (>4 months) of selinexor was feasible in 4 patients. PK and PD analyses showed dose-dependent increases in C_{max} / $AUC_{0\text{-}inf}$ ($T_{1/2}$ ~6 hrs) and increases in XPO1 mRNA. p53 accumulation and Flt3 protein down-regulation was observed in BM blasts of 10 patients after treatment with selinexor. Treatment with oral selinexor was associated with reductions in BM blast counts in 13 patients with post-baseline blast counts, and was observed across different AML subtypes (Figure 1). Overall response rate (ORR) including complete remission (CR), CR with incomplete hematological recovery (CRi), bone marrow CR (CRm), partial responses (PR) and morphological leukemia free state (MLFS) was 17% with a duration of responses of 6-13 weeks. Responses included: CR: 4 pts (8%); CRm in chloroma: 1 pt (2%); CRi: 1 pt (2%), MLFS: 1 pt (2%) and PR: 2 pts (4%). Twelve (23%) of the remaining pts have experienced stable disease for >30 days (including 1 pt for >10 months), 14 (27%) had progressive disease and 16 patients did not complete cycle 1 due to consent withdrawal, infections, deaths, or other reasons (31%). Infections were considered to be non-drug related, and there were no reported drug-associated Grade 5 events on study to date.

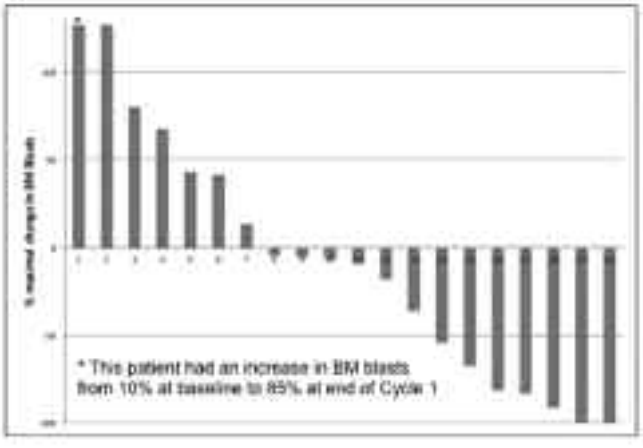

Figure 1. Maximum Change in Bone Marrow Blast Percentage vs. Baseline

Summary and Conclusion: Oral selinexor can be given over months and induce remissions in pts with heavily pretreated AML. A randomized study of selinexor *vs* available agents in older, chemotherapy-ineligible relapsed AML patients is being initiated.

S1354

PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE ASSESSED BY MULTIPARAMETRIC FLOW-CYTOMETRY IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background: Achievement of morphologic complete remission (CR) is a prerequisite for cure in acute myeloid leukemia (AML), but patients in morphological CR may still have variable levels of occult leukemia, defined as minimal residual disease (MRD). Over the last 2-3 decades, much effort has been made to develop accurate and sensitive methods that could determinate response to treatment more precisely than morphology, and to detect residual leukemia in patients considered in morphologic CR. MRD monitoring by multiparametric flow-cytometry (MFC) relies on expression of "leukemia-associated immunophenotypes" (LAIPs), defined as the presence of a combination of antigens and/or flow cytometric physical abnormalities that are absent or very infrequent in normal bone marrow (BM).
Aims: Aim of this study is to evaluate the prognostic impact of MRD assessed by MFC in childhood AML.
Methods: Patients younger than 18 years affected by *de novo* AML (diagnosis May 2003-May 2011), enrolled in AIEOP-LAM 2002 protocol, were eligible for this study. Bone marrow aspirates from 142 patients were collected at diagnosis (n=142), at the end of induction 1 (n=142), and at the end of induction 2 (n=94), according to samples availability. MRD was assessed during follow-up, by five-color MFC, through the detection of patient-specific leukaemia associated aberrant phenotypes, defined for each patient at diagnosis. We used during follow-up 1-3 combinations per patient and each combination included the following common antigen backbone CD45(ECD)/CD33(PEcy5)/CD34(PEcy7) and two specific antigens defined for each patient at diagnosis, according to patient specific LAIPs. SYTO16 combination (SYTO16/GLY-A/CD45/7AAD) was used to assess nucleated cells (SYTO16+) and to identify dead cells (7AAD+) in each sample.
Results: Among the 142 patients recruited to this study, at the end of induction 1 35.9% presented MRD≥1%, 11.3% MRD 0.1-1% and 48.6% MRD<0.1%; samples from 6 patients were not evaluable. We assessed the correlation between levels of MFC-MRD and disease-free-survival (DFS), by means of the Kaplan-Meier method. Presence of MRD≥0.1%, at the end of induction 1, was associated with a DFS of 35.29±7.23% at 6 years, whereas MRD<0.1% with a DFS of 73.16±5.62% at 6 years (p<0.01). MFC-MRD was assessed also after induction 2 in 94 (66.2%) of the 142 patients. We evaluate the correlation between levels of MFC-MRD at the end of induction 2 and DFS, by means of the Kaplan-Meier method. The 6 years DFS-probability, assessed in the 36 (38.3%) patients with MRD data ≥0.1% at the end of induction 1, was 45.4% (SE 16.7) for patients with MRD<0.1% at the end of induction 2, and 22.8% (SE 8.9) for patients who were MRD≥0.1% at the end of induction 2 (p 0.10).
Summary and Conclusion: Our study revealed a significant correlation between MFC-MRD at the end of induction 1, and the patient's outcome: MRD≥0.1% appeared to be predictive of poor outcome (MRD≥0.1%, at the end of induction 1, was associated with a DFS of 35.29±7.23% at 6 years, whereas MRD<0.1% with a DFS of 73.16±5.62% at 6 years). Our data suggest that MFC-MRD at the end of induction 1 provides important prognostic informations that may be used to a better stratification and to guide therapy of childhood AML.

Myeloproliferative neoplasms - Clinical 1

S1355

CALR MUTATION IS A STRONG INDEPENDENT FAVORABLE PROGNOSTIC VARIABLE IN PRIMARY MYELOFIBROSIS

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Background: Mutations in the calreticulin (CALR) gene were recently discovered in approx. 60% of patients (pts) with primary myelofibrosis (PMF) unmutated for JAK2V617F and MPLW515. In retrospective studies, CALR mutated PMF had better overall survival (OS) compared with JAK2V617F or MPLW515 mutated. A prognostic synergism between CALR and ASXL1 mutations was recently reported, highlighting the inferior OS associated with "CALR–ASXL1+" mutation status. We previously also reported that mutations in any one of ASXL1, EZH2, IDH1/2 and SRSF2 identify a High Molecular Risk category (HMR) in PMF predicting for worse OS and leukemia free survival (LFS).
Aims: In this study involving a series of 274 PMF pts from 3 Italian centers we aimed to investigate the prognostic role of CALR mutations in relation to the presence of additional subclonal mutations.
Methods: PMF diagnosis was according to 2008 WHO criteria and all pts provided an informed consent. Genotyping for CALR, JAK2V617F, MPLW515, EZH2, ASXL1, TET2, IDH1/2, DNMT3A, CBL, SRSF2 was performed in granulocytes using allele specific RTQ-PCR, HRM or direct sequencing. The prognostic value of the molecular variables with regard to OS was estimated by the Kaplan-Meier method and Cox regression.
Results: Pts median age was 58y. Median follow-up was 3.8y. Death occurred in 85 pts (31%). IPSS risk category: low-risk 45.5%, Int-1 25.9%, Int-2 16.5%, High-risk 12%. Frequency of mutations was: CALR 24.8%, *JAK2*V617F 56.2%; *MPL*W515 6.6%; *EZH2* 3.3%; *ASXL1* 19.3%; *TET2* 10.3%; *IDH1-2* 1.5%; *DNMT3A* 5.5%; *CBL* 6.0%; *SRSF2* 7.5%. Seventy-three pts (26.6%) were included in the HMR category, while 18 (6.5%) were unmutated for all genes assessed ("all mutations negative"). Median OS in the entire series was 11.7y; 23y in low-risk, 6.5y Int-1, 4.2y Int-2, 2.3y high-risk (P<0.0001). CALR mutated pts showed a significantly better OS with a median of 20.2y (HR 0.46, 95% CI 0.27-0.78) compared with WT pts (median 8.2y; P=0.003). IPSS-adjusted multivariable analysis confirmed the independent prognostic relevance of CALR mutations for OS (HR 0.14, 95% CI 0.27-0.86; P<0.0001) in the lower (low and intermediate-1) IPSS risk categories (median survival, 27.7y *vs* 21.7y, respectively for CALR+ and CALR wt; P=0.02) while the difference was borderline in the higher (intermediate-2 and high risk; 4.2y *vs* 2.6y, respectively; P=0.09) risk categories. HMR-adjusted multivariable analysis showed an independent prognostic role of CALR mutations for OS (HR 0.44, 95% CI 0.26-0.77; P<0.0001) in both HMR (17.7y *vs* 4.2y, respectively; P=0.008) and LMR (20.2y *vs* 8.2y, respectively; P=0.003) categories. Frequency of HMR-subclonal mutations was similar in CALR (22.2%) and JAK2V617F (23.0%) mutated patients. We analyzed the individual impact of any of the 10 genotyped genes in CALR mutated pts and found that the positive effect of CALR mutation was not influenced by any of the additional subclonal mutated genes. We also observed that "all mutations negative" pts constituted a worse prognosis group with OS similar to HMR+ category (9.2y and 6.7yr, respectively; P<0.0001 *vs* LMR, median survival 20.2y).
Summary and Conclusion: Overall, these results confirm the favorable outcome of CALR mutated PMF patients and show that additional subclonal mutations do not impair such positive impact, reinforcing the idea that CALR+ PMF is a distinct entity in terms of prognosis. The adverse outcome associated with "all mutations negative" pts suggests the presence of novel molecular abnormalities that remain to be identified.

S1356

CLINICAL EFFECT OF FOUNDING DRIVER MUTATIONS IN PATIENTS WITH PRIMARY MYELOFIBROSIS: A BASIS FOR MOLECULAR CLASSIFICATION AND PROGNOSTICATION

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Background: Primary myelofibrosis (PMF) is the Philadelphia-negative myeloproliferative neoplasm (MPN) with the worst prognosis, with a median survival of 6.5 years. Current prognostication at diagnosis is based on the International Prognostic Scoring System (IPSS), which includes anemia, age, constitutional symptoms, leukocytosis, and circulating blasts. The dynamic IPSS (DIPSS) can be used at any time during the clinical course of the disease. The vast majority of PMF patients has now a molecular marker: *JAK2* or *MPL* mutations are found in 60-70% of patients, whereas *CALR* (calreticulin gene) mutations account for the majority of the remaining cases. The molecular characterization of patients with nonmutated *JAK2*, *CALR*, and *MPL* remains an important unanswered question.
Aims: In a large cohort of PMF patients, we studied the impact of the three founding driver mutations (*JAK2*, *MPL*, and *CALR*) on clinical phenotype, risk of evolution into acute myeloid leukemia (AML) and overall survival (OS).
Methods: Inclusion in the current study required availability of clinical data at diagnosis to calculate IPSS and at least one DNA sample to assess mutation status of the above driver genes. A total of 629 patients with PMF were recruited from 4 centers. Diagnosis of PMF and that of AML were done according to WHO criteria. All patients were screened for *JAK2*, *MPL* and *CALR* mutations with methods previously reported. The 4 genotypic subgroups (*JAK2* mutated, *MPL* mutated, *CALR* mutated, and triple negative) were compared in terms of phenotype at diagnosis, cumulative incidence (CI) of leukemic evolution, and OS.
Results: Of 629 PMF patients studied, 407 (64.7%) carried *JAK2* (V617F), 141 (22.4%) carried *CALR* mutations, 25 (4%) carried *MPL* mutations, and 56 (8.9%) were triple-negative. Within *CALR* mutated patients, 72% had the 52-bp deletion (type 1 mutation): this frequency is significantly higher than that we previously found in patients with essential thrombocythemia (46%, *P*<.001). The clinical phenotype at diagnosis differed according to mutation status: in particular, *CALR* mutated patients had younger age, lower leukocyte count, higher platelet count, and lower IPSS and DIPSS risk distribution (*P*<.001 in all comparisons).The founding driver mutation had a major impact on both leukemic evolution and OS. Seventy-six out of 629 (12%) patients developed AML. Triple-negative patients had the highest CI of leukemic evolution whereas *CALR* mutated patients had the lowest CI (the 10-years CI of AML was 31.9% *vs* 9.5%, respectively, *P*=.028).In univariate analysis, the OS of *CALR* mutated patients was longer (median survival equal to 18 years) than that of *JAK2* mutated subjects (median survival 9 years, HR 2.3, *P*<.001), *MPL* mutated subjects (median survival 9 years, HR 2.6, *P*=.009), and triple negative subjects (median survival 4 years, HR 5.2, *P*<.001). The impact of the founding mutation on OS was independent of IPSS at diagnosis, DIPSS at diagnosis, and time-dependent DIPSS (max *P*=.021).
Summary and Conclusion: Accounting for *JAK2*, *CALR*, and *MPL* mutation status is of fundamental diagnostic and prognostic relevance in myeloid neoplasms with bone marrow fibrosis. The high frequency of *CALR* type 1 mutation in PMF suggests a particularly active role of the 52 bp-deletion in determining marrow fibrosis and extramedullary hematopoiesis. Patients with nonmutated *JAK2*, *CALR*, and *MPL* have a myeloid neoplasm that combines myelodisplastic features with bone marrow fibrosis and has a very poor prognosis with a particularly high risk of leukemic evolution.

S1357

CLINICAL AND MOLECULAR RESPONSE TO INTERFERON ALPHA THERAPY IN ESSENTIAL THROMBOCYTHEMIA PATIENTS WITH CALR MUTATIONS

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Background: Somatic mutations in the calreticulin encoding gene (*CALR*) were recently described in the majority of patients (pts) with myeloproliferative neoplasms (MPNs) with non-mutated *JAK2* gene. These mutations represent the second most frequent molecular marker for pts with essential thrombocythemia (ET) and myelofibrosis (MF). We, followed by other groups, have previously shown that interferon-alpha (IFNa) induces complete hematological responses in *JAK2* mutated MPN pts. We showed that IFNa may reduce the *JAK2*-mutated clone and induce complete molecular responses in selected pts. We have also reported that *TET2* mutated clones could be resistant to IFNa therapy.
Aims: As the impact of IFNa on clones with molecular lesions other than *JAK2* mutations is thus uncertain and wild type calreticulin is reported to be involved in the resistance to IFNa in viral hepatitis infection, we analyzed the impact of IFNa therapy in *CALR* mutated MPN pts.
Methods: Total DNA was extracted from blood samples using Qiagen blood DNA mini kits. The following analyses were performed in MPN pts treated with peg-IFNa-2a in our Hospital after informed consent. First, *CALR* gene mutations were searched using direct Sanger sequencing of the exon 9. Then *CALR* mutant allele burden was evaluated by fragment analysis, allowing to determine the peak area ratio between mutant and wild type alleles (GeneMapper software, Life technologies). Both sequencing and fragment analysis were performed on a 3500xL DX Genetic Analyser (Life technologies). Finally mutations in *TET2*, *ASXL1*, *EZH2*, *SRSF2*, *IDH1* and *IDH2* were searched through a Next Generation Sequencing (NGS) approach on a MiSeq instrument using a TruSeq custom amplicon approach (Illumina).
Results: We identified 35 ET pts treated by peg-IFNa-2a with no detectable *JAK2* or *MPL* mutations. Direct sequencing of the *CALR* gene exon 9 identified the presence of mutations in 26/35 pts, and sequential DNA samples taken before and during IFNa treatment were available in 14 of them. In 5 of these 14 pts, the mutation was the 52bp deletion (p.L367fs*46) and in 6 pts, the 5bp insertion (p.K385fs*47) while less frequent mutations were detected in 3 pts (p.Q365fs*50 and K375fs*49). The search for additional mutations by a NGS approach is ongoing, with other mutations detected in some pts. Complete results of NGS analyses will be presented at the meeting. Median IFNa treatment duration was 26.5 months (range: 10-96). All of the 14 pts achieved complete hematological response (ELN criteria). Using a quantitative fragment analysis approach we measured the mutant *CALR* allele burden (%*CALR*) in samples taken before, during and after treatment discontinuation. The median %*CALR* was 43% (range: 8-51) before IFNa and 19% (rage: 3-49) in the last available sample (p=0.018). Duration of treatment could influence the molecular response: the %*CALR* dramatically dropped by more than 90% in 4 pts with a long exposure to IFNa (median of 60 months), while no significant difference was observed in 7 pts with shorter exposure (median of 18 months). Finally, a re-increase of the %*CALR* from 18 to 46% was observed in a patient with evolution to post-ET MF 28 months after IFNa discontinuation.
Summary and Conclusion: IFNa therapy is highly effective in ET patients with *CALR* mutations, inducing a high rate of hematological and molecular responses. These results also suggest that CALR-mutant allele burden could be a useful biomarker for monitoring disease evolution and response to therapy.

S1358

ASSESSING THE SAFETY AND EFFICACY OF RUXOLITINIB IN AN OPEN-LABEL, MULTICENTER, EXPANDED-ACCESS STUDY IN PATIENTS WITH MYELOFIBROSIS: A 520-PATIENT UPDATE

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Background: Ruxolitinib (RUX) is a potent JAK1/JAK2-inhibitor that has demonstrated durable improvements in splenomegaly, myelofibrosis (MF)-related symptoms, and quality of life, and has been associated with improved survival in patients (pts) with MF. For countries with no access to RUX outside of a clinical trial, the phase 3b expanded-access JAK Inhibitor Ruxolitinib in Myelofibrosis Patients (JUMP) trial was designed to assess the safety and efficacy of RUX in pts with primary MF (PMF), post–polycythemia vera MF (PPV-MF), or post–essential thrombocythemia MF (PET-MF) who are treatment naive, had progressed, or are intolerant of prior therapy. As of December 2, 2013, 1784 pts have been enrolled in 25 countries.
Aims: To report the safety and efficacy of RUX in pts enrolled in the JUMP study.
Methods: Pts with PMF, PPV-MF, or PET-MF classified as high-risk, intermediate (int)-2 risk, or int-1 risk with an enlarged spleen (≥ 5 cm from costal margin) were included. The primary endpoint is to assess the safety of RUX. Additional endpoints include the proportion of pts with a >50% reduction in palpable spleen length, progression-free survival (PFS), leukemia-free survival (LFS), and overall survival (OS).

Results: 520 enrolled pts (PMF, n=313; PPV-MF, n=122; PET-MF, n=84; missing, n=1) with a median exposure of 11 months were evaluable at time of data cutoff (March 31, 2013). The median age was 68 years (range, 32-88 years) and 51.5% (n=268) were male. At time of data cutoff, most pts were ongoing or had completed treatment as per protocol, including transition to commercial drug (28.3% [n=145] and 42.9% [n=223], respectively); 29.2% (n=152) had discontinued treatment for other reasons. Due to adverse events (AEs), 59.1% of pts experienced a dose reduction/interruption. Overall, 14.6% of pts (n=76) experienced an AE that led to study drug discontinuation, most commonly due to anemia (1.9%, n=10) or thrombocytopenia (1.4%, n=7). The most common hematologic grade ≥ 3 AEs were anemia (32.5%, n=169) and thrombocytopenia (10.8%, n=56). Mean hemoglobin levels declined from baseline (BL; 107.2 g/L) to a nadir of 94.2 g/L at approximately 12 weeks of therapy and increased to 98.4 g/L by week 24. Rates of nonhematologic grade ≥ 3 AEs regardless of study drug relationship were low overall (**Table 1**). At week 24, 56.2% (n=182) and 24.0% (n=78) of pts experienced ≥ 50% reduction and 25% to 50% reduction in palpable spleen length from BL, respectively. Best response from BL in palpable spleen length by week 24 for each patient is shown in **Figure 1**. The estimated probability of PFS, LFS, and OS at 48 weeks was 0.88 (95% CI, 0.84-0.91), 0.91 (95% CI, 0.88-0.94), and 0.92 (95% CI, 0.89-0.94), respectively.

Table 1. Nonhematologic Grade ≥ 3 AEs Regardless of Study Drug Relationship (>1%)

Preferred Term	All Pts; N=520; n (%)
Pneumonia	19 (3.7)
Dyspnea	9 (1.7)
Diarrhea	8 (1.5)
Pyrexia	8 (1.5)
General physical health deterioration	7 (1.4)
Abdominal pain	6 (1.2)
Urinary tract infection	6 (1.2)
Arthralgia	6 (1.2)

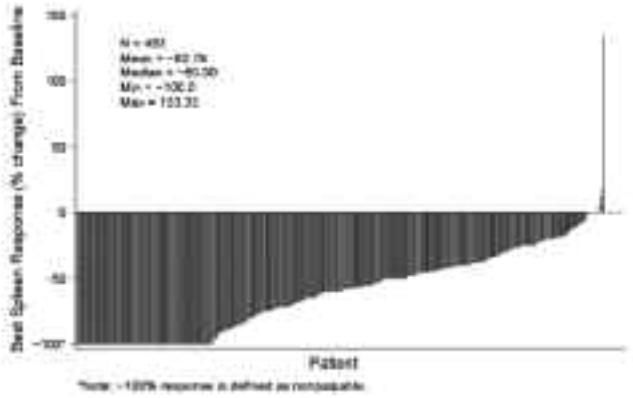

Figure 1. Best Spleen Response from Baseline by Week 24 for Each Patient (% Change in Palpable Spleen Length)

Summary and Conclusion: The JUMP study includes the largest cohort of MF pts treated with RUX reported to date. Consistent with RUX's mechanism of action, the most common AEs were anemia and thrombocytopenia; however, these AEs led to discontinuation in only 10 and 7 pts, respectively. As observed in other studies with RUX, the majority of pts in JUMP experienced spleen size reductions. The safety and efficacy of RUX in JUMP is consistent with the phase 3 COMFORT studies.

S1359

A PHASE I/II, OPEN-LABEL STUDY EVALUATING TWICE-DAILY ADMINISTRATION OF MOMELOTINIB (GS-0387, CYT387) IN PRIMARY MYELOFIBROSIS OR POST-POLYCYTHEMIA VERA OR POST-ESSENTIAL THROMBOCYTHEMIA MYELOFIBROSIS

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Background: Momelotinib (MMB) is a JAK1/2 inhibitor under investigation for the treatment of MF. Results from a phase 1/2 study identified a maximum tolerated dose of 300 mg orally once daily (QD).

Aims: To investigate if additional benefit might be achieved with MMB in a twice daily (BID) regimen, further dose escalation was carried out in this Phase 1/2 study. Remaining patients (pts) have reached a minimum of 12 mos on study as of this interim analysis, 1 Nov 2013.

Methods: Pts with MF categorized as IPSS high risk, int-2 risk, or int-1 risk with symptomatic splenomegaly or hepatomegaly, and platelet (plt) ≥ 50 x 10^9/L, were enrolled and provided written informed consent. Following a dose escalation phase, pts were treated on a dose expansion phase until loss of clinical benefit or intolerance. Spleen responses by physical examination were assessed for pts with baseline splenomegaly >5 cm and by MRI for all pts with spleens. Transfusion (txn) independence, hemoglobin (Hb) and symptoms were documented at baseline and subsequent visits.

Results: 61 pts were administered MMB at initial doses of 200 mg BID (n=54) or 250 mg BID (n=7); 47% pts had IPSS high risk and 40% int-2 risk. Median palpable spleen size was 14 cm and 32% pts were txn dependent. Prior treatment included JAK inhibitors in 23% of pts and immunomodulators in 26%. Median time on MMB was 335 days (range 2 - 674). The most common reasons for ending study were adverse events (AEs) (n=17), consent withdrawal (n=7) and disease progression (n=5). AEs were peripheral neuropathy (n=4), dysaethesias (n=1), declining performance status (n=2), elevated creatinine (n=1), prolonged QTc (n=1), acute coronary syndrome (n=1) and transaminase increase (n=2). Fatal AEs were CNS bleed (n=3), pneumonia (n=1) and MF progression (n=1), none drug related.

No dose limiting toxicity was identified at the 250 mg BID dose, but as it required dose interruptions and modifications most commonly due to thrombocytopenia (n=4), the safety committee chose 200 mg BID for dose-expansion. The most common ≥ Grade 3 AEs were thrombocytopenia (28%), pneumonia (12%), fatigue (12%), elevated lipase (8%), and anemia (7%). 49% of pts reported an AE on the first day, which generally consisted of G1/G2 dizziness, headache and/or hypotension which resolved without intervention. Nausea from all causes was reported in 34% of pts; 26% G1, 6% G2, 2% G3. Diarrhea from all causes was reported in 48% of pts; 33% G1, 11% G2, 3% G3. Cardiac disorders were reported in 21% of pts; including 3% heart failure, 5% atrial fib/flutter, 1 pt (previous heart transplant) with AV block and ventricular tachycardia, 1 pt with sick sinus syndrome, and 2 pts with G1 bradycardia. Additionally, 1 pt with prolonged QTc at baseline became more prolonged (G1). Peripheral neuropathy was reported in 46% of pts; 20% G1, 25% G2 and 2% G3. Only 1/12 pts who were txn independent with Hb ≥ 10gm/dL at baseline reported an AE of anemia (G1) in the first 6 mos. 4/53 pts with baseline plt count ≥ 75 x 10^9, reported a plt count<25 x 10^9.

Table 1.

EFFICACY ANALYSIS TABLE	Number of Patients (%)
Received at least one dose of MMB and underwent at least one evaluation for response	60 (100)
Spleen Response by physical examination	
Baseline palpable splenomegaly > 5 cm	30 (83.3)
≥ 50% reduction in palpable splenomegaly that lasted ≥ 8 weeks for baseline splenomegaly ≥ 10 cm: A (n= 30)	26
Resolution of palpable splenomegaly that lasts ≥ 8 weeks for baseline splenomegaly > 5 and < 10 cm: B (n = 12)	7
Spleen Response: A + B	33 (66.0)
Median time to onset of spleen response (n = 33)	43 days (8-511)
Median duration of spleen response (range) (n = 33)	288 (56 – 630 +)
Spleen Response by MRI	
Baseline MRI (pts without prior splenectomy)	38 (84.7)
≥ 35% reduction in spleen volume by MRI at 24 weeks	27 (48.6)
Anemia Response	
Transfusion dependent at baseline, Cole criteria (≥ 4 units for the 12 weeks prior and ≥ 1 unit in 28 days prior to the first dose of MMB)	19 (31.7)
Achieved transfusion independence ≥ 12 weeks: A (A/19)	6 (31.6)
Median time to onset of transfusion independence, Cole criteria (range) (n = 6)	136 days (1-239)
Median duration of transfusion independence, Cole criteria (range) (n = 6)	Not reached (85 – 64 + days)
Transfusion independent with Hb < 10 g/dL at baseline	8 (13.3)
Rise in Hb ≥ 2 g/dL for ≥ 12 weeks: B (n=8)	1 (12.5)
Overall anemia response A + B (A + B/27)	7 (25.9)
Constitutional Symptoms (MFSAF) Best response for patients with baseline symptom and at least one follow-up assessment	**Patients with ≥ 50% decrease in baseline**
Total Symptom Score, (n = 32)	20 (55.6)
Abdominal discomfort (n = 39)	32 (82.1)
Abdominal pain (n = 29)	20 (69.7)
Bone Pain (n= 30)	25 (83.3)
Itching (n = 25)	25 (100)
Night Sweats: (n=33)	32 (91.4)
Early Satiety: (n=48)	34 (70.8)
Worst Feeling of previous 24 hrs (n= 54)	32 (59.3)
TSS ≥ 50% decrease in baseline at 6 months (n = 25)	12 (48.0)

Summary and Conclusion: MMB at a 200 mg BID dose has an acceptable toxicity profile, and is effective in reducing splenomegaly, in ameliorating

constitutional symptoms, and further demonstrates beneficial effect on anemia. Although comparisons between small single arm studies should be interpreted with caution, no additional therapeutic benefit was observed compared to previous reports at the total daily dose of 300 mg. The results of this study support selection of the 300 mg QD capsule formulation as the starting dose for the phase 3 study of MMB in pts with MF

Chronic myeloid leukemia - Clinical 2

S1360

OVERALL SURVIVAL AND PROGNOSIS IN 2190 PATIENTS WITH FIRST-LINE IMATINIB TREATMENT CONSIDERING DEATH DUE TO CHRONIC MYELOID LEUKAEMIA AS THE ONLY EVENT

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Background: The IN-study section of the European Treatment and Outcome Study (EUTOS) registry comprises data on 2410 patients with chronic myeloid leukaemia (CML) who were enrolled between 2002 and 2006 in prospective, controlled clinical trials. All patients had Philadelphia chromosome-positive chronic-phase (CP) CML and were on first-line imatinib-based treatment within half a year after diagnosis.

Aims: To describe long-term survival and identify variables with a prognostic influence on overall survival (OS) when death due to CML is considered as the only event.

Methods: Survival was censored at the time of allogeneic stem cell transplantation (SCT) in first CP. Only death after recorded disease progression was regarded as death due to CML. Cumulative incidence probabilities (CIPs) of death due to CML were estimated, treating death due to any other cause as a competing event. All prognostic factors were measured at baseline. Their influence on the cause-specific hazard of dying from CML was analyzed using Cox regression, allowing fractional polynomials for continuous variables. Candidate variables were age, sex, spleen size enlargement, haemoglobin, platelets, leukocytes, and percentages of blasts, eosinophils, and basophils in peripheral blood. Risk groups of the final model were identified by using the minimal p-value approach with adjustment for multiple testing. Overall level of significance was 0.05.

Results: Patients of study groups in Germany, France, Italy, Spain, the Netherlands, and the Nordic study group had sufficient follow-up with a common median observation time of 6.4 years; 2190 patients had complete data for all candidate prognostic factors. Allogeneic SCT in first CP was performed in 92 patients (4%, 24 died). Without prior SCT in first CP, 193 patients died, 8-year CIP was 11% [95%>confidence interval (CI): 9-13%]. In 86 cases (45% of 193), cause of death was CML (not due to CML: n=96, 50%, unknown cause: n=11, 6%). Eight-year CIP due to CML was 4% [95%>CI: 3-5%] and 7% [95%>CI: 5-8%] due to other causes including unknown. Higher age, more blasts, a bigger spleen size enlargement, and low platelet counts significantly increased the hazard of dying from CML. The four factors were combined in a new prognostic model. Use of the minimal p-value approach resulted in three risk groups with statistically significantly different CIPs due to CML. Eight-year CIPs were 2% [n=1082, 95%>C.I.: 1-3%], 5% [n=759, 95%>C.I.: 4-7%], and 11% [n=349, 95%>C.I.: 8-15%]. The risk groups according to the Sokal score had 8-year CIPs of 3% [95%>C.I.: 2-4%], 4% [95%>C.I.: 3-6%], and 7% [n=497, 95%>C.I.: 5-10%]. The Euro score resulted in three groups with 8-year CIPs of 4% [95%>C.I.: 3-5%], 3% [95%>C.I.: 2-4%], and 12% [n=221, 95%>C.I.: 8-17%]. The EUTOS score suggested two groups with 8-year CIPs of 4% [95%>C.I.: 3-5%], and 9% [n=230, 95%>C.I.: 5-14%].

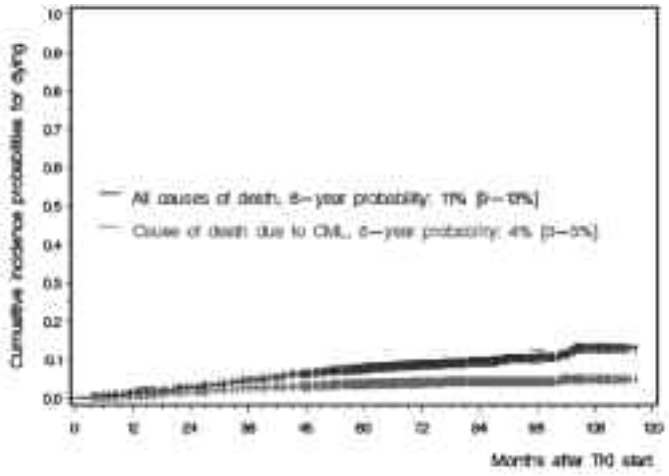

Figure 1.

Summary and Conclusion: For assessment of comparability between patient samples, prognostic models built from baseline variables remain important. In relation to other significant variables, in the new prognostic model, the importance of age was distinctly reduced when compared to models where all causes of death counted as an event. The risk groups also had significantly different CIPs when all causes of death or only progression were considered as event. In comparison to other scores, only the new model identified three risk groups with pairwise significantly different CIPs. The new model led to the largest patient groups with an 8-year CIP above 10%. Independent data for further comparisons are collected.

S1361

EARLY MOLECULAR RESPONSE PREDICTS ACHIEVEMENT OF UNDETECTABLE BCR-ABL IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) TREATED WITH NILOTINIB: 3-YEAR FOLLOW-UP OF ENESTCMR

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Background: ENESTcmr demonstrated that switching to nilotinib resulted in deeper and faster molecular responses (MRs) in CML-CP pts with detectable BCR-ABL on long-term imatinib therapy.
Aims: We present 3-y results, with a focus on predictors of response in nilotinib-treated pts.
Methods: Pts treated with imatinib for ≥ 2 y with complete cytogenetic response but detectable BCR-ABL were randomized to nilotinib 400 mg twice daily (n=104) or to continue imatinib (400 or 600 mg once daily; n=103). Crossover from imatinib to nilotinib was allowed for detectable BCR-ABL at 2 y or treatment failure/confirmed loss of response at any time. The association between early MR and achievement of confirmed (in 2 consecutive samples) undetectable (by RQ-PCR with sensitivity of ≥ 4.5 logs) BCR-ABL by 2 y was analyzed in the 91 nilotinib-treated pts with detectable BCR-ABL at study start who remained on study and had available samples at 3 mo. Confirmed undetectable BCR-ABL by 2 y was analyzed by (1) BCR-ABL level at 3 mo (≤ 0.005% or >0.005%) and (2) whether BCR-ABL level at mo 3 was half of baseline (BL) BCR-ABL (halving time ≤ or >90 d). The BCR-ABL ≤ 0.005% threshold was chosen because it represented the lowest quartile BCR-ABL value at 3 mo in nilotinib-treated pts.
Results: Switching to nilotinib continued to result in higher rates of $MR^{4.5}$ ($BCR\text{-}ABL^{IS}$ ≤ 0.0032%) *vs* remaining on imatinib (cumulative incidence of $MR^{4.5}$ by 3 y in pts without $MR^{4.5}$ at BL, 47% v 33% in the nilotinib and imatinib arms, respectively; *P*=.045; intention-to-treat analysis). Median time to $MR^{4.5}$ was 2 y in the nilotinib arm and was not reached in the imatinib arm by 3 y. 46 pts in the imatinib arm crossed over to nilotinib. Including only responses up to crossover in patients without $MR^{4.5}$ at BL, 47% of pts in the nilotinib arm *vs* 24% in the imatinib arm achieved $MR^{4.5}$ by 3 y (*P*=.0003). At 2 y, 52 pts on nilotinib and 78 pts on imatinib had detectable disease; 4/52 who continued nilotinib, 0/35 who continued imatinib, and 11/43 who crossed over from imatinib to nilotinib achieved undetectable BCR-ABL by 3 y. The rate of $MR^{4.5}$ in pts without $MR^{4.5}$ at BL was higher in pts randomized to nilotinib (33% by 1 y) than in pts who crossed over from imatinib to nilotinib with similar follow-up (21%). The safety profiles of nilotinib and imatinib were comparable to the 1-y report. By 36 mo, selected cardiovascular events of interest were experienced by 12 pts in the nilotinib arm (ischemic heart disease [IHD], 7; cerebrovascular event [CVE], 3; peripheral arterial disease [PAD], 7), 2 pts in the imatinib arm (IHD, 1; CVE, 1), and 2 pts who crossed over from imatinib to nilotinib (IHD, 1; PAD, 1). Mean BCR-ABL level at mo 3 in the 91 nilotinib-treated pts with detectable BCR-ABL at study start who remained on study and had evaluable samples was 0.08%. 19 of 91 (21%) achieved confirmed undetectable BCR-ABL by 2 y. Pts with BCR-ABL ≤ 0.005% at 3 mo had significantly higher rates of undetectable BCR-ABL by 2 y than those with BCR-ABL >0.005% at 3 mo (Figure A), as did pts with a BCR-ABL halving time of ≤ 90 d (*vs* >90 d) (Figure B).

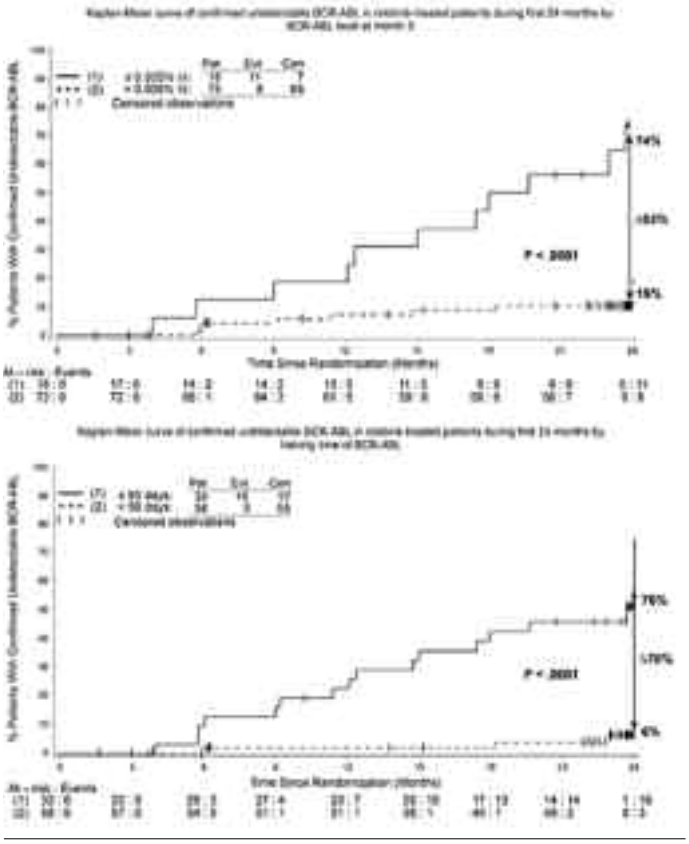

Figure 1.

Summary and Conclusion: With 3 y of follow-up, switching to nilotinib in pts with minimal residual disease on long-term imatinib therapy continued to result in deeper and faster MRs *vs* remaining on imatinib. Achieving either a threshold of BCR-ABL ≤ 0.005% at 3 mo or a rapid decrease in BCR-ABL from BL (halving time ≤ 90 d) was significantly associated with confirmed undetectable BCR-ABL by 2 y in nilotinib-treated pts—an entry criteria for treatment-free remission trials.

S1362

LONGER-TERM FOLLOW-UP OF THE IMPACT OF BASELINE (BL) MUTATIONS ON PONATINIB RESPONSE AND END OF TREATMENT (EOT) MUTATION ANALYSIS IN PATIENTS (PTS) WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CP-CML)

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Background: In CML, the presence of BCR-ABL kinase domain (KD) mutations, including low-level (LL) mutations, can predict clinical responses to second-line BCR-ABL tyrosine kinase inhibitors (TKIs), and sequential treatment with TKIs can lead to development of highly TKI-resistant compound mutations (≥2 mutations in the same BCR-ABL allele). Ponatinib is a potent BCR-ABL TKI that has *in vitro* activity against all BCR-ABL mutants tested and suppresses the emergence of any single mutation at clinically achievable concentrations (40 nM with ≥30 mg/d).
Aims: We evaluated the impact of BL single, LL, and compound mutations on responses to ponatinib and EOT mutations in CP-CML pts in the phase 2 PACE trial.
Methods: Pts with CP-CML (94% ≥2 prior TKIs; 60% ≥3 prior TKIs) resistant or intolerant to dasatinib and/or nilotinib (N=203) or with T315I at BL (N=64) were enrolled, gave informed consent, and were included in the efficacy analysis. The primary endpoint was major cytogenetic response (MCyR) by 12 mos. Median follow-up (7 Oct 2013) for the safety population (N=270) was 27.4 (0.1-36.3) mos. Next generation sequencing (NGS) was conducted on all BL samples (N=267) with the Ion Torrent PGM allowing read lengths ≤400 bp for detection of compound mutations; mutations observed at a frequency >1% are reported. Sanger sequencing (SS) was conducted on both BL and EOT samples.
Results: By NGS at BL, 266 mutations (substitutions in the ABL KD [M237-E507]) were detected in 163 (61%) pts; 105 (39%) mutations were LL mutations not detected by SS. 75 unique single mutations were detected by NGS. 27 of the 75 were also detected by SS, and all 27 have been associated with resistance to TKIs other than ponatinib. Of the 48 LL mutations detected only by NGS, 5 have previously been associated with resistance to TKIs. 12% of pts had only LL mutations. Overall, no mutations were detected in 39% of pts, 1 mutation in 38%, and ≥2 mutations in 23%. Compound mutations were detected in 15% of pts overall. 48 unique compound mutations were observed; T315I, F317L, and F359C/I/V were the most commonly observed components of compound mutations. Ponatinib responses were seen in pts with each of the 20 unique single mutations present in ≥2 pts at BL by NGS. Responses were observed regardless of overall NGS BL mutation status (**table**). Of the 123 pts who discontinued, 95 had successful mutation assessments by SS at or near the EOT visit. 7 pts had mutations at EOT that were not detected by NGS at BL; 5 involved compound mutations and 2 involved gain of T315I. Of 30 pts without MCyR who remained on study at time of analysis, 1 gained a mutation (T315I) at 1 yr. Of 3 pts who lost MCyR but remained on study at the time of analysis, 0 had gains at time of MCyR loss. Of the 53 pts who had mutations detected post-BL, the same mutations were detected at BL in 38 pts by SS and in 45 pts by NGS.

Table 1.

Summary and Conclusion: Responses to ponatinib were observed regardless of BL mutation status. Response rates tended to be lower in pts without mutations, suggesting that BCR-ABL independent resistance mechanisms may be involved. In general, ponatinib activity was not adversely affected by the presence of compound mutations at BL. Rarely, the development of compound mutations was observed at EOT, with one of the involved mutations observed at BL or by history. No single mutation that consistently confers primary and/or secondary resistance to ponatinib in CP-CML has been observed to date. NCT01207440.

S1363

ATHEROTHROMBOTIC RISK ASSESSMENT DURING TYROSINE KINASE INHIBITORS TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS: NEW INSIGHT?

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Background: Peripheral Arterial Occlusive Disease (PAOD) has been reported in chronic myeloid leukemia (CML) patients (pts) treated with second generation Tyrosine Kinase inhibitor (TKI) nilotinib (NIL).
Aims: To explore potential underlining mechanisms, we investigated genetic and biochemical traits associated with vascular events, in CML pts treated with TKIs.
Methods: 110 CML pts, 58 on imatinib (IM) and 52 on NIL (median treatment time 84 mos, range 12-180, and 56 mos, range 3-228, respectively), all in complete cytogenetic response, were studied. Pts were screened for PAOD a/o other atherothrombotic episodes and evaluated for: traditional cardiovascular risk factors (TCRF) (Diabetes Mellitus, Dyslipidemia, Blood Pressure, Body Mass Index, Smoke, Familiarity); sCD40L, Endogenous Thrombin Potential (ETP); oxidized LDL (oxLDL) level; IL6, IL10 and TNFα pro/anti-inflammatory cytokines network; intron 4 IVS4-14 A>G polymorphisms of OLR1 (rs3736235), encoding for the oxidized LDL receptor 1 (LOX1) to evaluate the distribution of genotypes AA (cardiovascular low risk), AG and GG (cardiovascular high risk).
Results: The distribution of classical risk factors showed a slight prevalence of dyslipidemia in the NIL cohort. Similarly, the presence of 3 or more TCRF was more frequent in the NIL group. In the IM cohort 3/58 (5%) pts experienced an atherothrombotic event (1 PAOD, 2 carotid occlusion major than 50%), while in the NIL cohort 14/52 (27%) atherothrombotic events were documented (9 PAOD, 5 acute coronary syndrome) (p=0.00011). LOX-1 polymorphism was evaluated in all 110 pts and genotype frequency respected the Hardy-Weinberg equilibrium with other populations of ancestral Caucasian origin. However, when considering the genotype frequency according to TKI treatment, we found a slight excess of homozygotes A/A in the IM group and a significant excess of homozygotes G/G in the NIL treated cohort. Interestingly the homozygotes G/G clustered in the NIL sub-group with history of atherothrombotic events during treatment. Multivariate analysis showed that once corrected for age, sex, BMI and each applicable, biochemical and genetic data available at the moment of event recording or clinical observation if event-free; the single influencing risk factor was the G/G homozygosis for IVS4-14A/G of OLR1 in the NIL group (Fig.1). No significant influence was detected for each single traditional risk factor, despite the slight increase of dyslipidemic subjects in the NIL group (Fig.1). Furthermore, the clustering of 2 or ≥3 TCRF was not associated with the increased risk of cardiovascular events during treatment with both TKIs (Fig. 1). In addition, we found significant differences in many biochemical parameters evaluated: oxLDL, sCD40L level and ETP were significantly higher in NIL *vs* IM treated group, while IL10 level, inversely related to oxLDL, sCD40L and ETP, was significantly lower.
Summary and Conclusion: Our data suggest no influence of classical risk factors in atherothrombotic risk during TKI treatment, while an unbalance of pro/anti-inflammatory cytokines network observed in NIL pts, together with genetic pro-atherothrombotic predisposition conferred by LOX1, may have a role in the increased incidence of vascular events. With the ultimate intent to achieve a "personalized" TKI treatment, we are conducting a prospective study, in newly diagnosed CML pts treated front line with any TKIs, in order to identify a genetic/biochemical tool able to early detect pts at potential increased atherothrombotic risk.

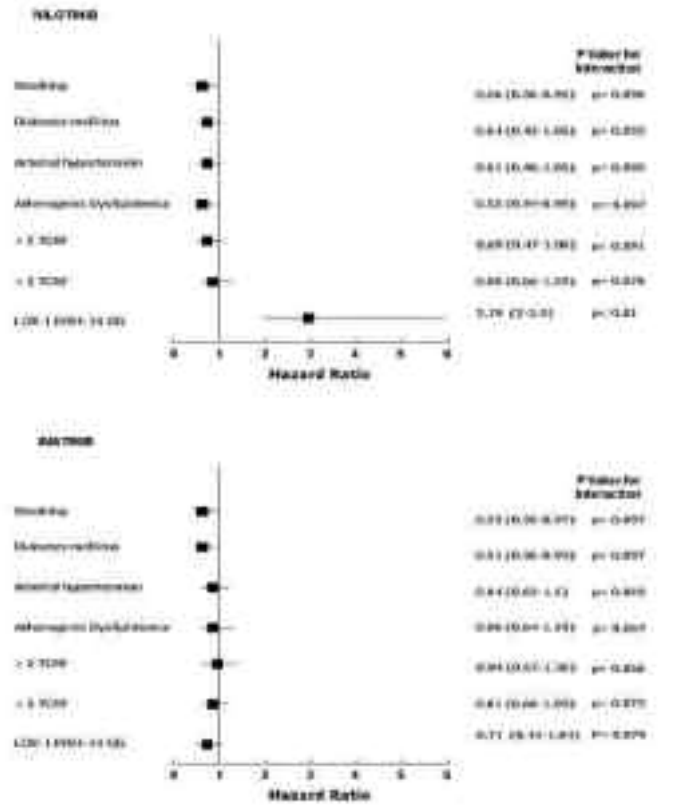

Figure 1.

S1364

CRIZOTINIB IS AN ALLOSTERIC ABL-INHIBITOR ABLE TO TARGET BOTH NATIVE BCR/ABL AND BCR/ABL-T315I IN VITRO AND IN VIVO MODELS OF PH+ LEUKEMIA

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Background: Targeting BCR/ABL with tyrosine kinase inhibitors (TKIs) is a proven concept for the treatment of Philadelphia chromosome-positive (Ph+) leukemias. Resistance attributable to either kinase mutations in BCR/ABL or non-mutational mechanisms remains the major clinical challenge. With the exception of Ponatinib, all approved TKIs are unable to inhibit the "gatekeeper" mutation T315I. However, a broad spectrum of kinase inhibition increases the off target effects of TKIs and may be responsible for cardiovascular issues of Ponatinib. Thus, there is an urgent need for additional therapy options for resistant Ph+ leukemias. Crizotinib, a kinase inhibitor designed to target c-MET and ALK for the treatment of lung cancer, exhibits activity also against the ABL-kinase in cell free systems.

Aims: We investigated the therapeutic potential of Crizotinib for the treatment of Ph+ leukemia patients with advanced and therapy-resistant disease.

Methods: Inhibition of BCR/ABL kinase and *in vitro* efficacy of Crizotinib were investigated i.) in Ph+ and Ph- leukemia cell lines (K562, SupB15, BV173 and Jurkat, U937, respectively); ii.) in primary patient-derived long term culture (PD-LTCs) of Ph+ALL patients with different levels of responsiveness to TKI and one harboring the T315I; iii.) in Ba/F3 cells rendered factor independent by the expression of native BCR/ABL or BCR/ABL-T315I. Furthermore we compared the *in vivo* efficacy of Crizotinib with that of Ponatinib in syngeneic mouse models of BCR/ABL- or BCR/ABL-T315I-driven CML or ALL. Based on the particular binding properties to both c-MET and ALK we investigated the mechanisms by which Crizotinib inhibits the BCR/ABL kinase in an AlphaScreen ABL/myristoil-peptide interaction-displacement assay.

Results: Here we show that Crizotinib not only inhibited autophosphorylation of BCR/ABL in all tested cell lines but also efficiently suppressed growth of Ph+ cell lines with an IC50 of 50-100nM, whereas Ph- cell lines did not respond to concentrations even >1μM. Furthermore K562 underwent erythroid differentiation upon Crizotinib to the same extent as Imatinib. It abolished factor independent growth of both BCR/ABL- and BCR/ABL-T315I-positive Ba/F3 cells. The growth suppression by Crizotinib (IC50 50-100nM) in Ph+ PD-LTCs was completely independent of the responsiveness/resistance to other TKIs or to the presence of T315I. This might be due to the fact that Crizotinib boundn to the myristoil binding pocket (MBP) of ABL even with an higher affinity as compared to GNF-2 as revealed by an AlphaScreen drug/protein interaction assay. The *in vitro* efficacy of Crizotinib was confirmed *in vivo* in both syngeneic models of BCR/ABL- or BCR/ABL-T315I-driven CML and in BCR/ABL-driven ALL. At 100mg/kg o.d. for 14 days it significantly prolonged survival to nearly exactly same extent as Ponatinib at 25mg/kg o.d for 14 days, without apparent toxicity.

Summary and Conclusion: These findings strongly indicate that Crizotinib is an allosteric inhibitor of BCR/ABL and its resistance mutants and suggest the clinical evaluation of Crizotinib for the treatment of advanced and therapy-resistant Ph+ leukemia.

Stem cell transplantation - Experimental

S1365

CIRCULATING MIR-586 PARTICIPATES IN OCCURRENCE OF ACUTE GRAFT-VERSUS-HOST-DISEASE BY DOWN-REGULATING INDOLEAMINE-2,3-DIOXYGENASE

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Background: Indoleamine-2,3-dioxygenase (IDO) is able to catalyze the first and rate-limiting step in the catabolism of tryptophan, which is the essential amino acid for T cell proliferation. It played important roles in immunosurppression associated with maternal-fetal immune tolerance, tumor immunity, autoimmunity and chronic infection. During acute graft-*versus*-host-disease (aGVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT), donor T cells will be activated and lead to tissue injury. IDO has been proved to be involved in the occurrence of aGVHD through its immunosurppressive function. Our previous work also demonstrated that plasma IDO level was correlated with the severity of aGVHD. Increased IDO expression would serve as a protective molecular during aGVHD. However, the exact regulatory mechanism of IDO is still unclear. MicroRNAs (miRNAs) are a class of small non-coding RNA that negatively regulate gene expression by translational repression or induction of mRNA degradation. Recently, circulating miRNAs have been reported to be the promising biomarkers for various kinds of diseases.
Aims: This study is to find the potential regulatory miRNA of IDO and investigate the correlation between plasma miRNA expression and aGVHD.
Methods: The potential regulatory miRNA for IDO was identified through bioinformatics analysis using TargetScan, Miranda and DIANA-microT. The regulatory function of target miRNA on IDO protein and gene was confirmed by Western Blot analysis and luciferase assay. The plasma of 25 patients undergoing allo-HSCT was prospectively collected at +7 day(d), +14d, +21d, +30d, +45d, +60d, +90d and the occurrence of aGVHD. Real-time quantitative PCR (RQ-PCR) analysis was performed to examine the miRNA expression of plasma at different time points posttransplant. Receiver operating characteristic (ROC) analysis was employed to determine the optimal cut-off value for predict the occurrence of aGVHD.
Results: Through bioinformatics analysis, miRNA-586 was found to be the putative regulatory miRNA for IDO. The intracellular IDO protein would be reduced when miRNA-586 was transfected into interferon-gamma-treated Hela cells. While, the mRNA expression level of IDO was not affected obviously. In addition, luciferase assay in 293T cells showed that miRNA-586 was able to bind directly to the promoter region of IDO gene and affect its transcription. However, when the potential binding sites were mutated, this phenomenon could not be observed. Among the 25 patients who underwent allo-HSCT, 10 patients developed into aGVHD and 15 patients did not. Based on their miRNA-586 expression levels at designed time points, we found that the levels of miRNA-586 at +7d posttransplantation were significantly different between these two groups (P=0.001). The results of ROC analysis showed that the patients whose miRNA-586 levels were higher than $4.5x10^4$copies/ul were more prone to develop into aGVHD (AUC=0.898,P<0.001).
Summary and Conclusion: Our study suggested that miRNA-586 might participate in occurrence of aGVHD by down regulating IDO and might be a putative target for novel aGVHD therapy. The plasma level of miRNA-586 at +7d after allo-HSCT would be a good biomarker for predicting the occurrence of aGVHD.

S1366

SERUM MIR-29A IS UP-REGULATED IN ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO HSCT) AND ACTIVATES DENDRITIC CELLS (DCS)

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Background: aGVHD is one of the most frequent and lethal complications after allo HSCT, underscoring the need to develop novel therapies. To achieve this goal, aGVHD mechanisms needs to be further elucidated. Recently it was reported that miRNAs modulate aGVHD.We hypothesize that serum miRNAs expression is deregulated in aGVHD and could play a role in aGVHD pathogenesis.
Aims: To asses serum miRNAs expression in aGVHD and dissect their functional role.
Methods: We performed serum miRNA expression analysis using deep-sequencing (Solid Platform) from allo HSCT recipients samples. Peripheral blood samples were collected weekly until day 100+ and at the time of clinical diagnosis of aGVHD. A mouse model of aGVHD (B6 donor into F1 recipients) was used to assess serum miRNA expression in animals with aGVHD. Functional studies were performed using miR-29a and miR-16 Dotap formulations using mouse dendritic and T cells. Cytokines were measured using ELISA.
Results: We included 10 patients with aGVHD (bowel n=2; skin (n=5) and both skin/bowel (n=3)). Conditioning regimens were mainly non-myeloablative (n=9) using unrelated donors (n=9). Allo HSCT patients with no aGVHD, matched for age, disease, conditioning regimen, donor and timing of sample collection were used as controls. We compared miRNA expression between all patients with aGVHD (n=10) and controls (n=7) using class comparison. Among the 7 miRNAs up-regulated in aGVHD samples we found miR-29a (Fold change (FC) >2, p<0.01). Since miR-29a is involved in immune regulation we validated this miR by RT-PCR in the B6-F1 model of murine aGVHD (miR-29a levels were 4.9 higher in aGVHD mice (n=6) than controls (n=4) p<0.01). Since our group reported before that miR-29a binds as ligands to TLR7/8, we hypothesized that serum miR-29a could bind to TLR7/8 of APCs activating NFkB and enhancing alloreactive responses during aGVHD. First, we examined whether extracellular miR-29a could activate dendritic cells (DCs). B6D2F1 splenocytes were stimulated with Dotap formulations (mimicking exosomes) of miR-29a. We found that CD69 expression measured by FACS is significantly elevated in CD11c+ DCs/CD4+ and CD8+ T cells treated with miR-29a compared to controls (Dotap alone or Dotap-miR16). To investigate whether T cells could be activated by the miR alone, untouched resting T cells from mouse spleen were isolated and stimulated with Dotap-miR-29a or controls. CD69 was not up-regulated indicating that the activation of T cells was dependent on APC activation. To further confirm that miR-29a could activate DCs, we isolated murine DCs and repeated the above experiment. We found that miR-29a stimulation of DCs but not controls induced the up-regulation of canonical DC maturation and activation markers, CD40, CD80 and CD86. Furthermore, miR-29a Dotap treatment of DCs alone stimulated the release of TNFα (114.2±14 pg/ml *vs*. mir16-Dotap 26.98±2. pg/ml, p<0.01) and IL-6 (103.83±7pg/ml *vs*. 37.01±1 pg/ml mir16-Dotap, p<0.05) in the supernatant. To show that the release of pro-inflammatory cytokines by miR-29a is through the TLR7 pathway, we repeated the experiment using DCs isolated from TLR7-/- mice, and saw no upregulation of activation/maturation markers and no release of pro-inflammatory cytokines. Interestingly, B6D2F1 dendritic cells stimulated with miR-29a elicited a stronger alloreactive proliferative response from CFSE labeled B6 T cells as opposed to control treated DCs underscoring the importance of miR-29a in driving an alloreactive response.
Summary and Conclusion: Our results indicate that serum miR-29a is up-regulated during aGVHD and activates DCs, likely by direct binding to TLR7/8. NFkB activation by miR-29a results in the release of TNF-a and IL-6 and elicits a stronger allo-reactive T cell proliferative response.

S1367

ALLOGENEIC (ALLO) HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN PATIENTS OLDER THAN 60 YEARS: COMPARISON OF HAPLOIDENTICAL T-REPLETE HSCT FOLLOWED WITH POST-TRANSPLANT HIGH DOSE CYCLOPHOSPHAMIDE

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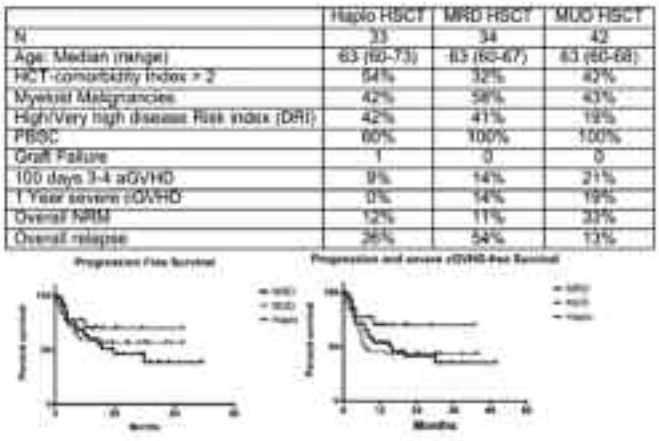
Background: The identification of a donor has always limited the extent of allo HSCT. Recently it has been shown by different teams that a haploidentical donor could be a valid option to perform allo HSCT given adapted immunosuppression is used. Notably the use of PT-HDCy), after T-replete HSCT following reduced intensity (RIC) or non-myeloablative (NMAC) conditioning, has been associated with promising results. However little data exist concerning elderly population when this population is characterized by a lack of HLA matched sibling and a higher incidence of severe GVHD and non-relapse mortality.
Aims: Using this strategy in a joined program between two institutions we recently transplanted 33 patients over the age of 60 years and compare their outcome with patients of the same age transplanted from a MRD or MUD.
Methods: 70% of the patients in haplo group were prepared with Flu-TBI 2gy (NMAC) while all patients in other groups received the same RIC (Fludarabine (150 mg/m2) –Busilvex (2 days) –rabbit ATG (2 days)). Patients in haplo group received post graft Immunosuppression with PT-HDCy (50 mg/kg on D 3 and 4) followed with CSA and MMF while patients in other groups received either CSA (70%) or Tacrolimus starting on D1.
Results: Patients in the haplo group have trends to present higher comorbidities and more severe diseases (Table). Haplo and MRD HSCT patients present with a similar lower NRM than MUD patients while Haplo patients present a relapse rate intermediate between MRD and MUD. Overall there is a trend for better PFS after Haplo HSCT (Figure 1) which is even higher when analyzing the progression and severe cGVHD free survival (Figure 2).



	Haplo HSCT	MRD HSCT	MUD HSCT
N	33	34	42
Age: Median (range)	63 (60-73)	63 (60-67)	63 (60-68)
HCT-comorbidity Index > 2	64%	32%	42%
Myeloid Malignancies	42%	59%	43%
High/Very high disease Risk index (DRI)	42%	41%	19%
PBSC	100%	100%	100%
Graft Failure	1	0	0
100 days 3-4 aGVHD	9%	14%	21%
1 Year severe cGVHD	0%	14%	19%
Overall NRM	12%	11%	33%
Overall relapse	36%	54%	13%

Figure 1.

Summary and Conclusion: We conclude that T-replete Haplo HSCT after RIC and followed by PT-HDCy seems associated with promising results notably as compared with MUD HSCT. The low rate of severe aGVHD and the absence of severe cGVHD are likely to conduct to lower complications and better quality of life. The reduction of donor search duration and the absence of graft acquisition fees represent potential additional benefits. in this persepcetive, the place of Haplo HSCT in patients beyond 60 years of age should now be prospectively addressed.

S1368

ALLOGENEIC BONE MARROW TRANSPLANTATION INDUCES MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS), WHICH PREVENT T CELL PROLIFERATION IN VITRO BUT ARE UNABLE TO SUPPRESS T CELL FUNCTIONS AND GVHD IN VIVO

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Background: Allogeneic bone marrow transplantations (BMT) are always associated with pro-inflammatory responses due to conditioning regimens and the development of alloantigen-specific T cells. These activated, donor-derived T cells attack recipient organs leading to tissue destruction and the development of graft-*versus*-host disease (GVHD). GVHD-induced inflammation is associated with enhanced T cell proliferation and cytotoxicity and increased serum levels of pro-inflammatory cytokines. Inflammatory responses frequently induce the development of myeloid-derived-suppressor cells (MDSCs), a heterogeneous population of immature myeloid-derived cells able to prevent T cell activation and proliferation. Murine MDSCs are characterized by the co-expression of CD11b and Gr-1, the up-regulation of arginase-1 and iNOS, and most importantly their ability to inhibit T cell-mediated immunity.
Aims: Therefore, we analyzed whether GVHD development induces MDSCs able to suppress T cell functions *in vitro* and *in vivo*.
Methods: GVHD was induced in two different murine allogeneic BMT models by transplantation of allogeneic BM and T cells. During the course of disease the induction of MDSCs in bone marrow and spleen of transplanted mice was analyzed and MDSCs were characterized phenotypically by flow cytomety, molecularly by the expression of iNOS and arginase-1 RNA, both molecules required for T cell suppression, and functionally by their capability to inhibit T cell functions *in vitro* and *in vivo*.
Results: In the BM and spleen of transplanted mice MDSCs were detectable as early as 5 days after transplantation. Numbers of MDSCs increased continuously up to day 10 and ranged between $6x10^6$ and $2x10^7$ MDSCs /spleen depending on the BMT model used. Interestingly, at this time point the numbers of MDSCs in the spleen were three times higher than the numbers of allogeneic T cells. MDSCs declined after day 10 but were still detectable more than 40 days after BMT. Despite the high numbers of MDSCs mice developed GVHD indicating that these MDSCs might probably not be fully activated. However, MDSCs isolated from the spleens of transplanted mice exhibited high expression of arginase-1 and iNOS and most importantly efficiently prevented allogeneic T cell proliferation *in vitro*. To test their functionality *in vivo*, we isolated MDSCs from GVHD-developing mice at day 10 after BMT and adoptively transferred these MDSCs in mice receiving allogeneic BM cells and spleen cells. Surprisingly, although these MDSCs efficiently prevented T cell proliferation *in vitro*, they were unable to prevent GVHD *in vivo*. However, the transfer of MDSCs, which were generated *in vitro* by incubating bone marrow cells with GM-CSF, totally inhibited clinical GVHD.
Summary and Conclusion: In summary, the development of GVHD is strongly correlated with a fulminant induction of donor-derived MDSCs, which prevent T cell activation and proliferation *in vitro*. However, these MDSCs are not functional *in vivo* showing that the presence of MDSCs in pathological conditions does not necessarily reflect their functionality. Future studies will show, whether treatment with growth factors or cytokines can further mature and activate MDSCs *in vivo* in such a way that they prevent GVHD development.

S1369

THE ACUTE GRAFT VERSUS HOST DISEASE CAN BE EFFECTIVELY PREVENTED BY TOLL-LIKE RECEPTOR 7-MEDIATED TOLERANCE IN A MISMATCHED TRANSPLANTATION MOUSE MODEL

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Background: Graft *vs* Host Disease (GvHD) is the leading cause of non-relapse mortality and morbidity following allogeneic stem cell transplantation (alloHSCT) adversely affecting the outcome. Tissue damage in GvHD is caused by alloreactive T-cell-mediated immune attack, thus immunosuppression is commonly used as anti-GvHD prophylaxis, increasing patients' susceptibility to opportunistic infections. Toll-like receptors (TLRs) are innate immune receptors able to detect pathogen-associated molecular patterns (PAMPs) and endogenous self-antigens, implicated in the development of infectious and autoimmune diseases, respectively. The conditioning-induced loss of intestinal track integrity, followed by LPS-TLR4 interaction, is a key event for acute GvHD initiation. Polymorphisms of the TLR4/9 encoding genes in alloHSCT recipients have been associated with the GvHD severity, while TLR tolerance induction by direct low-dose TLR agonist injections to the donors or recipients prevent experimental autoimmunity.
Aims: In order to address whether TLR tolerance induction could also prevent alloreactivity and aGvHD, by a clinically applicable approach, we investigated the ex-vivo TLR tolerance induction to donor lymphocytes.
Methods: We investigated the *in vitro* TLR2,4,7 tolerance induction in mouse splenocytes (mSPLCs) repeatedly exposed to low-dose TLR-specific agonists followed by an high-dose challenge. A non-specific high-dose challenge was also used to check for cross-tolerance among TLRs. The induced hyporesponsiveness was assayed by TNFα quantification in mSPLCs culture supernatants and the optimal dose/duration for each agonist to induce maximum tolerance was determined. The capacity of the ex-vivo TLR7-tolerized donor lymphocytes to prevent aGvHD was investigated in a fully mismatched transplantation mouse model (C57BL/6 to Balb/c irradiated recipients) where aGvHD appears within 45 days post-transplantation. A 10-point murine GvHD scoring system based on 5, daily evaluated, parameters was used for the clinical assessment of aGvHD.
Results: Maximum TLR2,4,7 tolerance induction was achieved in mSPLCs *in vitro* by a 3 day-exposure of cells to LPS, R848 and Pam3CSK4 (10ng, 14mM and 1000ng/ml respectively, $p<0.001$). TLR7-mediated "desensitization" of mSPCs by R848 was also associated with a strong cross-tolerance effect to TLR2/4 as it was demonstrated by the hyporesponsiveness of TLR7-tolerized mSPLCs to Pam3CSK4 or LPS challenge. In a mismatched transplantation model, the recipients were administered : T-cell depleted bone marrow cells (TCD-BM) without or with mSPLCs (Groups I, II, respectively) and TCD-BM+mSPLCs tolerized with Pam3CKS4, LPS and R848 (Groups III,IV and V respectively). The successful "desensitization" of the transplanted mSPCs was checked before transplantation in Groups III-V by high-dose specific challenge. No animal from Groups II,III and IV survived beyond day 25 post-transplantation, whereas Groups I,V showed significantly lower aGvHD score ($p<0.001$), rapid weight recovery ($p<0.05$) and higher survival rates ($p<0.001$) at the end of the experiment. Upon sacrifice, histopathology in skin and gastrointestinal tract sections demonstrated severe aGvHD lesions in Groups II,III, IV whereas normal findings were seen in Groups I,V. Significantly higher IFNγ mRNA levels were measured in PBMCs of Groups II-IV over Groups I,V. When mSPLCs from sacrificed, at day20, representative animals from all groups were challenged with R848, group V T-cells demonstrated a strong hyporesponsiveness whereas normal TLR activation was observed in mSPLCs from all other groups.
Summary and Conclusion: In summary, the ex-vivo tolerance induction to TLR7 (but not TLR2 or 4) in donor lymphocytes could serve as a clinically applicable tool for aGvHD prophylaxis.

Thrombosis and vascular biology

S1370

MORTALITY AND CARDIOVASCULAR DISEASE AFTER A FIRST EPISODE OF VENOUS THROMBOEMBOLISM IN YOUNG AND MIDDLE-AGED WOMEN

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Background: Cardiovascular disease is one of the leading causes of death in the developed world. Patients with a history of venous thromboembolism (VTE) seem to have increased risk of myocardial infarction and stroke. Little is known about this risk in young and middle aged women.
Aims: To evaluate the risk of arterial cardiovascular disease and death in women, aged 18-64 years, after a first episode of VTE.
Methods: We performed a cohort study including 1438 women with a previous first episode of VTE (exposed) and 1406 age-matched women without any history of VTE (unexposed). The cohort was derived from TEHS, a Swedish population-based case-control study on risk factors for VTE, and the women were recruited in Sweden 2002-2009. During 2011 a letter was sent to the participants in TEHS as an invitation to participate in this follow-up. Information of myocardial infarction and ischemic stroke was obtained from a questionnaire or from data recorded in the Swedish Patient Register. Data on death was obtained from the Cause of Death Register. Comparisons between exposed and unexposed were calculated in a Cox regression model and are presented by hazard ratios (HRs). Adjustment was made for age, smoking, BMI and occurrence of diabetes mellitus.
Results: 2118 women (mean age 47 ± 13 years) accepted participation in this study. The median follow-up time was 70 months. 38 (3.5%) of the exposed and 12 (1.2%) of the unexposed had died during the follow-up period. The incidence of myocardial infarction and stroke was higher among the exposed than the non-exposed (2.2 % *vs* 1 %, p=0.02). The adjusted HR for myocardial infarction and stroke was 2.3 (95% CI 1.0-5.2, p 0.04) for women with a previous episode of VTE compared with unexposed. The hazard ratios were similar in patients with VTE provoked by cast/surgery, oestrogen treatment or unprovoked VTE.
Summary and Conclusion: In our study young and middle-aged women have increased risks of death, myocardial infarction and stroke after a first event of VTE. Better assessment of cardiovascular risk factors and life style intervention could be beneficial for this patient group to decrease the risk of cardiovascular disease in the future.

S1371

INHIBITION OF COMPLEMENT-MEDIATED THROMBOTIC MICROANGIOPATHY WITH ECULIZUMAB IMPROVES HEMATOLOGICAL AND RENAL OUTCOMES IN ADULT PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare and life-threatening condition. It is caused by genetic abnormalities in the complement system, leading to chronic, uncontrolled complement activation. This results in systemic thrombotic microangiopathy (TMA), ischemia and severe damage to organs. Outcomes with plasma exchange/plasma infusion (PE/PI) are suboptimal in aHUS, as up to 65% of patients have permanent renal damage or die. Eculizumab is a terminal complement inhibitor and is the first and only approved treatment for aHUS.
Aims: To report efficacy and safety data on eculizumab from an ongoing study; the largest clinical trial in aHUS conducted to date.
Methods: This is an open-label, prospective, multicenter, multinational single-arm study in adults (≥18 years) with aHUS. Inclusion criteria included platelet count<150 × 10^9/L, hemoglobin ≤ lower limit of normal, lactate dehydrogenase (LDH) ≥1.5 × upper limit of normal (ULN) and serum creatinine ≥ULN at screening. Patients with Shiga toxin□producing *E. coli* (STEC-HUS) or ADAMTS-13 activity<5% were excluded, and identification of a complement mutation was not required for admission. Eculizumab was administered intravenously at 900 mg/week for 4 weeks, 1200 mg in Week 5 and 1200 mg every 2 weeks thereafter. The primary endpoint was proportion of patients with complete TMA response at 26 weeks (platelet and LDH normalization and<25% increase in serum creatinine from baseline).
Results: Of 41 patients treated with eculizumab (mean age 40.3 years, 68% female, 73% newly diagnosed), 38 (93%) completed 26 weeks of treatment. At baseline, 20 patients (49%) had no identified complement mutation, CHFR1/3 polymorphism or CFH antibody and mean (SD) platelet count and serum creatinine was 119.1 (66.1) × 10^9/L and 411 (264.6) µmol/L respectively. Eculizumab was initiated within 2 weeks (median) of patient's current manifestation and was associated with improvements in hematologic and renal outcomes (Table). Of the 24 patients on dialysis at baseline, 20 (83%) were able to stop dialysis during the study; 5 within 3 weeks prior to the first dose of eculizumab. Of the 35 patients receiving PE/PI at baseline, 26 (74%) were able to discontinue PE/PI. The most common adverse events (AEs) were headache (37%) and diarrhea (32%); most AEs were mild or moderate. Two patients had meningococcal infections; both recovered and one continued with eculizumab.

Table 1. Efficacy outcomes through 26 weeks' treatment with eculizumab in patients with aHUS (n=41)

Efficacy outcome	Results
Complete TMA response, n (%) [95% CI]	30 (73) [57.1–85.8]
Hematologic normalization, n (%) [95% CI]	38 (88) [73.8–95.9]
Platelet count normalization, n (%) [95% CI]	40 (98) [87.1–99.9]
Mean (SD) increase in platelet count from baseline, × 10^9/L	135 (114); p<0.0001
Mean (SD) increase in eGFR from baseline	29.3 (25.6); p<0.0001
eGFR increase ≥15 mL/min/1.73 m² from baseline, n (%)	22 (54)

CI, confidence interval; eGFR, estimated glomerular filtration rate; SD, standard deviation; TMA, thrombotic microangiopathy

Summary and Conclusion: In adult patients with aHUS, sustained inhibition of complement-mediated TMA with eculizumab led to clinically meaningful improvements in key hematologic and renal outcomes. There were no unexpected safety concerns with eculizumab. No patients died. These results support the importance of early and accurate differential diagnosis and rapid initiation of eculizumab in adult patients with aHUS.

S1372

IMPROVING OUR UNDERSTANDING OF ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS): INITIAL PATIENT CHARACTERISTICS FROM AN INTERNATIONAL REGISTRY

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Background: Atypical hemolytic uremic syndrome (aHUS) is a life-threatening genetic disease; in most cases, it is caused by chronic, uncontrolled complement activation. It causes thrombotic microangiopathy, which leads to ischemia and severe end-organ damage. Collecting data on the epidemiology and outcomes of treatment for this rare disease will be imperative to maximize future patient care.
Aims: To enhance our understanding of the natural history and progression of aHUS by creating an international registry of patients with a clinical diagnosis of aHUS. The registry will also provide long-term safety and efficacy data on eculizumab, a terminal complement inhibitor. Here, we report baseline characteristics of the first 211 patients enrolled into the registry.
Methods: The aHUS Registry is an observational, non-interventional, multicenter, global registry (NCT01522183) initiated in April 2012. Patients with a clinical diagnosis of aHUS are eligible for inclusion regardless of age, treatment modality and availability of a genetic diagnosis. Patients with severe deficiency of ADAMTS13 activity (<5%) and evidence of STEC-HUS are excluded. Data on demography, medical history and disease management (including efficacy and safety outcomes) are collected at enrollment and then prospectively at 6-monthly intervals.
Results: As of 18 September 2013, 211 patients from 12 countries in Europe, the USA and Australia had been enrolled into the registry. Baseline characteristics are summarized in the Table. Approximately half of the patients are female and approximately half are adults. A genetic complement mutation

or complement autoantibody was identified in 59% of the screened patients. Many have renal impairment, with 43% having received dialysis and 16% having received a kidney transplant. A total of 43% have received plasma exchange/infusion prior to registry entry, and approximately half have received eculizumab.

Table 1. Baseline characteristics of the first 211 patients enrolled into the aHUS Regi September 2013

	Eculizumab treatment		
	Yes (N=104)	No (N=107)	Total (N=211)
Mean (SD) age at diagnosis, years	23.9 (21.4), n=94	17.1 (16.8), n=75	20.9 (19.6), n=169
Mean (SD) age at initial symptoms, years	23.6 (21.4) n=93	17.1 (16.4) n=76	20.8 (19.5) n=169
Age at enrollment, n (%)			
<2 years	17 (16.3)	2 (1.9)	19 (9.0)
≥2 to <5 years	8 (7.7)	7 (6.5)	15 (7.1)
≥5 to <12 years	15 (14.4)	18 (16.8)	33 (15.6)
≥12 to <18 years	5 (4.8)	16 (15.0)	21 (10.0)
≥18 years	55 (52.9)	55 (51.4)	110 (52.1)
N/A	4 (3.8)	9 (8.4)	13 (6.2)
Female patients, n (%)	58 (55.8)	45 (42.1)	103 (48.8)
Stated family history of aHUS, n yes (%)	16 (15.4)	20 (18.7)	36 (17.1)
Complement mutation/auto-antibody, n/N screened (%)	47/88 (53)	46/89 (52)	93/157 (59)
Prior kidney transplant, n (%)	14 (13.5)	20 (18.7)	34 (16.1)
Prior dialysis, n (%)	58 (55.8)	33 (30.8)	91 (43.1)
Prior plasma exchange/infusion, n (%)	59 (56.7)	31 (29.0)	90 (42.7)

Summary and Conclusion: Patient registries are useful for collecting real-world data, particularly for rare conditions. The aHUS Registry will enhance our understanding of aHUS and provide useful safety and efficacy data on the use of eculizumab in this condition. Ultimately, we hope this will improve the management of patients with aHUS. We encourage physicians to enroll eligible patients into the registry.

S1373

DOMAIN SPECIFICITY AND INHIBITORY POTENTIAL OF ANTI-ADAMTS13 ANTIBODIES AT PRESENTATION AND RELAPSE IN THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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Background: Acquired TTP is a life-threatening autoimmune disease associated with the development of autoantibodies (auto-Ab) against the metalloprotease, ADAMTS13. Little is known about the pathogenicity of auto-Ab directed against different domains and how domain specificity alters between presentation and remission/relapse.
Aims: To develop novel assays to determine domain specificity of anti-ADAMTS13 IgG and investigate the relationship with clinical outcomes using UK TTP Registry samples.
Methods: Full-length ADAMTS13 and ADAMTS13 fragments [MDTCS (N-terminal domains); TSR2-8 and CUB1/2 (C-terminal domains)] were expressed in HEK293T cells and purified. Microtitre plates were coated with ADAMTS13 or ADAMTS13 fragments, incubated with diluted TTP plasmas and bound IgG detected. Domain specificity was further analysed by pre-incubation of samples with soluble ADAMTS13 fragments to compete for ADAMTS13 binding.
Results: In a cohort of 60 acquired TTP patients at initial presentation, all had auto-Ab that recognised ADAMTS13 N-terminal domains (MDTCS). 17/60 patients also had auto-Ab against TSR2-8 and 17/60 against CUB1/2 domains respectively, whereas 26/60 had auto-Ab that only recognised MDTCS. In a preliminary analysis of 37 patients, there was no correlation between auto-Ab specificity and total anti-ADAMTS13 IgG titre; age; number of plasma exchanges to remission and, in contrast to previous reports, anti-C-terminal auto-Ab were not associated with lower platelets at presentation. 24% (4/17) patients with anti-C-terminal and 14% (2/14) with only anti-N-terminal auto-Ab at presentation relapsed after rituximab. 8 relapse patients were investigated further: 6/8 exhibited altered auto-Ab domain specificity upon relapse, most frequently with loss of anti-C-terminal reactivity. One patient with persistently high anti-ADAMTS13 IgG and normal ADAMTS13 activity during remission had predominantly anti-TSR2-8 antibodies, suggesting these were non-inhibitory. Functional analysis of domain specific antibodies suggests that anti-spacer auto-Ab are the primary pathogenic species.
Summary and Conclusion: These novel assays enable analysis of the auto-Ab repertoire in acquired TTP and how this may change with relapse. Our findings suggest that there is continual development of the autoimmune response during treatment that can result in altered domain specificity at relapse.

S1374

PREDICTIVE VALUE OF ADAMTS-13 ACTIVITY IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disorder associated with a severe deficiency of the VWF-cleaving protease ADAMTS-13 causing persistence of highly adhesive ultra-large VWF (ULVWF). Several studies are evaluating the predictive value for disease recurrence of ADAMTS-13 activity and its inhibitors.
Aims: To characterize the levels of ADAMTS-13 activity and its inhibitors and establish the prognostic values of these biomarkers for recurrences in TTP patients.
Methods: Between Jan 2001 and Jan 2014, 52 consecutive patients (39F/13M) with clinical diagnosis of TTP were prospectively enrolled after informed consent. Venous blood samples were obtained from 46 patients with idiopathic TTP (35 at the acute phase and at subsequent time points until clinical and hematological remission, and 11 patients at complete remission only), 3 patients with drug-induced TTP (2 thienopyridine-associated and 1 fludarabine-associated) and 3 with bone marrow transplantation (BMT)-associated TTP. ADAMTS-13 activity (by chromogenic assay, Technoclone), inhibitors (by mixing studies), and anti-ADAMTS-13 antibodies (by ELISA kit, Technoclone) were measured in plasma samples.
Results: A complete ADAMTS-13 activity deficiency (*i.e.*<5%) was detected in plasma from the 35 idiopathic TTP patients at the acute phase. In 30/35 patients the deficiency was associated to inhibitory activity against ADAMTS-13 (range: 52-100%) and anti-ADAMTS-13 antibodies (range: 26-209 U/ml). At remission, 16 patients showed normal (*i.e.* >50%), 5 moderate (*i.e.* 20-50%), 2 severe (*i.e.* 5-20%), and 4 complete deficiency (*i.e.*<5%) of ADAMTS-13 activity. In the majority of cases, the normalization or the partial recovery of ADAMTS-13 activity was associated to the reduction in both the inhibitory activity and the anti-ADAMTS-13 antibodies. All of the 6 patients with ADAMTS-13 activity<20% at clinical remission had at least one relapse, as compared to only 9 out of the 21 patients with ADAMTS-13 activity >20% ($p<0.05$). A similar pattern, though not statistically significant, was observed with ADAMTS-13 activity inhibition: *i.e.* relapses occurred in 67% of patients with a detectable inhibition during remission, compared to 37% of patients with no inhibition. In 5 TTP patients, we could not detect any inhibitory activity and anti-ADAMTS-13 antibodies at the acute phase, at clinical remission, and upon relapse. These patients were therefore identified as possible carriers of a true constitutive ADAMTS-13 deficiency. The 3 patients with BMT-associated TTP showed moderate reduction in plasma ADAMTS-13 activity, without measurable anti-ADAMTS-13 antibodies. Differently, all of the 3 patients with drug-associated TTP, showed severe ADAMTS-13 deficiency in the acute phase with anti-ADAMTS-13 antibody positivity. These 3 patients had normal ADAMTS-13 activity on remission.
Summary and Conclusion: Our data demonstrate that, during the acute phase, the measurement of ADAMTS-13 does not distinguish the idiopathic TTP from the drug-induced form, but helps to identify the BMT-associated TTP. Importantly, during remission, the persistent deficiency of ADAMTS-13 activity is predictive of recurrences in these patients. Due to the high risk of relapse, ADAMTS-13 monitoring is recommended in TTP patients follow-up.

Acute lymphoblastic leukemia - Biology 2

S1375

EXPRESSION OF MEF2C AT DN1/DN2 STAGES IS REQUIRED FOR PROPER PROGRESSION THROUGH THE DN2/DN3 STAGES OF T-CELL DEVELOPMENT

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Background: The transcription factor myocyte enhancer factor 2C (MEF2C) is essential to the development of many tissues, including muscle, brain, and the immune system. In the immune system, Mef2c plays a role in the development of both myeloid and lymphoid cells. Mef2c guides megakaryocyte development and directs granulocyte-monocyte progenitors towards the monocytic cell fate. In the lymphoid lineage, Mef2c plays roles in B-cell development and the activation of mature B-cells. To date, no function has been identified for Mef2c in normal T-cell development. In T-cell acute lymphoblastic leukemia (T-ALL), MEF2C has recently been identified as a driving oncogene in patients arrested at an immature developmental stage. This immature cluster is equivalent to early thymic progenitor ALL (ETP-ALL). Understanding the genetic and molecular-biological basis of T-ALL subtypes is essential for the design of future tailored therapies in patient care to improve cure while minimizing adverse late-treatment effects.
Aims: One of our goals is therefore to understand the oncogenic role of MEF2C that results in ETP-ALL. Identification of MEF2C targets normally activated during very early thymocyte development can give insight in oncogenic mechanisms activated by prolonged *MEF2C* expression in ETP-ALL. In this study, we investigate a role for Mef2c in normal T-cell development.
Results: First, we assessed *MEF2C* gene expression in human T cell development stages from *in vitro* OP9-DL1 co-cultures as well as in murine thymocyte subsets. We collected gene expression data from 10 flow-sorted co-culture fractions ranging from human umbilical cord blood-derived hematopoietic stem cells to CD4+CD8+ double-positive (DP) subsets. *MEF2C* is expressed in the early CD34+CD7+, CD34-CD7+ and CD34-CD7+CD5+ fractions while not expressed in the more mature CD7+CD5+CD1+CD4-CD8- and CD4+CD8+ DP stages. These observations are in line with our observation of murine *Mef2c* expression in early T-cell development (DN1-DN2) and with recent gene expression data on mouse thymocyte subsets (Mingueneau *et al.*, 2013). Next, we analyzed the effect of ablation of the *Mef2c* gene on T-cell development *in vivo* using Mef2c conditional knockout mice. These mice were crossed with Lck-Cre or CD2-Cre mice. Lck-Cre is expressed in the DN2 and subsequent thymocyte stages, while CD2-Cre is expressed during the entire T- and B-cell development. As expected, the CD2-Cre-mediated deletion of Mef2c impaired B-cell development in the bone marrow and reduced the number of peripheral B-cells. Remarkably, deleting Mef2c by either CD2-Cre or Lck-Cre impaired T-cell development at the DN2-DN3 developmental stage resulting in an accumulation of DN2-DN3 thymocytes. Accordingly, the DN4 and DP thymocyte percentages were reduced. In addition, the number of peripheral CD4 and CD8 T-cells was severely reduced in the absence of Mef2c, with a more pronounced reduction of CD8 T-cells in comparison to CD4 T cells.
Summary and Conclusion: In summary, we conclude that Mef2c is critical for normal early T-cell development and acts at the DN1-DN2 stage.

S1376

TARGETED RESEQUENCING OF 115 GENES IDENTIFIES ASSOCIATION BETWEEN JAK3 AND EPIGENETIC MUTATIONS IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) arises as result of a multistep oncogenic process involving overexpression of specific transcription factors, deletion of *CDKN2A*, and mutations of many different genes including *NOTCH1*, *FBXW7* and *PHF6*. Next-generation sequencing has recently led to the identification of additional T-ALL driver genes. However, the frequency of these mutations and their co-occurrence remains unknown.
Aims: The aim of this study is to gain insight into the spectrum and association of gene mutations in T-ALL.
Methods: We investigated two independent series of T-ALL (n=80 and n=75). Customized biotinylated oligonucleotide probes (Haloplex, Agilent) were used to select the target regions. We performed 100-bp paired-end sequencing on an Illumina HiSeq2000 instrument. Analysis of the sequence reads was performed using the NextGENe® software. For a variant call we required a read depth of 20 and an allele frequency of 20%. Polymorphisms annotated in dbSNP135 were excluded. The targeted regions comprised 115 genes previously identified as mutated in ALL or candidate oncogenes. Statistical analyses were performed using SPSS.
Results: In the first cohort of 80 T-ALL (including 34 adult cases) we found 356 single nucleotide variants (SNV) and small indels. The median number of mutations per patient was 4. Gene mutations previously identified by Sanger sequencing were also detected by this targeted resequencing approach, validating the quality of the data. Deletions of *CDKN2A, CDKN2B, PHF6, PTEN* and *PTPN2* were also detected based on reduced read depth. Twenty-seven genes were mutated in more than 5% of the cases. As expected, the highest mutation rate was found for *NOTCH1* (55%). Mutation frequencies of *PHF6* (18%), *FBXW7* (14%), *BCL11B* (10%) and *WT1* (7.5%) were in the range of reported frequencies. Interestingly, genes newly mutated in T-ALL included *TDRD6* (6%), *TET3* (6%), *ODZ2* (5%) and *CNOT1* (5%). In the second cohort (n=75, including 10 adult cases) we identified 314 SNV and indels, with a median of 3 mutations per patient. Twenty-two genes were mutated in more than 5% of the cases. In this series, genes newly mutated in T-ALL included *CREBBP* (12%) and *ODZ2* (8%). In both cohorts, the *JAK-IL7R* axis was confirmed to be an important oncogenic pathway as mutations in the *JAK1-JAK3-IL7R* genes were found in 24.5% (38 of 155) of T-ALL. We found that 33% of mutant cases carried two different mutations in *JAK3*. Most significant associations in cohort 1 included positive correlations between mutations in *CNOT1*/*CNOT3* and *BCL11B* genes (*p=0.007*) and mutations in *JAK1*/*JAK3* and *PHF6* (*p=0.002*). In cohort 2, this relationship was not statistically significant, but it was clear that mutations of *JAK1/JAK3* were enriched in *PHF6* aberrant cases (30.8% *versus* 16.1% of cases). A positive correlation between *JAK1*/*JAK3* and *SUZ12* mutations (*p=0.004*) was found in cohort 2. Conversely, *JAK1/JAK3* mutant cases were negatively associated with *TAL/LMO* related patients (cohort 1 *p=0.014*, cohort 2 *p=0.003*).
Summary and Conclusion: Our comprehensive sequence analysis in 155 T-ALL cases has identified the *JAK1-JAK3-IL7R* signaling complex as a potential therapeutic target in 25% of T-ALL. High levels of heterogeneity regarding the spectrum of mutations were observed, with 40 genes being mutated in at least 5% of cases, for which specific associations were also detected.

S1377

ABT-199 MEDIATED INHIBITION OF BCL2 AS A NOVEL THERAPEUTIC STRATEGY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is a high-risk subtype of ALL with survival rates that gradually improved over the last years mainly through introduction of intensified chemotherapy. However, the clinical outcome of resistant or refractory T-ALL remains extremely poor.
Aims: In this study, we investigated whether targeting BCL2 by the specific inhibitor ABT-199 might serve as a new therapeutic strategy in human T-ALL.
Methods: Two independent T-ALL patient cohorts as well as independent sets of sorted normal T-cell populations were analyzed by gene expression profiling and/or western blot analysis. IC_{50} values for ABT-199 were determined in a panel of T-ALL cell lines and in a series of genetically well-characterized primary T-ALL samples. Xenografts of luciferase positive human T-ALLs were used to evaluate the *in vivo* sensitivity of human T-ALL towards ABT-199 in immunodeficient NSG mice.
Results: Gene expression profiling analysis in a large panel of primary T-ALL samples confirmed that high expression of anti-apoptotic *BCL2* is a hallmark of immature subtypes of T-ALL. As normal T-cell development serves as the conceptual framework for the understanding of T-ALL biology, we wondered whether high *BCL2* expression in immature T-ALL is merely a reflection of the spatiotemporal regulation of BCL2 during T-cell development. Indeed, *BCL2* expression is high in $CD34^+$ T cell progenitors and gradually decreases upon differentiation with the lowest values in $CD4^+$ $CD8^+$ double positive T-cells. Interestingly, *BCL2L1* expression showed an opposite trend, suggesting an anti-apoptotic switch from BCL2 to Bcl-xL during T-cell maturation. Next, we determined the IC_{50} values for ABT-199 in a panel of 11 human T-ALL cell lines and observed a strong negative correlation between the IC_{50} values and *BCL2* mRNA (Spearman r=-0.85, p-value=0.0015) and BCL2 protein (Spearman r=-0.7, p-value=0.0204) levels. Mature T-ALL cell lines showed modest responses

towards ABT-199 treatment with IC_{50} values ranging from 0.2 to 10μM. However and most notably, the cell line LOUCY, which shows a transcriptional program highly related to early immature T-ALLs, was highly sensitive towards ABT-199 treatment (IC_{50}=13.9nM). As expected, induction of apoptosis upon ABT-199 treatment in LOUCY cells was associated with a strong induction of caspase activity. Subsequently, we selected primary T-ALL samples that represent major molecular genetic subtypes of T-ALL in order to determine *in vitro* ABT-199 sensitivity of immature, *TLX1*/*TLX3*, *HOXA* or *TAL1/LMO2* positive T-cell leukemias. Most (5 out of 6) *TAL1/LMO2* positive leukemias showed limited response to ABT-199 treatment. In contrast, half of the immature, *TLX3* and *HOXA* positive primary leukemias were highly sensitive to ABT-199 with low IC_{50} values (<50nM). Thus, it is clear that ABT-199 sensitivity is not uniform within a particular molecular genetic T-ALL subtype. Therefore, we hypothesize that BCL2 dependency in human T-ALL is initially determined by its cell of origin, but will ultimately be defined by the additional cooperative genetic defects that occur in each T-ALL patient sample. Finally, we performed xenograft experiments using luciferase positive LOUCY cells in which leukemic growth of LOUCY cells was visualized using bioluminescence. Treatment of established xenograft tumors with 100 mg ABT-199/kg for 4 days resulted in a significant reduction of leukemic burden (p-value=0.0295).
Summary and Conclusion: In conclusion, our study highlights BCL2 as an attractive molecular target in specific subtypes of human T-ALL, which could be exploited by the BH3-mimetic drug ABT-199.

S1378

A MECHANISTIC ROLE FOR MIR-126, A HEMATOPOIETIC STEM CELL MICRORNA, IN ACUTE LEUKEMIAS

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Background: We recently showed that miR-126 expression faithfully identifies the engrafting fraction of bone marrow (BM) and cord blood (Gentner et al, Sci Transl Med 2010), in which it regulates hematopoietic stem cell (HSC) pool size by modulating cell cycle progression. Antagonizing miR-126 expands HSC by enhancing PI3K/AKT signaling, without causing their exhaustion or transformation (Lechman *et al.*, Cells Stem Cell 2012).
Aims: Here we adress the role of miR-126 in human and murine leukemogenesis.
Results: By reconstituting mice with lentivirally transduced BM cells ectopically expressing miR-126, we noted differential effects in HSC and progenitors: while miR-126 overexpressing (126OE) HSC were more quiescent and outcompeted by HSC with physiologic miR-126 expression, progenitor subsets were increased in number and proliferated more upon 126OE. Unexpectedly, 40% of mice (n=49) developed vector-marked, high-grade neoplasms in a 1 year time window (n=15 B cell precursor NPL, n=4 myeloproliferative NPL), while not a single vector-related malignancy was observed in mice transplanted with control vector transduced cells (n=30). Next, we reconstituted mice with BM cells carrying a tetracycline-regulated, conditional miR-126OE vector (n=26). B cell NPL developed in 40% of mice that were kept off doxycycline (miR-126 ON configuration; n=15), while all mice treated with doxycycline (miR-126 off; n=11) remained tumor-free over >8 months. Remarkably, mice with end-stage leukemia (WBC 150/nl, Hb 4g/dl, Plt 20/nl) fully recovered after a short course of doxycycline treatment (n=9), demonstrating that miR-126 is an oncogenic driver lesion. Mechanistically, miR-126OE significantly reduced apoptosis in B cell precursors and blocked differentiation at the immature B cell stage, and miR-126 withdrawal in established tumors rapidly triggered apoptosis. At least part of this effect was due to interference of miR-126 with p53 activation by directly targeting its upstream regulator Cdkn2aip. To establish the relevance of miR-126 in human disease, we measured miR-126 expression in blasts from 16 adult patients with acute lymphoblastic leukemia (ALL). miR-126 was highly expressed in most studied ALL cases (Phil+: n=11, Phil-: n=5) and was in the range of levels found in CD34+ HSC. We then perturbed miR-126 expression by transducing primary ALL blasts from 3 representative patients with lentiviral vectors overexpressing or knocking down miR-126 (miR-126OE or KD, respectively) and compared *in vitro* growth, apoptosis and engraftment potential in NSG mice with control vector transduced cells. Transduction efficiency was similar among groups (mean: 40%). Both miR-126OE and KD significantly increased apoptosis and reduced engraftment in all 3 diseases. We then collected the ALL graft from the BM of the transplanted mice and performed secondary transplantation. Using different readouts (%transduced cells at engraftment, limiting dilution assay, survival analysis), we confirmed a strong disadvantage of ALL blasts in which miR-126 expression was perturbed. These data strongly argue for an obligate dependence of ALL on a specific miR-126 expression level. Most interestingly, when analyzing miR-126 expression with single cell resolution using a lentiviral miR-126 reporter vector, we found substantial intrapatient heterogeneity, with 7 out of 12 patients showing 2-4 distinct cell clusters that maintained their specific miR-126 expression when purified and xenotransplanted. These clusters might correspond to ALL subclones present in individual patients.
Summary and Conclusion: We conclude that miR-126 is pathogenetically relevant in ALL and warrants further investigation as a biomarker and a therapeutic target.

S1379

LOW CELLULAR ENERGY METABOLISM IS ASSOCIATED WITH INCREASED LEUKEMIA INITIATING POTENTIAL IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In acute lymphoblastic leukemia (ALL) leukemia initiating cells (LICs) have been considered to be organized in an hierarchical fashion with only few immature cells capable to propagate leukemia upon transplantation onto immunodeficient mice. Recent data demonstrating LIC-activity also in more committed cells are supportive of a stochastic model in which all cells would possess stem cell properties. However, as a common strategy these studies are based on assessing engraftment potentials of cellular sub-populations defined by expression of distinct surface marker profiles. Nevertheless, so far the nature of leukemia initiating cells in ALL is still unclear. Aberrant energy metabolism has been described to be characteristic for cancer cells including recent reports on alterations in the oxidative state of malignant cell's, describing lower levels of reactive oxygen species (ROS) in breast cancer and myeloid leukemia cells associated with stem cell properties.
Aims: In order to further characterize LICs in ALL we investigated the cellular redox state of primary leukemia cells and analyzed whether energy metabolism of ALL cells determines leukemia initiating cell activity.
Methods: Levels of reactive oxygen species (ROS) were analyzed by detection of oxidation-specific fluorescence of chloromethyl-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) in patient derived xenograft B-cell precursor (BCP) ALL samples. In combination with ROS levels, leukemia cells were stained for their DNA and RNA content allowing determination of distinct cell cycle phases. Moreover, cellular sub-fractions sorted according to high or low ROS levels were analyzed for their potential to repopulate immunodeficient mice using our NOD/SCID/huALL xenograft model.
Results: First, we analyzed cellular energy metabolism along with cell cycle distribution and assessed ROS levels in cellular sub-fractions defined by distinct cell cycle phases, which had been functionally characterized with respect their NOD/SCID mouse repopulating activities. Most interestingly, low ROS levels indicating low oxidative state were identified in leukemia cell fractions of early G0/G1 cell cycle phases, which also showed increased NOD/SCID repopulating activity in functional assays. In contrast, leukemic cell populations of late G2/M cell cycle phases characterized by prolonged engraftment and lower leukemia initiating capacity, displayed increased ROS levels. Vice versa, analysis of cell cycle distribution with respect to energy metabolism showed that ROS^{low} cells are allocated to early cell cycle whereas the majority of ROS^{high} cells originate from later cell cycle phases, suggesting that the ALL cell's oxidative state is indicative for its leukemia initiating activity. To further functionally address this hypothesis, two patient-derived leukemia samples were investigated. Cells were sorted according to high or low levels of ROS and transplanted onto NOD/SCID mice. Both sub-fractions led to leukemia engraftment. Most interestingly, ROS^{low} cells showed an higher activity to engraft and repopulate recipient animals as compared to ROS^{high} ALL cells as shown by significantly prolonged leukemia free survival of mice transplanted with ALL cells of an high oxidative state.
Summary and Conclusion: In conclusion, our data indicate that all cells in ALL show LIC activity, with cells of low energy metabolism representing the driving leukemia initiating cell compartment, thus pointing to redox modulation as a potential therapeutic target in ALL.

Red cell biology

S1380

MTORC1 PATHWAY IS ACTIVATED BY BOTH SCF AND EPO AND REGULATES RIBOSOME BIOGENESIS DURING ERYTHROPOIESIS

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Background: Erythropoiesis requires the renewal of erythroid progenitors with cell size maintenance and their maturation with size reduction during 3 last rapid cell divisions. The first step is supported by the Stem Cell Factor (SCF) which promotes the proliferation and survival of the progenitors and the second step depends on erythropoietin (EPO). Inherited Diamond Blackfan Anemia (DBA) and acquired *5q-* syndrome are characterized by defective ribosome biogenesis resulting in a profound anemia, confirming the important role of ribosome in erythropoiesis. Indeed, active ribosome biogenesis under the control of the mTORC1 pathway has been implicated in the regulation of cell growth before division and maintenance of cell size. Thus, we suspect that ribosome biogenesis may stop for cells to maturate with size reduction at each division.
Aims: The aim is to study the regulation of ribosome biogenesis by SCF and EPO signals during erythropoiesis and to investigate the regulatory role of ribosome biogenesis for differentiation.
Methods: We used two *in vitro* systems of expansion and differentiation of erythroblasts (E) deriving either from human $CD34^+$ of cytapheresis, or from mouse fetal liver. Structure of the nucleolus was studied by electron and immunofluorescence microscopy. Ribosomal biogenesis was quantified by a pulsed SILAC (Stable Isotopic Labeling by Amino acids in Culture cell) proteomic assay as percentage of ribosome renewal, and also by detection and quantification of ribosomal RNA (rRNA) precursors by FISH and by qPCR. Signaling pathways were studied by western blot.
Results: Proerythroblasts (proE) maintained under SCF and EPO, added together, demonstrate a maximal ribosome biogenesis with a rate of renewal of 60% and 50% every 14h or 24h in murin and human erythroblasts, respectively. Ribosome biogenesis decreases with the disappearance of proE and basophilic E. A dramatic reduction of nucleolus size and changes of structure is also observed at the stage of orthochromatophilic E suggesting that it becomes non-functional. Compared to SCF or EPO alone, the effect of the two cytokines is additive and responsible for a strong activation of mTORC1 pathway as judged by the phosphorylation of p70S6K1. This later kinase has been directly involved in the regulation of ribosome biogenesis by promoting rDNA transcription. By contrast, SCF and EPO had no additive effect on the phosphorylation of 4-EBP1, another mTORC1 substrate which is implicated in the initiation of 5'TOP mRNA translation initiation. Moreover, specific inhibition of p70S6K1phosphorylation by rapamycin leads to a decrease of ribosome biogenesis and an acceleration of erythroid differentiation.
Summary and Conclusion: This study shows that optimal ribosome biogenesis upon activation of mTORC1 pathway by SCF and EPO is required to maintain immature erythroblasts. The extinction of mTORC1 pathway drives cell to terminal erythroid differentiation and our results show that this effect could be caused directly by a drastic decrease of ribosome biogenesis. Our results show that p70S6K1 activity is a key player of the erythroid differentiation process.

S1381

CIRCULATING EXTRACELLULAR DNA MEDIATES VASO-OCCLUSIONS IN SICKLE CELL DISEASE

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Background: It was recently reported that circulating extracellular DNA (cirDNA) is increased in sickle cell disease (SCD). CirDNA apears to be associated to intravascular hemolysis and cell-free heme in plasma. But nothing is known of the physiopathological impact of cirDNA in SCD, nor its exact source.
Aims: Here, we aimed to define a role and possible origin of cirDNA in SCD. In particular, we hypothesized that cirDNA may be connected with and favor red blood cell (RBC) aggregation, vaso-occlusive crises (VOC) and ischemic events.
Methods: A blood collection from SCD patients and matched healthy volunteers was built. Platelet-free plsama, RBCs and neutrophils were separated by centrifugation. We cultured human neutrophils *in vitro* and monitored NETs formation. We also quantified extracellular DNA with a fluorescent intercalant probe. We used our model of renal vaso-occlusions induced by infusion of heme-loaded RBC microparticles in humanized SAD transgenic mice which share many features of human SCD.
Results: In SCD patients at steady state, cirDNA was increased by 300% over health, and a further 100% during VOC. Interestingly, cirDNA failed to increase when patients were treated with hydroxyurea, suggesting that increases in hemolysis and cirDNA may be connected during SCD. Next, we treated cultured neutrophils and endothelial cells with purified heme. Heme induced neutrophil extracellular traps (NETs) and endothelial apoptosis, with release of genomic DNA in supernatants. Next, we purified neutrophil DNA, added it to whole blood and studied RBC aggregation by laser-assisted optical red blood cell aggregometry. DNA induced RBC aggregation in SCD blood, but not healthy blood. Conversely, DNase-1 added to SCD blood collected during VOC reduced RBC aggregation back to steady state levels. Next, we triggered renal vaso-occlusions in SAD transgenic mice, and cirDNA levels were increased by 200% within 30 minutes. Moreover, one intravenous bolus of DNase-1, administered after inducing the occlusions, accelerated renal reperfusion back to normal levels in under 20 minutes. In contrast, spontaneous reperfusion with vehicle injection took over 90 minutes.
Summary and Conclusion: Our data reveal a novel mechanism of vascular occlusion in SCD: CirDNA may bind RBCs in blood, promote their aggregation and favor small vessel occlusions and reduced tissue perfusion. CirDNA and NETs may thus bridge intravascular hemolysis, inflammation and ischemic tissue injury in SCD. Interestingly, DNase digestion of plasma DNA and NETs may prove an unexpected but powerful novel pharmaceutical approach to manage vaso-occlusive events.

S1382

THE REGULATORY ROLE OF THE SECOND TRANSFERRIN RECEPTOR IN ERYTHROPOIESIS

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Background: Transferrin receptor 2 (TFR2) is a transmembrane protein, expressed in the liver and in the erythroid compartment, mutated in hemochromatosis type 3. Hepatic TFR2 is an activator of the master iron regulator, hepcidin (*HAMP),* while erythroid TFR2 is a partner of the erythropoietin receptor (EpoR), required for its efficient transport to the cell surface. In erythroid precursors *TFR2* silencing delays the erythroid differentiation. The *Tfr2*$^{-/-}$ mice lacking the hepcidin inhibitor *Tmprss6* shows iron deficiency and erythrocytosis, which is absent when *Tfr2* is specifically deleted in the liver suggesting that TFR2 is a limiting factor for erythropoiesis particularly in iron-deficiency.
Aims: To clarify the erythroid function of Tfr2 we generated and analyzed a mouse model specifically lacking *Tfr2* in the bone marrow (*Tfr2*^{BMKO}).
Methods: Lethally irradiated wild type (wt) male mice were transplanted with bone marrow cells from wt or *Tfr2*$^{-/-}$ donors. We evaluated the hematological parameters from 2 to 6 months after bone marrow transplantation (BMT) in mice fed a normal or iron-deficient (ID) diet, the iron parameters, the hepatic iron-genes expression and the rate of differentiation and apoptosis of the erythroid precursors at sacrifice.
Results: *Tfr2*^{BMKO} mice show Transferrin Saturation (TS), liver (LIC) and spleen (SIC) iron content comparable to wt controls. The ID diet induces a similar decrease of TS and SIC in both genotypes, while LIC was unchanged. The expression of *Bmp6,* the iron *Hamp* activator, is similar in wt and *Tfr2*^{BMKO}, compatible with the normal iron burden of *Tfr2*^{BMKO} mice. At each time point *Tfr2*^{BMKO} mice have higher Hb levels and RBCs count, but lower MCV and MCH than wt controls, a condition more evident in iron-deficiency. Four months after BMT *Tfr2*^{BMKO} have a higher proportion of nucleated erythroid precursors and reduced apoptosis of late erythroblasts than wt controls, in the presence of comparable Epo levels. At this time *Hamp* is lower in *Tfr2*^{BMKO} than in wt mice. Iron-deficiency increases the proportion of erythroid precursors, reduces apoptosis and increases Epo levels in wt mice, while the phenotype of *Tfr2*^{BMKO} animals remains unchanged.
Summary and Conclusion: Erythroid Tfr2 is not involved in systemic iron metabolism and in iron-mediated *Hamp* modulation as its hepatic counterpart. The reduced *Hamp* expression in *Tfr2*^{BMKO} animals 4 months after BMT irrespective of Epo levels, is likely related to the erythroid expansion and reduced apoptosis of late erythroblasts. Tfr2 behaves as a modulator of erythropoiesis in keeping with its function as an EpoR partner. By modulating the Epo sensitivity of the erythroid precursors, Tfr2 might adjust the erythrocyte number in order to balance their production according to the available iron, particularly in iron-deficiency. The lack of a phenotype change in iron-deficient *Tfr2*^{BMKO} mice further supports the hypothesis that Tfr2 is required for the correct erythroid response to iron deprivation. Ongoing full characterization of *Tfr2*^{BMKO} mice erythropoiesis and evaluation of the Epo-EpoR signaling pathway at different maturation stages of the erythroid precursors will help to clarify the underlying mechanisms.

S1383

MONOCYTE CHEMOATTRACTANT PROTEIN (MCP-1) IS A NOVEL MODIFIER OF TISSUE IRON LEVELS AND A PREDICTOR OF DISEASE SEVERITY IN HEREDITARY HEMOCHROMATOSIS

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Background: Most patients with the iron overload disorder Hereditary Hemochromatosis (HH) are homozygous for the p.Cys282Tyr mutation in the *HFE* gene, but only a small percentage develops severe clinical symptoms. Environmental or genetic factors are thought to modify tissue iron overload and disease severity in these patients.
Aims: To identify and validate genetic modifiers of systemic iron levels
Methods: In a search for genetic modifiers of iron levels we performed a targeted RNAi screen for genes that affect transferrin uptake. Validation experiments were performed in MCP-1 knock-out mice and a phenotypically well characterized cohort of HH patients.
Results: We identified the monocyte chemoattractant protein (MCP-1), a chemokine known to be decreased in the serum of HH patient, as a critical suppressor of transferrin uptake in human cells. We next analyzed whether MCP1-deficiency alters iron homeostasis in MCP-1 knock-out mice. Interestingly, the lack of MCP-1 causes decreased serum iron levels and transferrin saturation, massive iron-overload in the spleen as well as mild iron accumulation in the liver. The iron imbalance in MCP-1-deficient mice is unlikely explained by an aberrant immune cell composition of the spleen or by an impairment of the hepcidin/ferroportin regulatory system: hepcidin mRNA expression is unaltered, splenic ferroportin protein expression is strongly increased in MCP-1 deficient mice, as would be expected as a consequence of iron overload. Consistent with our findings in the cellular assays, we observe inappropriately high levels of TfR1 in the MCP-1-depleted spleen paralleled by an increased expression of globin/heme biosynthesis genes and Epo production. This observation raises the possibility that augmented TfR1-mediated iron uptake from the plasma may be responsible at least in part for splenic iron accumulation. Finally, MCP-1 levels were shown to modify disease severity in patients with HH. Among individuals homozygous for the p.Cys282Tyr *HFE* mutation MCP-1 levels negatively correlate with liver iron overload and clinical manifestation of HH at the time of diagnosis.
Summary and Conclusion: Taken together our results suggest that appropriate MCP-1 expression is required to prevent excessive iron accumulation and that a low MCP-1 profile may be used as a novel biomarker predicting symptomatic disease in HH. These findings broaden the growing spectrum of homeostatic roles of MCP-1 that reach beyond its originally identified functions in immune cells recruitment.

S1384

SOLUBLE TRANSFERRIN RECEPTOR 2 IS SHED FROM PLASMA MEMBRANE AS AN EARLY SIGNAL OF IRON DEFICIENCY

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Background: Transferrin receptor-2 (TFR2) is a transmembrane protein, homologous to the iron importer transferrin receptor-1 (TFR1), with a restricted expression to hepatocytes and erythroblasts. TFR2 is the proposed sensor of diferric transferrin (holo-TF) with a regulatory role in iron homeostasis. The manifestation of *TFR2* mutations, responsible for type 3 hemochromatosis in humans, and *Tfr2* inactivation in mice (*Tfr2-/-*), is systemic iron overload with inappropriately low levels of hepcidin, the master regulator of body iron distribution. The binding of holo-TF favors the receptor recycling and its stabilization on the cell surface. In cultured hepatoma cell lines TFR2 interacts with hemochromatosis proteins HFE and HJV to activate hepcidin expression. In erythroid cells TFR2 interacts with erythropoietin receptor (EpoR) for efficient transport of EpoR to the cell surface. Both *TFR2*-hemochromatosis patients and *Tfr2-/-* mice have no evident hematologic abnormalities. However, *TFR2* silencing in human erythroid progenitors delays their terminal differentiation.
Aims: Since TFR1 is released into plasma as a marker of iron deficiency and erythropoietic activity, we searched for a soluble form of TFR2 and investigated its features and function.
Methods: The supernatants of TFR2-transfected cells (HeLa, HuH7, CHO-Trvb-0) and TFR2 competent cells (UT7 and CD36+ human erythroid progenitors) were analyzed by western blot for the presence of soluble TFR2 (sTFR2) and its modulation in response to cell iron changes. We studied the function of sTFR2, evaluating its activity on hepcidin promoter in a luciferase assay in hepatoma cell lines (HuH7 and Hep3B) and its effect on Epo-EpoR interaction in erythroid cells.
Results: sTFR2 is released in the culture media and the shedding is inhibited in a dose dependent manner by holo-TF. The ligand might interfere with the cleavage likely changing TFR2 folding; the $TFR2^{G679A}$ variant, that abolishes the ligand binding motif, releases sTFR2 even in the presence of holo-TF. sTFR2 shedding occurs at the membrane level; a mutated dynamin, that blocks endocytosis, increases sTFR2 release, while brefeldin-A, that blocks intracellular trafficking, inhibits TFR2 shedding. To define the protease involved in the cleavage we tested different protease inhibitors: sTFR2 decreases in the presence of the proconvertase inhibitor cloromethylchetone (CMK). The overexpression of furin (that cleaves HJV) and PCSK7 (responsible for TFR1 shedding) did not modify the amount of sTFR2 suggesting that a different CMK-sensitive protease is involved in the process. Since the cleavage site remains unknown we generated an artificial soluble TFR2 (sTFR2*) spanning the entire ectodomain (696 aa) compatible with the size of the released form. sTFR2* interacts with the iron related proteins HJV and HFE and partially reduces both basal and HJV-mediated hepcidin promoter activation *in vitro*. sTFR2* does not affect EpoR expression or Epo binding in erythroid cells.
Summary and Conclusion: We identified a previously unrecognized soluble form of TFR2 that is shed from plasma membrane both in transfected hepatoma cells and in TFR2-expressing erythroid cells, a process regulated by the ligand holo-TF. We suggest distinct local effects for hepatic and erythroid sTFR2. When transferrin is unsaturated sTFR2 could interfere with the hemochromatosis proteins in the liver, with a modest decoy function on hepcidin activation. At the same time, because of TFR2 low affinity for holo-TF and its interaction with EpoR, the release of erythroid sTFR2 might signal iron deficiency earlier than sTFR1.

PUBLICATION ONLY

Acute lymphoblastic leukemia - Biology

PB1385

CD45 ANTIGEN NEGATIVITY CORRELATES WITH PTPRC MUTATION

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Background: The protein tyrosine phosphatase CD45, encoded by the *PTPRC* gene, is a well known regulator of B- and T-cell receptor signaling. *PTPRC* loss-of-function mutations have been described together with activating mutations of *JAK1*, *IL7R alpha* and *LCK* in T-ALL cell lines, and the consequent down-regulation of CD45 expression resulted in increased downstream signals. CD45 negativity has been described in a small proportion of pediatric T-cell acute lymphoblastic leukemias (T-ALL). We have recently identified a 49 year-old CD45 negative male T-ALL patient. He was a prednisone poor responder and showed a slow blast clearance after induction therapy. To our knowledge, this represents the only adult case so far reported.
Aims: To get insights into the *PTPRC* mutational status we used a combined analysis that could be useful to define a peculiar and rare T-ALL subset.
Methods: Diagnosis of T-ALL was established according to the World Health Organization. Flow cytometry analysis on bone marrow cells was conducted using FACSCanto II instrument (Becton Dickinson, San Josè, CA). CD45 expression was investigated using a PE-conjugated CD45 monoclonal antibody (clone 2D1) (BD Biosciences). gDNA samples of matched tumor (bone marrow) and germline (salivary) were analyzed by whole exome sequencing (WES, Illumina HiSeq2000). Whole transcriptome analysis (RNAseq) was also performed to identify alternative transcripts, fusion transcripts, single nucleotide variants (SNVs) and to quantify gene expression levels. Normal thymus cells from healthy donors were used as negative controls. Validation of SN*Vs* was performed by Sanger sequencing.
Results: Flow cytometry analysis on bone marrow cells revealed an 84% infiltration by T-lineage blast cells: CD7/CD99/TdT/cyCD3/CD5+, with a partial expression of CD2 and CD34, and negative for CD3/CD1a/CD4/CD8/TCRαβ/TCRγδ and for myeloid antigens. The CD45 antigen was also absent. Conventional cytogenetics revealed a normal karyotype, while molecular biology failed to show any recurrent fusion transcripts nor any specific TCR gene rearrangement. WES identified an essential splice site mutation at the end of intron 15-16 of *PTPRC* gene (c.1721-2A>G, *ref.* ENST00000442510) with a variant allele frequency of 0.8125. Validation by PCR and Sanger sequencing confirmed that the *PTPRC* mutation was somatically acquired. RNAseq data indicated that in the majority of mRNA's introns 15 and 16 were retained, while this was not the case for other T-ALL cases or normal thymus cells. In addition, splicing of exon 15 to the middle of exon 16 or a newly used exon was observed, all indicative of erroneous splicing. Notably, a *JAK1* R724H mutation was also detected by WES and RNAseq.
Summary and Conclusions: Overall, this study highlights that the absence of CD45 can be associated with *PTPRC* mutations. The low *PTPRC* expression, together with the lack of CD45 antigen, lead to speculate that none of these alternative transcripts will produce normal proteins but rather unstable proteins prone to be degraded. The study of additional CD45 negative T-ALL cases would help to better define the role of these phenotypic features on the clinical behavior of this peculiar and rare subset. Given the role of CD45 in the regulation of JAK/STAT signaling, further investigations are warranted to clarify if cells harboring CD45 lesion might be sensitive to JAK inhibitors.

PB1386

UPREGULATION OF MIR-181A/C PREDICTS POOR PROGNOSIS IN PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: MicroRNAs (miRNAs) play an important role in lymphoid differentiation, and aberrant miRNA expression has been demonstrated as a frequent event in lymphoid malignancies.
Aims: The aim of this study was to investigate the expression status of miR-181a/c and its clinical significance in adult de novo T-cell acute lymphoblastic leukemia (T-ALL) patients.
Methods: The expression levels of miR-181a/c in bone marrow lymphoid cells were measured in 86 newly diagnosed T-ALL patients and 20 cases of normal healthy donors by real-time quantitative polymerase chain reaction. The prognostic values of miR-181a/c in T-ALL were also analyzed.
Results: Compared with normal controls, upregulation of miR-181a/c in the bone marrow of T-ALL patients with statistically significant differences ($P<0.001$) was found. miR-181a/c upregulation was identified in 62 of 86 (72.1%) T-ALL patients. The patients with miR-181a/c upregulation had lower hemoglobin level than those without miR-181a/c upregulation (64 *versus* 79 g/L, respectively, p=0.008). Moreover, multivariate analyses revealed that miR-181a/c upregulation was associated with worse complete remission (CR) rates (p=0.01), relapse-free survival (RFS; p=0.02), and overall survival (OS) (p=0.03) in T-ALL patients.
Summary and Conclusions: miR-181a/c upregulation is a common event, and its association with poor CR rates, RFS, and OS. These findings suggest that miR-181a/c upregulation may be used as an unfavorable prognostic marker in T-ALL.

PB1387

Abstract withdrawn

PB1388

OPTIMIZATION OF LENTIVIRAL TRANSDUCTION IN PATIENT-DERIVED ACUTE LEUKEMIA CELLS GROWING IN MICE

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Background: Acute leukemia is a frequent disease in children and adults and remains difficult to treat in many patients. Novel therapeutic options are intensively desired and require preclinical testing in adequate model systems. For preclinical testing of new treatment strategies our lab uses the individual xenograft mouse model to amplify primary patient cells in severely immunocompromised mice. We recently established genetic manipulation of patient-derived xenograft (Pdx) cells using third-generation lentiviral vectors to express various transgenes in Pdx cells (Terziyska *et al.*, PLOS one, 2012).
Aims: Nevertheless, lentiviral transduction remains challenging in leukemia cells in general and in patient-derived cells in particular, as these cells are reluctant towards *in vitro* growth. Here, we aimed at improving lentiviral transduction in order to obtain higher transduction efficiencies in Pdx cells.
Methods: Expression of fluorescent proteins and cell surface markers (*e.g.* NGF-receptor) allow flow cytometry-based cell enrichment and sample purification over magnetic columns using magnetic beads. Luciferase expression enables direct visualization of Pdx cells growing in mice by bioluminiscence *in vivo* imaging. Lentivirus production was improved to achieve titers of 10^{9-10} infectious units/mL on B-ALL Nalm6 cell line cells by using pre-coated flasks for culture of HEK-293T cells followed by concentration of the virus supernatant by factor 150 - 200. Using feeder cells, *in vitro* co-culture enabled prolonged culture of transduced Pdx cells *in vitro* in parallel to re-transplantation into NSG mice. Irradiated mouse stromal cell lines EL08 or OP9 allowed *in vitro* culture until the transgene was expressed.
Results: Lentivirus production was improved and the transduction protocol for Pdx cells was optimized by increasing multiplicity of infection (virus to cell ratio) to 1.000 - 1.500 and culturing Pdx cells on laminin-coated plates during infection. If 2 transgenes were to be expressed concomitantly, simultaneous transduction with 2 small constructs was more efficient than a single transduction with a large construct. Although the Pdx cells did not cycle in the co-culture, they produced the transgene, e. g., a fluorescent protein, which could be measured in flow cytometry usually 3 - 6 days after transduction. The data obtained after co-culture nicely correlated with data obtained upon transplantation of lentivirally transduced Pdx cells into mice and were thus predictive for the transduction rate measured after *in vivo* cell amplification. In some, but not all samples and constructs, *in vitro* co-culture even enabled enrichment of transgenic cells directly after lentiviral transduction and spared one passage of cell amplification in mice.
Summary and Conclusions: Taken together and upon optimization of virus production, cell infection and cell culture, transduction efficiencies are now high enough to allow enrichment to above 95% transgenic Pdx cells at best even within a single mouse passage.

PB1389

THE LEVELS OF IKZF1-DELETION IN CHINESE ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The deletion of IKZF1 has been shown to be a hallmark of ALL and early studies suggest it is associated with a poor prognosis. Most IKZF1 intragenic deletions encompass exons 4 to 7, 4 to 8, 2 to 7 and 2 to 8 (thereafter named Δ4-7, Δ4-8, Δ2-7 and Δ2-8, respectively). The deletion breakpoints for these alterations are usually located within a few nucleotides, suggested the feasibility of detecting the levels of IKZF1-deletion based on real-time quantitative polymerase chain reaction (RQ-PCR).
Aims: To detect the levels of IKZF1-deletion in the leukemic cells from Chinese adults with acute lymphoblastic leukemia by using the multiplex RQ-PCR.
Methods: This study involved untreated 328 adult ALL patients at Peking University People's Hospital from 2007-2012. Leukemia cells from 412 ALL patients were obtained at diagnosis and/or relapse from bone marrow or blood samples. In order to detect IKZF1 Δ4-7, Δ4-8, Δ2-7 and Δ2-8 alterations, DNA from the samples were examined by using the multiplex RQ-PCR, multiplex fluorescent PCR and sequence analysis. Plasmid standards of albumin (ALB) gene and IKZF1 Δ4-7 deletions were prepared, and six serial plasmid dilutions (10^7-10^2 copies) were amplified by RQ-PCR to construct a standard curve for the absolute quantitative assessment of copy number. The IKZF1 deletion copy number in 100 ALB copies was used as the IKZF1-deletion gene content.
Results: The results for amplified the IKZF1 Δ4-7 deletion plasmids revealed that all correlation coefficients were above 0.99, and the sensitivity of detection was one copy. In eight DNA samples of IKZF1 Δ4-7-positive patients, the Ct value was in the linear range for a tenfold dilution series (10^0-10^{-5}) and the deletions were detected with a sensitivity of 10^{-4} or 10^{-5}. High correlation coefficients allowed accurate assessment of the quantity of IKZF1 deletions in unknown samples. The detection rate of IKZF1-deletion in untreated 328 ALL patients was 36.28%, including 66 (68.75%) with BCR-ABL1-positive ALL, 46 (22.44%) with BCR-ABL1-negative ALL. In 119 IKZF1-deletion positive specimens, the average IKZF1-deletion copies/ALB copy was 138.54% (0.11%–697.93%). Eighteen IKZF1-deletion-positive patients (84 samples in total) were followed up after treatment, among them, 14 patients in hematologic remission continued to test negative for IKZF1-deletion within 9–66 weeks of follow-up. In three relapsed cases and in one case with no remission, the IKZF1-deletion copies/ALB copies (%) value was obvious increase as compared with the baseline (Figure1). Sequence analysis and multiplex fluorescent PCR revealed that the 119 positive cases included 80 (67.2%) cases with type Δ4-7, 29 (24.4%) cases with type Δ2-7, seven (5.9%) cases with type Δ4-8, three (2.5%) cases with type Δ2-8, and eight cases with deletion of both alleles. Patients with 284 B-ALL subtype had a higher IKZF1-deletion rate (118/284, 41.5%) than those (1/44, 2.3%) with 44 T-ALL subtype (P<0.01). IKZF1 deletions were significantly associated with higher initial white blood cell counts in the peripheral blood(P<0.01),but not with age, gender, blast in the bone marrow, hemoglobin and platelet counts in the peripheral blood.
Summary and Conclusions: The results demonstrated that multiplex RQ-PCR was a reliable and sensitive method for detecting IKZF1 deletions and for the quantitative analysis of minimal residual diseases. Multiplex RQ-PCR should be considered as a routine screening assay for IKZF1 deletions in adult ALL.

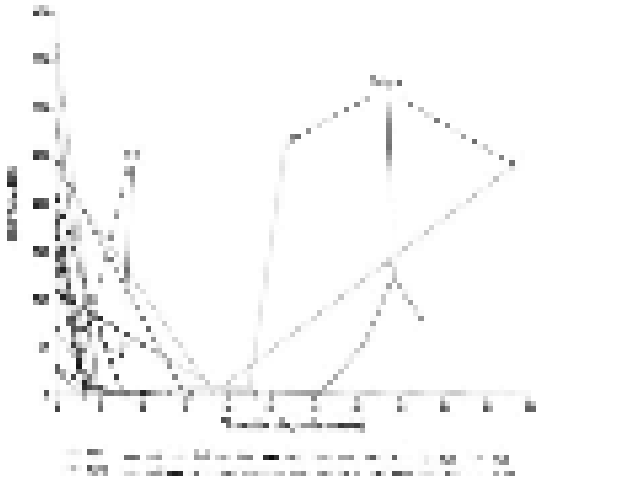

Figure 1. IKZF1 detected copies/ALB copies (%) at diagnosis, after induction therapy, and during follow-up in ALL partients with IKZF1 detection. NR, no hematological remission; Relapse, hematological relapse.

PB1390

GENOMIC ANALYSIS OF NOTCH MUTATIONS IN A CASE OF ALAGILLE SYNDROME WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Alagille syndrome (ALGS), or arteriohepatic dysplasia, is a congenital multisystem disease due to Notch signalling pathway mutations, most commonly affecting JAG1 (ALGS type 1), and more rarely NOTCH2 (ALGS type 2), leading to hepatic, lung, renal and ocular dysfunction (chronic cholestasis, peripheral pulmonary artery stenosis, dysplastic kidneys pigmentary retinopathy), and skeletal abnormalies (minor vertebral segmentation, characteristic facies, posterior embryotoxon/anterior segment defects). ALGS is an autosomal dominant disease, but it is characterized also by variable penetrance and clinical expression and somatic/germline mosaicism. A 20-year-old man with ALGS was admitted to the University Hospital of Verona because of pancytopenia. Following analyses led to the diagnosis of Philadelphia chromosome/bcr-abl-negative, CD10-positive, B-lineage acute lymphoblastic leukemia (common B-ALL).
Aims: The occurrence in the same patient of both congenital Notch pathway mutations (ALGS) and acquired neoplastic disease related to the Notch signalling (B-ALL) is quite an exceptional event that offers a great opportunity to investigate the role of Notch in the predisposition to, onset and development of leukemia. In order to identify the genetic components involved in this complex phenotype, we sequenced the exome of a bone marrow sample collected from the patient.
Results: By genome interpretation with Knome pipeline applied to the reference genome UCSC hg19, we found missense variants both in NOTCH2 (E38K) and JAG1 (P871R) genes that are mainly involved in the syndrome, although their effect on protein function was predicted not to be deleterious. We detected putative damaging mutations in genes such as PAX5 (R38H) and NOTCH1 (K1821N) which might be strongly related to the observed disease. In fact, PAX5 is a member of PAX protein family of transcription factors implicated into regulation of early development, that binds NOTCH2 and likely altering its functionality. On the other hand, NOTCH1 is involved in cell growth and proliferation and thus the predicted alteration of function of the corresponding protein may have an important role in neoplastic transformation.
Summary and Conclusions: Overall, this study provides novel insights in the pathogenetic development of B-ALL in a patient with dysregulated Notch signalling due to ALGS. On the basis of the genetic abnormalities found and here discussed, we suggest a model that may explain the molecular link among the different signalling pathways involved in the development and relapse of B-ALL.

PB1391

EVALUATION OF NEW MARKERS FOR FLOW CYTOMETRIC MONITORING OF MINIMAL RESIDUAL DISEASE IN CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common cancer of childhood. Initial diagnostics of this disease is currently established with multiparametric flow cytometry, *e.g.* using EuroFlow Consortium approach. Flow cytometry is also successfully applied for treatment monitoring, through the detection of minimal residual disease (MRD) at different treatment timepoints. The initial response to the induction treatment, ie. degree of reduction of leukemic cell burden during the first 4-6 weeks of chemotherapy as measured by MRD is particularly crucial for stratification of patients into risk groups.
Aims: The aim of the study was to assess the expression of newly identified markers of blast cells in childhood BCP-ALL, CD304 and CD86 which are not expressed by normal B-cell precursor cells. The stability of expression of these markers was assessed in terms of usefulness for MRD monitoring.
Methods: Bone marrow samples of 99 consecutive children with BCP-ALL were stained at initial diagnosis with four 8-color combinations of monoclonal antibodies, including the new markers CD304 and CD86. In 53 patients, bone marrow samples were analysed at day 15 of the induction treatment and in 25 patients also at day 33.
Results: The expression of CD304 was observed in 56.4% of patients at diagnosis. High expression of CD304 was observed in 27.4% of patients

whereas in 29.0% of patients the expression of CD304 was weak. The expression of CD86 was found in 32.7% of patients at diagnosis. High expression of CD86 was observed in 12.7% of patients while in 20.0% of patients the expression of CD86 was low. The examination of bone marrow samples at day 15 and 33 of the induction treatment revealed that the level of expression of both CD304 and CD86 during treatment was similar as compared to the diagnosis. No cases of disappearance of either of the two examined antigens were found.

Summary and Conclusions: Both tested markers appeared to be useful for MRD monitoring, particularly CD304. More accurate definition of the phenotype of leukemic blasts enables more precise assessmet of leukemic blasts numbers during early treatment timepoints, which is important for proper stratification of BCP-ALL patients into risk groups.

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PB1392

BOTANICAL ALKYL HYDROQUINONE HQ17(3) IS CYTOTOXIC TO T(9;22) PHILADELPHIA CHROMOSOME SUP-B15 ALL CELLS THROUGH INDUCING OXIDATIVE STRESS AND IRON-DEPENDENT AUTOPHAGY-ASSOCIATED LYSOSOMAL EVENTS

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Background: Acute lymphoblastic leukemia with Philadelphia chromosome (Ph^+-ALL)(t(9;22)*BCR-ABL*) is a very high risk (VHR) hematological neoplasm. Multiple genetic lesions together with constitutively active BCR-ABL oncoprotein contribute to a very aggressive clinical cause. Tyrosine kinase inhibitors (TKIs) fail to convey long-term control of the disease. Searching for agents with higher specificity to leukemias and investigating the molecule mechanisms involved in the selective ibhibitory effects on leukemic cells will help to find new anti-leukemic therapeutics for the Ph^+-ALL. HQ17(3) [10'(Z),13'(E),15'(E)-heptadecatrienyl-hydroquinone], a natural product isolated from the sap of *Rhus succedanea*, exhibited very effective cytotoxic effect on the TKI-resistant Ph^+-ALL cell line SUP-B15 (IC_{50}: 1.9 μM), but spared normal peripheral blood mononuclear cells.

Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced cytotoxic effects in Ph^+-ALL SUP-B15 cells.

Methods: HQ17(3)-treated and control SUP-B15 cells were stained and analyzed by flow cyteometry: 1) membrane lipid disturbance was analyzed by Annexin V/PI stain, 2) DNA fragmentation was defined as sub-G1 fraction of cellular DNA content after the PI staining, 3) mitochondrial membrane potential (MMP) loss were stained by DiOC6(3). Pan-caspase inhibitor (zVAD-fmk), receptor interacting protein 1 (RIP1) inhibitor (necrostatin-1, Nec-1), iron-chelator (deferroxamine, DFO), 3-methyladenine (3MA) or chloroquine (CQ) (autophagy inhibitors), and lysosomal protease inhibitors were used in combination with HQ17(3) in some experiment. Acridine orange stain and confocal microscopy are used to visualize the changes of lysosomes in the presence of HQ17(3). Autophagic flow in response to HQ17(3) was revealed by accumulation of LC3B-II visualized by western blot analysis.

Results: Introduction of HQ17(3) induced rapid (within 24 hours) and extensive cell death characterized by losing plasma membrane integrity (PI^+) concomitant with PS exposure (Annexin V^+), which was not prevented by zVAD-fmk and/or Nec-1, indicating a caspase-independent necrotic death program. HQ17(3)-induced cell death displays MMP loss and profound nuclear DNA fragmentation that could be attenuated by ROS scavengers. ROS production/oxidative stress account for important part of the cell demise. DFO abolished the HQ17(3)-induced cell death, suggesting iron-dependent event(s) is critical for the HQ17(3)-induced cell destruction. Both the number and size of acidic vesicles are significantly increased 4 hours after treatment of HQ17(3) then diminished when cell death is evident. Application of AEBSF (serine protease inh.) and/or pepstatin/CA074-Me (cathepsin D/B inh.) did not rescue cells from death, thus rule out the lysosomal membrane permeability (LMP)-mediated death program. Autophagic flow was enhanced in HQ17(3)-treated cells. Further, autophagy inhibitors (3MA and CQ) showed a modest protective effect.

Summary and Conclusions: Naturally-derived HQ17(3) displayed selective, and significant cytotoxicity in Ph^+ ALL SUP-B15 cells. HQ17(3) induces extensive iron-dependent and autophagy-related lysosomal events followed by necrotic-like cell demise. The cell death program is caspase-independent, and different from necroptosis or LMP-mediated cell death. ROS production contributes to this potent cell destruction process. These results suggest that agents selectively induce or sustain ROS in leukemic cells may induce autophagy-associated cell death, and would potentially augment the treatment for VHR-ALL with t(9;22) translocation.

PB1393

INTRA-TUMORAL HETEROGENEITY OF T-ALL LEUKEMIC BLASTS AT DIAGNOSIS AND FOLLOW-UP; IMPLICATIONS FOR MINIMAL RESIDUAL DISEASE MONITORING

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Background: Heterogeneity of T-cell acute lymphoblastic leukemia (T-ALL) blasts may compromise minimal residual disease (MRD) monitoring, the most important prognostic tool in T-ALL. PCR is currently used for MRD monitoring, but potentially both PCR- and flow cytometry (FC)-based MRD methods might miss blast subpopulations that differ from the dominating clone at diagnosis, which is important if subpopulations have divergent chemo-sensitivity.

Aims: We aimed to investigate the intra-tumoral heterogeneity of the leukemia-associated immunophenotype (LAIP) in T-ALL patients and the implications for MRD detection.

Methods: We investigated the LAIP by 8-color FC in diagnostic bone marrow (BM) samples from 49 T-ALL patients (NOPHO ALL-2008). 22 of these were also analyzed for diversity of T-cell receptor (TCR) gene-rearrangements in flow-sorted blast subpopulations. MRD LAIP markers were evaluated by PCR TCR-marker detection in flow-sorted cells (61 follow-up BM samples from 30 pts). LAIP modulation patterns seen by FC during early induction treatment were further evaluated in 18 patients MRD-positive >0.1% (PCR-MRD day29).

Results: >80% of the T-ALL patients had at diagnosis heterogeneous LAIPs with bimodal marker expression, most often of CD1a, CD4, and TdT. Dominant TCR clonal gene rearrangements were generally conserved across the phenotypically diverse blasts (21 out of 22 patients), except in one patient. We did not detect an association between a high number of blast subpopulations at diagnosis (>two markers bimodally expressed) and high MRD level (PCR-MRD day29 >0.1%). The percentage of predicted LAIP-defined sorted MRD cells being PCR-positive was: 94% in patients with fully informative LAIP and 76% in patients with partly informative LAIPs (not all blasts having aberrant marker expression). The percentage of cells classified as normal cells being PCR-negative when sorted was: 93-95% (in patients with informative LAIP) or 62-75% (in patients with partly informative LAIP).

At early follow-up, we observed significant LAIP changes in several markers, including loss of CD1a and TdT and CD4 decrease.

Summary and Conclusions: Intra-tumoral heterogeneity of immaturity and T-linage markers was common in T-ALL. The phenotypically diverse blasts generally had invariable dominant TCR gene-rearrangements; accordingly PCR-MRD would detect all subpopulations. When all blasts of heterogeneous LAIPs were informative, MRD identified by FC was highly concordant with cells positive for TCR MRD markers. The T-ALL blasts showed changes in LAIP at early follow-up, most significant in reduction of immaturity markers, which can affect MRD by FC. Whether changes in heterogeneous LAIPs at follow-up are due to divergent subpopulation chemo-sensitivity or modulations of expression in surviving blasts need to be elucidated.

PB1394

CHARACTERIZATION OF SET-NUP214 REARRANGEMENT IN T-ACUTE LYMPHOBLASTIC LEUKAEMIA. SINGLE CENTRE EXPERIENCE

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Background: SET-NUP214 rearrangement was first reported in 1990, but with increasing strong association with T-ALL. Recently it was reported that this specific rearrangement could not be by itself leukemogenic.

Aims: We present two cases of acute leukaemia with SET-NUP214 rearrangement and complex karyotype.

Methods: To characterize the SET-NUP214 rearrangement 41 adult ALL patients were screened using hemaVisoin kit (DNA Technology, Denmark) and two were found SET-NUP214 positive with incidence of 4.9% which is equal to previous study. The first patient, female, 48 years old, was admitted to the hospital due to general lymphadenopathy. Her blood count indicated anaemia and leukopenia and film examination showed 59% large size blasts. Bone marrow biopsy and biopsy from the cervical node have been done. The diagnosis of early T ALL was made (CD7+, CD5 dim, sCD3-, cCD3+, CD4-, CD8-, CD34+, HLADR+, CD117+, MPO+). Cytogenetic analysis showed complex karyotype deletion of chromosomes 13, 17, 12 and 18. The second patient, male, 45 years old, was

admitted to the hospital due to easy tiredness and osteoalgia. His blood count indicated lymphocytosis and film examination revealed 63% large size blasts. His bone marrow biopsy was dry tap. His computed tomography revealed general lymphadenopathy. The diagnosis of early T ALL was made by both bone marrow biopsy and immunophenotypic analysis of peripheral blood (CD7+, CD38+, CD34+, CD3+, CD4-, CD8-, CD33+, CD1a-).

Results: In both cases, a single SET-NUP214 transcript was amplified by RT-PCR and sequencing of the fusion transcript revealed fusion of the exon 7 of SET to exon 18 through exon 32 of NUP214. This fusion transcript is identical to that described in the previously published cases (Image 1). In order to further investigate this rare rearrangement we performed array-CGH analysis. Apart from del(9)(q34.11-34.11) which generated the SET-NUP214 rearrangement both patients were found to harbour multiple genomic losses and gains. Twenty additional genomic regions on chromosomes 2, 6, 8, 9, 11, 12, 16, 17 were deleted in the first patient and thirteen genomic imbalances located on chromosomes 1, 4, 6, 7, 9, 12, 13, 17, 18, 20 were identified in the second patient. Both patients shared del (17)(q11.2), del(6)(q16.1-q21) and del(12)(p12.1-13.1) –spanning the ETV6 and CDKN1B gene loci– , that have been also described in other published cases with SET-NUP214. The female patient received combined chemotherapy followed by transplantation from her fully matched sibling. After one year she relapsed and died during the induction therapy. The male patient received also combined chemotherapy followed by transplantation from a fully matched unrelated donor. He died six months after the transplantation.

Summary and Conclusions: SET-NUP214 rearrangement is recently reported to early T ALL and to acute undifferentiated leukaemia. The reported cases are still few to make inferences but since this rearrangement is insufficient to develop leukaemia in mice, it needs additional chromosomal aberrations to induce leukemogenesis. It seems that the complex karyotype and possibly the ETV6 and CDKN1B deletions, detected in the many cases with SET-NUP214 are critical to the phenotype and to prognosis of this type of acute leukaemia.

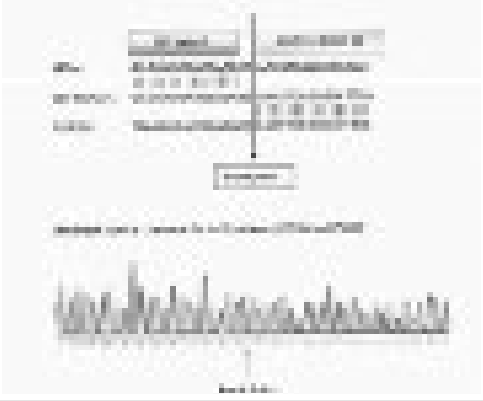

Figure 1.

PB1395

EVIDENCE OF ANTITUMOR ACTIVITY OF MACROPHAGES IN CULTURE

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Background: For a long time macrophages are considered as the main cells defending the organism from infection agents, but their role in the struggle against tumor isn't always evident. Study of macrophage-lymphocyte contacts in blood leukocyte cultures of patients with different diseases revealed that macrophage-lymphocyte rosettes (MLRos) formation *in vitro* represents immune reactivity of organism. Their low indices show decrease of immunity, whereas their high level reflects immune conflict in the course of the disease. [Shvelidze T., Saralidze T., Saralidze N., Mokhevishvili L. (2013) Atlas of Hematology, Preclinical Diagnosis. //www.e-bookland.ge/medical/ATLAS-of-HEMATOLOGY]. Our previous investigations showed that amount of MLRos in patients with malignant solid tumor diseases equaled 54,5±1,1%, while in healthy donors the general amount of MLRos is 37,4±2,2%; among them macrophages contacted with 1 lymphocyte composes 20,7±1,1%, macrophages contacted with 2 lymphocytes - 11,8±1,0%, and macrophages contacted with 3 and more lymphocytes 4,8±0,7%. Though in patients with malignant solid tumor macrophages were contacted with one or rarely with two lymphocytes, increase of total amount of MLRos was considered as reaction of immunocompetent cells to abnormal for organism tumor cells. In patients treated with a-interpherone MLRos increased to 81,0+4%, and macrophages were contacted with 3 and more lymphocytes that confirmed activation of immune system. This data coincided with improvement of the patients.

Aims: Based on the abovementioned results we decided to estimate the role of macrophages in the cases of acute lymphoblastic leukemia.

Methods: For this aim was used a method of cultivation of blood and bone marrow cells worked out by Saralidze T., Shvelidze T. (1998), that gives opportunity to monitor hemopoiesis in norm and pathological states.

Results: Foresaid method supports proliferation of macrophages in norm and proliferation of blast cells in the cases of acute lymphoblastic leukemia according to the type and form of the disease. In peripheral blood and bone marrow leukocyte cultures (3 -9 –days) of the patients with acute lymphoblastic leukemia proliferation of blast cells was accompanied by formation of macrophage-lymphocyte and blast-macrophage rosettes. Besides lysis and degradation of blast cells contacting with macrophages without their endophagocytosis (that is characteristical for the digestion of strepto- and staphylococci by the macrophages) was observed. These facts point on the antitumor role of macrophages in the cases of acute lymphoblastic leukemia in culture. *in vitro* proliferation and mitosis of macrophages was confirmed by incorporation of H^3-thymidine in them.

Summary and Conclusions: Multiplication of macrophages *in vitro* and their antitumor action against blast cells in the cases of acute lymphoblastic leukemia, makes it possible to think about formation of individual macrophage banks for clinical use.

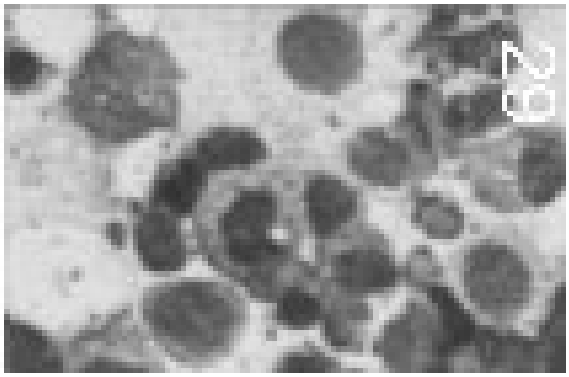

Figure 1. Abundance of lymphoid blast cells. Contact of lymphoid cells with macrophage – blast-macrophage rosette (center). 5-day-culture of bone marrow aspirate of a patient with acute B lymphoblastic leukemia. May-Grünwald-Giemsa stain. X 1000.

PB1396

THE FREQUENCY OF HLA -A, B, DRB1 ALLELES ACCORDİNG TO RISK GROUPS IN CHILDREN WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMİA

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Background: Previous studies have demonstrated some significant differences in HLA allele frequencies in leukemic patients and normal subjects.

Aims: The purpose of this study is to evaluate the frequencies of HLA class I (A, B) and class II (DRB1) alleles in patients with acute lymphoblastic leukemia and compare to unrelated healthy subjects in Central Anatolia of Turkey.

Methods: This study was performed in 90 children with ALL, whose ages were ranging between 1-18 years. Twenty nine of 90 patients had standard risk group (SRG) of ALL, 37 moderate risk group (MRG), and 24 high risk group (HRG) respectively according to Berlin Frankfurt Münster (BFM) standards. We have typed for HLA-A, B, DRB1* alleles in patients with ALL and 90 unrelated normal subjects in Central Anatolia of Turkey. PCR-SSO low resolution method (Luminex technology) was used for HLA typing.

Results: Allele frequencies of HLA-A*01,HLA-A*29 and DRB1*07 were higher in patients with ALL compared to the control group (p=0.008, p=0.032, and p=0.000, respectively). On the contrary, HLA-B*08 and DRB1*08 alleles frequencies in patients with ALL lower than controls (p=0.010, p=0.016, respectively). DRB1*04 allele was higher in HRG ALL and MRG ALL than in SRG ALL (p=0.009). DRB1*07 allele was higher in SRG ALL than in HRG and MRG ALL (p=0.007). The most observed haplotype was A*02, B*35, DRB1*13 (p=0.023) in patients with ALL. We could not find any haplotypes negatively associated with ALL. The most observed homozygous allele was A*24 (p=0.043) in the presented cohort.

Summary and Conclusions: These results suggest that HLA-A*01, A*29, DRB1*07 alleles may play a presumptive predisposing factor in ALL , whereas HLA-B*08 and DRB1*08 alleles have been found to be negatively associated with ALL. In addition, DRB1*04 allele has been found as associated with HRG and MRG ALL. Also, DRB1*07 allele may play a presumptive predisposing factor for SRG ALL.

Acute lymphoblastic leukemia - Clinical

PB1397

Abstract withdrawn

PB1398

HIGHER INCIDENCE OF HYPERDIPLOIDY AND BETTER PROGNOSIS FOR HIGH HYPERDIPLOIDY IN OUR COHORT OF PEDIATRIC B-ACUTE LYMPHOBLASTIC LEUKEMIA: A FLOW CYTOMETRY BASED PILOT STUDY FROM NORTHERN INDIA

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Background: The numerical and structural genetic aberrations are an important prognostic indicator in pediatric ALL. DNA ploidy assessment forms an important method to detect numerical genetic abnormalities and patients are categorized into favorable or unfavorable risk groups based on high hyperdiploid (>51 chromosomes) or hypodiploid (<46 chromosomes) DNA index respectively.
Aims: To standardize and note the frequency of DNA ploidy abnormalities in pediatric B-ALL and to correlate the same with response to chemotherapy.
Methods: DNA ploidy assessment was done in 40 pediatric B-ALL cases confirmed on bone marrow and immunophenotyping before start of chemotherapy. 40 non-leukemic controls were run with each sample. Samples were processed using DNA ploidy kit reagents (Cycletest DNA ploidy kit; BD biosciences) and analysis was done on a flow cytometer (LSR-II; BD biosciences). The DI (DNA index) was calculated using the Mofit software. The results were compared with standard risk criteria and chemotherapy outcome (modified UK-ALL protocol).
Results: A hyperdiploid cell line was noted in 33/40 (82.5%). 7/40 (17.5%) cases had diploid cell line. None of the cases showed a hypodiploid population. DI of 1.01-1.15 (Hyperdiploid A) was seen in 17/33 (51.5%), DI between 1.16-1.6 in 11/33 (33.3%) and 4/33 (15.2%) had a DI>1.90 (tetraploidy range). Cases with DI >1.16 had significantly better response to treatment than with those having diploid DI or DI between 1.06-1.16 ($p<0.05$). Significant prognostic factors on Cox analysis were DI>1.16, age between 2-9 years, WBC count< 50,000 and Hb<9.0.

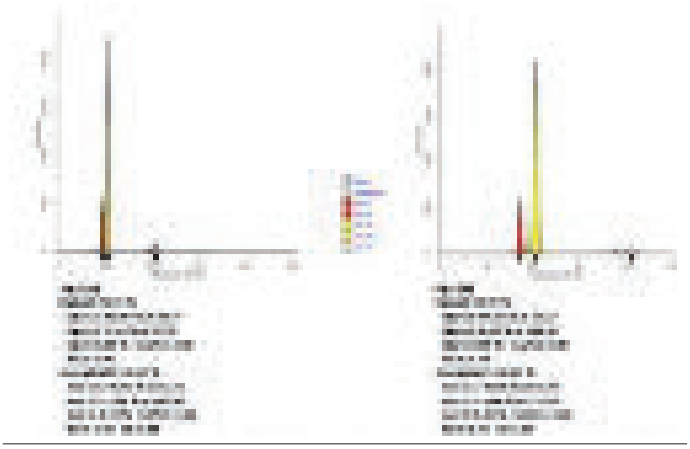

Figure 1. a) and b) showing Hyperdiploid A and Hyperdiploid B cases with DI 1.08 and 1,19 respectevely.

Summary and Conclusions: There is a relatively high frequency of hyperdiploidy cell line in our cohort of B-ALL patients. Cases with DI>1.16 and WBC count <50,000 at presentation are likely to respond better to chemotherapy with less risk of relapse. However, prospective studies on a large cohort of B-ALL cases in our population are needed to derive definitive conclusions and make individualized treatment decisions.

PB1399

SAFETY AND TOLERABILITY OF INTRATHECAL ADMINISTRSTION OF LIPOSOMAL CYTARABINE IN COMBINATION WITH DEXAMETHASONE AS CNS INVOLVEMENT PROPHYLAXIS AND TREATMENT IN LEUKEMIA/ LYMPHOMA PATIENT

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Background: Prophylaxis or treatment of central nervous system (CNS) involvement is a part of therapy of adults with acute lymphoblastic leukemia (ALL) and aggressive non Hodgkin lymphoma (NHL) patients. The treatment strategy includes cytostatics and steroids administered intrathecally (i.t.) by lumbar puncture. In the past few years, the new formulation of slow-release liposomal cytarabine (DepoCyte, Mundipharma, Cambridge, UK) was introduced. The drug is characterized by prolonged activity in the cerebro-spinal fluid that may increase compliance in prophylaxis and reduce of the total number of i.t. injections in treatment of leukemia/lymphoma meningitis. One of the most common adverse reaction connected to liposomal cytarabine administration is arachnoiditis. However, its incidence can be significantly reduced by concurrent steroids administration. Additionally, steroids given orally or intra venous prevent arachnoiditis but have very limited antineoplastic activity for tumor cells located in CNS.
Aims: The purpose of our study was to evaluate safety and tolerability of liposomal cytarabine and dexamethasone intrathecal administration.
Methods: Thirty patient (15 with ALL and 15 with aggressive NHL) aged 19-76 years (median 49) were treated i.t. with a total 121 (median 3 for a patient) single doses of 50mg liposomal cytarabine. Fifty three lumbar punctures also included 4 mg of dexamethasone administration. All of the patients received additionally steroids orally (dexamethasone or prednisone) for minimum 5 days.
Results: In the whole group no serious adverse event was present. Only in two cases (both in arm without dexamethason i.t.) mild headache were noted without long –term neurological side effects.
Summary and Conclusions: In conclusion, concurrent administration of dexamethasone with liposomal cytarabine intrathecally is feasible and well tolerated manner of CNS prophylaxis or treatment in ALL and aggressive NHL patients. The effectiveness of such treatment strategy needs further evaluation.

PB1400

EVALUATION OF SERUM LEVELS OF SELECTED CYTOKINES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA USING BIOCHIP ARRAY TECHNOLOGY

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Background: Cytokines have been studied as markers of immune system activation in various diseases including hematological malignancies. Alterations in this interacting functional network may have direct effect on the malignant cells or have indirect effect on leukemogenesis through altered functions of bone marrow stromal elements. The knowledge gained from multi-analytical determination of cytokines could allow better diagnosis and management of hematological malignancies, since cytokines or their receptors may also represent a target for specific anticancer therapy at the molecular level. Recently, some studies reported the possible diagnostic and prognostic use of cytokine levels in newly diagnosed acute leukemias.
Aims: The aim of our study was to evaluate serum levels of selected cytokines in patients with newly diagnosed acute lymphoblastic leukemia (ALL) and in healthy subjects using the innovative biochip array technology. This approach allows simultaneous detection of multiple cytokines from a single sample.
Methods: Serum samples of 21 newly diagnosed ALL patients (median age 46, range 24 - 75 years, 17 males and 4 females, 20 B-ALL, 1 T-ALL) and 15 healthy subjects (median age 41, range 25 - 58 years, 11 males and 4 females) were analyzed. We evaluated serum levels of the following cytokines: interleukin-5 (IL-5), interleukin-15 (IL-15), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-1 alpha (MIP-1 alpha), soluble IL-2 receptor alpha (sIL-2R alpha), soluble IL-6 receptor (sIL-6R), soluble tumour necrosis factor receptor I (sTNFR-I), soluble tumour necrosis factor receptor II (sTNFR-II), matrix metalloproteinase-9 (MMP-9). All analytes were measured by biochip array technology using chemiluminescent sandwich immunoassays applied to the Evidence Investigator analyzer (Randox). Probability values (p)<0.01 were considered statistically significant.
Results: In newly diagnosed ALL patients, we found significant increase in serum IL-15 (1.74±0.97 ng/L *vs.* 0.81±0.16 ng/L; p=0.0008), MIP-1 alpha (6.36±3.26 ng/L *vs.* 2.68±1.47 ng/L; p=0.0003), sIL-6R (2.29±1.80 mcg/L *vs.* 0.89±0.39 mcg/L; p=0.006), sTNFR-I (0.96±0.50 mcg/L *vs.* 0.25±0.07 mcg/L; p=0.000005), sTNFR-II (0.73±0.51 mcg/L *vs.* 0.29±0.14 mcg/L; p=0.003). Serum levels of other evaluated cytokines were without significant differences.
Summary and Conclusions: Our results indicate that serum levels of some cytokines (IL-15, MIP-1 alpha, sIL-6R, sTNFR-I, sTNFR-II) are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. Further investigation is needed to establish if the alterations observed in the levels of these molecules could be used as a prognostic indicator for ALL.
The work was supported by a long-term organization development plan 1011 (FMHS).

PB1401

HIGH-HYPERDIPLOID CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA, SINGLE CENTRE EXPERIENCE

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Background: High hyperdiploidy (HeH) (51-67chromosomes), characterized by non-random gain of chromosomes, is a distinct subgroup of pediatric B-cell precursor acute lymphoblastic leukemia (ALL) which is generally associated with favorable prognosis.
Aims: In order to address the important issue of clinical and genetic factors associated with HeH and their relation with established favorable prognostic factors we performed a retrospective analysis of patients with HeH who were treated for B-cell precursor childhood ALL in our department in the last 5 years and their cytogenetic analysis was available.
Methods: Prettreatment bone marrow samples were cultured and analyzed by standard cytogenetic methods. Targeted analyses for t(1;19), t(9;22), der(11q23), and t(12;21) by FISH, or reverse transcriptase-polymerase chain reaction (PCR) were carried out. A total of 20 children (11 girls and 9 boys) displayed G-banded karyotypes with 51-67 chromosomes that were also informative for molecular studies, were included for analysis.
Results: The most frequently gained chromosomes were X (100%), 21 (95%), 14 (55,5%), 17 (55,5%), 6 (50%), 4 (38,8%%), 18 (38,8%) and 10 (38,8%). Triple trisomy (+4, +10,+17) were identified in 33,33% cases. The triple trisomy-positive cases had a median of 57 chromosomes (range, 51-65), whereas the negative cases had a median of 54 chromosomes (range, 51-57) Translocation-positive high hyperdiploidy (t-HeH) was identified in only 2 cases with t(12;21). None of the HeH cases had evidence of extramedullary (central nervous system, mediastinal mass or testes) leukemia at diagnosis. Median age at diagnosis was 4.7 years (range: 1.5-13 years). Median WBCx109/L was 2.100 (range: 1200-17000). Only 3 cases were stratified as high-risk patients because they were prednisone poor responders and their minimal residual disease (MRD) in bone marrow on day 15 was LOG-1 but on day 33 they all achieved complete remission with MRD of LOG-4. All patients are in continuous complete remission with event-free and overall survival of 100%.
Summary and Conclusions: Our results are consistent with literature that HeH is the largest cytogenetic subgroup in childhood B-cell ALL and is associated with other favorable clinical features. The impact of triple trisomy (+4, +10,+17) in the context of high hyperdiploidy warrants further investigation in order to explore the potential of treatment deescalation allocating less intensive therapies and reducing toxicity in the frame of international collaborative studies.

PB1402

EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: DIFFERENT CHARACTERISTICS BETWEEN ACUTE MYELOID LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Extramedullary relapse (EMR) of Acute Leukemia (AL) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a contributor to post-transplant mortality and remains poorly understood, especially the different characteristics of EMR between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients.
Aims: In order to investigate the incidence, risk factor and clinical outcome of EMR for AML and ALL, we performed a retrospective analysis on 362 patients with AL who underwent allo-HSCT at the First affiliated Hospital of Soochow Univesity from January 2001 to March 2012.
Methods: We retrospectively studied 362 patients with AL, who underwent allo-HSCT at our center during these 11 years.
Results: (1) Compared with AML, ALL patients had a higher incidence of EMR (12.9% *vs* 4.6%, p=0.009); (2) The most common site of EMR was central nervous system (CNS) especially in the ALL group; (3) Multivariate analyses showed that the risk factors of EMR for AML patients included: advanced disease status at HSCT, hyperleukocytosis at diagnosis, a history of extramedullary (EM) leukemia prior to HSCT and conditioning regimen (total body irradiation [TBI]–based). While the top risk factors of EMR for ALL patients included hyperleukocytosis at diagnosis, adverse cytogenetics, and peripheral blood stem cell (PBSC) as stem cell source; (4) The prognosis of EMR of AL was poor. However, the estimated 3-year overall survival (OS) in AML patients with EMR was significantly lower than that in ALL patients (0 *vs* 18.5%, p=0.000).
Summary and Conclusions: The prognosis of EMR of AL was poor, and the treatment options were very limited. The characteristics of EMR post allo-HSCT between AML and ALL patients were different in our study which maybe suggested that there were different pathogenetic mechanisms for EMR of AML *versus* ALL after allo-HSCT. More studies are needed to investigate the pathogenetic mechanism, define risk factors, and establish treatment guidelines.

PB1403

SECONDARY NEOPLASMS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A NATIONWIDE POPULATION-BASED STUDY IN TAIWAN

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Background: Acute lymphoblastic leukemia (ALL) in the most common leukemia in children, but it is less common in adults. With the improvement of survival in last decades, secondary neoplasms (SNs) were observed. In childhood ALL, it had been reported the increased cumulative incidence of SN with long time follow-up. CNS tumor is the most common SN and associated with cranial irradiation. However, only one study reported the SN of solid tumor in adult ALL. For investigation of the small number of adult ALL, using the nationwide population-based dataset has an advatage on this issue.
Aims: To evaluate the risk of SN among adult ALL patients.
Methods: Patients who had been newly diagnosed with ALL between 1997 and 2011 were recruited from the Taiwan National Health Insurance database. Those who had age<20 years, antecedent or combined malignancies, subsequent hematologic malignancies were excluded. Standardized incidence ratios (SIRs) of SNs were calculated by comparing with the cancer incidence in the general population. Risk factors for cancer development were analyzed by Cox proportional hazards models. Effects of chemotherapy, radiotherapy, stem cell transplantation were regarded as time-dependent variables to prevent immortal time bias.

Table 1. Risk factors for SN among adult ALL after adjusting for competing mortality (N=1,381).

	Univariate analysis		Multivariate analysis	
Variables	HR (95% CI)	p value	HR (95% CI)	
Age≥ 40	0.45 (0.11–1.79)	0.257	0.43 (0.06–3.20)	0.409
Sex (male)	0.44 (0.11–1.77)	0.249	0.39 (0.09–1.69)	0.206
Comorbidities				
COPD	1.65 (0.35–7.91)	0.530		
ESRD	7.06 (0.92–53.98)	0.060	27.62 (1.42–538.30)	0.029
Dyslipidemia	0.73 (0.09–5.75)	0.769		
Treatment				
Anthracyclines	0.58 (0.16–2.14)	0.415		
Akylating agents	0.92 (0.26–3.29)	0.893		
Antimetabolites	2.03 (0.48–8.61)	0.336		
Etoposide	1.59 (0.40–6.29)	0.508		
Asparaginase	0.85 (0.24–3.07)	0.807		
Radiotherapy (except TBI)	5.98 (1.72–20.80)	0.005	6.68 (1.41–31.76)	0.017
TBI	2.51 (0.50–12.55)	0.262		
HSCT	3.03 (0.73–12.64)	0.128		

Chronic obstructive pulmonary disease (COPD); End-stage renal disease (ESRD); Total body irradiation (TBI);hematopoietic stem cell transplantation (HSCT).

Results: During the 15-year study period, 1,391 adult ALL patients were recruited. Overall, only night patients developed SNs with a follow-up of 3,100 person-years. The SIR for all cancers was 1.08 [95% confidence interval (CI) 0.50–2.06]. Four SNs developed within five years and the other five SNs developed thereafter. These SNs included cancers of breasts (3), head and neck (2), CNS (1), lung and mediastinum (1), uterus (1), and bladder (1). Multivariate analysis showed ESRD and the treatment of radiotherapy were independent risk factors.
Summary and Conclusions: In conclusion, we demonstrate that the incidence of SN is relatively low among the adult ALL patients. To treat these patients should firstly consider the treatment intensity by the risk group of their underlying disease rather than the risk of SN.

PB1404

EVALUATION OF ATTENTION DEFICIT AMONG CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The cognitive late effects experienced by many survivors of pediatric acute lymphoblastic leukemia (ALL) are well-established. The most commonly reported deficit is difficulty with attention. The factors, which cause attention deficit were not well evaluated.
Aims: In this study, we aimed to determine the frequency of attention deficit in ALL patients, who were treated with modified BFM 2000 protocol and the risk factors for the attention deficit.
Methods: 64 ALL patients between ages of 8-14 years, who were treated with modified BFM 2000 protocol and 30 healthy children between ages of 8-14 years were enrolled in this study. There were 36 girls and 28 boys in the patient group and the mean age was 10.63±2.26. There were 21 girls and 9 boys in the control group and the mean age was 10.80±1.85. Attention deficit in patient and control groups were assessed by the Benton Visuel Retention Test. Attention deficit was evaluated also according to the ALL risk group, cell type, central nervous system involvement, radiotherapy and high dose methotrexate therapy. Descriptive analysis was done. Paired groups were compared with independent t test, chi-square test and Fisher exact test.
Results: The prevalance of attention deficit was found to be% 32 in the patient group and%13 in the control group. The difference was statistically significant. Attention deficit was found to be significantly high among T-ALL patients, patients with central nervous system involvement and patients having high dose methotrexate therapy.
Summary and Conclusions: Attention deficit may be related to the disease itself or secondary to chemotherapy. Infiltration of cerebrospinal fluid by leukemic cells, intravenous perfusion of high dose methotrexate and having T-ALL were found to be risk factors for attention deficit among ALL patients.

PB1405

T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN DAILY HEMATOLOGICAL PRACTICE

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Background: Adult patients with T-cell acute lymphoblastic leukemia (T-ALL) or T-cell lymphoblastic lymphoma (T-LBL) form a subgroup of patients with inferior outcome compared to patients with B-cell precursor lymphoid malignancies.
Aims: Aim of this analysis was to describe features and outcome of patients with T-ALL or T-LBL treated using two similar protocols including consolidation by an allogeneic or autologous stem cell transplantation.
Methods: All adult patients diagnosed with T-ALL or T-LBL at our centre between 1997 and 2013 were included into this analysis. We describe baseline features of these patients, treatment options and treatment outcomes. The data were analysed for response and relapse rate and factors affecting survival.
Results: A total number of 44 patients aged 17 to 77 years (median age 31 years) at the time of diagnosis were included into this analysis; thirty (68%) patients with T-ALL and fourteen (32%) with T-LBL, thirty-three (75%) men and 11 (25%) women. Older, less intensive treatment protocol was used in 29 (66%) cases, whereas a newer, slightly more intensive one was used in 15 (34%) patients. Allogeneic hematopoietic stem cell transplantation (SCT) was performed in 6 (14%) patients, autologous SCT followed by maintenance therapy in 9 (20%) patients. Thirty-seven (88%) out of 42 evaluable patients achieved a complete hematologic remission (CR) in the median time of 28 days (range 9 to 121 days). Twenty-two (55%) patients in CR or partial remission (PR) eventually relapsed. The prognosis of these relapsed patients was extremely dismal; nineteen (86%) of them died irrespectively of the salvage treatment used. During the follow-up period with a median of 16.4 months (range 1 to 173 months) twenty-five (57%) patients died. Most common causes of death were disease progression (48%), infection (32%) and non-infectious treatment toxicity (8%). Nineteen (43%) patients remain alive in follow-up. Five-year progression-free (PFS) and overall survival (OS) in the whole cohort were 30% and 37%, respectively. The survival was not influenced by age, diagnosis (T-ALL *vs.* T-LBL) nor the treatment protocol used. As expected, the most significant risk factor for shorter OS was occurrence of relapse; 5-year OS in patients with relapse *vs.* without relapse was 14% *vs.* 87%, respectively, and median OS was 14.5 months *vs.* not reached, respectively, p<0.002. Allogeneic SCT was superior to chemotherapy alone; 5-year PFS 63% *vs.* 18% (p=0.02), 5-year OS 63% *vs.* 21% (p=0.01). Autologous SCT followed by maintenance therapy offers similar non-inferior survival improvement with 5-year PFS 45% and 5-year OS 67%. This difference remains statistically significant even in the transplant-eligible subgroup of patients under the age of 35.
Summary and Conclusions: Adult patients with T-cell acute lymphoblastic leukemia or T-cell lymphoblastic lymphoma form a less common subgroup of patients with inferior outcome. Autologous stem cell transplant followed by maintenance therapy offers a promising option for transplant-eligible patients without a donor. The prognosis of relapsed patients is extremely poor and these patients are therefore candidates for further analyses.

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PB1406

DIFFICULTIES OF MINIMAL RESIDUAL DISEASE MONITORING BY NESTED PCR IN TEL-AML1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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Background: The TEL/AML1 (ETV6/RUNX1) fusion gene secondary to t(12;21)(p13;q22) is the most frequent chromosomal structural abnormality in childhood ALL and can be found in up to 28% of B-lineage ALL patients, especially in younger children. It was also observed in up to 1% of newborns, as it occurs a prenatal event in utero. Multiple studies demonstrated the independent, favorable prognostic factor value, with a 5-year event-free-survival (EFS) of up to 97.6% and an overall survival (OS) of 98.9%. Despite this general trend, up to 20% of ALL TEL-AML1 patients relapse. There is no consensus regarding the value of TEL-AML1 positivity, using highly sensitive PCR tecniques for minimal residual disease (MRD) monitorinhg, as negative predictive factor for EFS and OS.
Aims: To analyze the results of MRD monitoring using Nested-PCR and RT-PCR for TEL-AML in pediatric acute lymphoblastic leukemia and to evaluate the impact of MRD Nested-PCR positivity on clinical and hematological outcome.
Methods: We analyzed 19 patients with TEL-AML ALL admitted in Fundeni Clinical Institute, Bucharest Romania between 2009-2013; for TEL-AML detection we used a multiplex PCR technique and for MRD monitoring the Nested-PCR; for positive Nested-PCR probes we continued with quantitative RT-PCR. All patients were treated according to BFM-ALLIC 2004 protocol; the MRD analysis has been performed at day 33, day 78, at the beginning of II protocol and every 3 months during maintenance protocol.
Results: Nineteen children, 9M/10F, median age 3y, range 2-14y were included in this study. The clinico-biological aspects at diagnosis showed: 19/19 cases L1 morphology, immunophenotype B common: 17 cases, preB: 2 cases, cytogenetic analysis: normal kariotype 17 cases, 2 abnormal kariotypes: 47 XY, mosaicism 46 XX and 46XY; 15 patients were assigned to standard risc group and 4 to the intermediate group. The Nested-PCR MRD monitoring showed: on day +33- 2 positive results, on day +78-1 positive result, and before protocol II – 2 positive results. We also observed positive results in at least one determination in 15 patients during maintenance protocol. The RT-PCR, performed in positive Nested-PCR cases, showed the presence of 1 or maximum 2 TEL-AML copies, without hematological relapse. All patients are in complete hematological remission during a median follow -up of 23 months.
Summary and Conclusions: The TEL-AML1 abnormality is associated with a favorable outcome. The MRD monitoring with Nested PCR for TEL-AML1 was very sensitive, being positive even in the presence of only 1 copy of the molecular abnormality; there is no correlation between MRD positivity using Nested-PCR and hematological or clinical relapse during a medium time of follow-up, suggesting that this approach is of limited value in predicting outcome.

PB1407

ESTIMATION OF CLINICAL EFFICIENCY OF BFM-ALL-2000 AND BM-ALL-2008 PROTOCOLS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Detection of minimal residual disease (MRD) has the value of evaluating the therapy in many hematologic malignancies, including acute lymphoblastic leukemia (ALL).
Aims: Estimation of clinical efficiency of BFM-ALL-2000 and BM-ALL-2008 protocols in children with ALL.
Methods: 19 patients with ALL were monitored for MRD after the achievement of complete remission (33 day after therapy). 8(42.1%) of them were treated with the BFM-ALL-2000 program (first group), and 11(57.9%) - with the BM-ALL-2008 (second group). Using multi parametric flow cytometry we compared the levels of MRD in patients of BM and PB after induction and consolidation, respectively.
Results: After induction and consolidation therapy in the first group persons the cut-off value of residual leukemic cells in BM and PB which correlated with outcome was 1.18×10^{-4}. Three of 8 (37.5%) patients with $>1.5\times10^{-4}$residual leukemic cells in PB after induction had a relapse, whereas the five patients (62.5%) with lower levels (0.48×10^{-4}) did not (p=0.0001). After therapy in the second group persons, 2(18.2%) patients had a level of MRD $>1.5\times10^{-4}$ and 1(9.1%) had a relapse; eight (72.7%) out of the remaining eleven patients, whose levels of MRD were below 1.5×10^{-4}, are still relapse-free (p=0.0001). In multivariate analysis, PB MRD status at the end of consolidation was found to have a significant effect on relapse-free survival (p=0.036). It`s remarkable that the treatment of the BM-ALL-2008 program had resulted in a rapid decline in MRD after induction therapy.
Summary and Conclusions: These preliminary results indicate that: BM-ALL-2008 program does not concede on clinical efficacy of accepted BFM-ALL-2000 program. Furthermore it is characterized by a rapid decline in MRD after induction therapy.

PB1408

CLINICAL SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE IN YOUNG ADULTS WITH STANDARD RISK/ PH-NEGATIVE PRECURSOR B-ACUTE LYMPHOBLASTIC LEUKEMIA: RESULTS OF PROSPECTIVE STUDY

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Background: Clinical risk classification is inaccurate in predicting outcome in adult patients with acute lymphoblastic leukemia (ALL), sometimes resulting in patients receiving inappropriate chemotherapy or stem cell transplantation (SCT).
Aims: To identify complementary markers suitable for further treatment stratification in patients with standard-risk (SR)/ philadelphia-negative (Ph-negative) precursor B-ALL
Methods: we evaluated the predictive value of minimal residual disease (MRD) after induction and consolidation chemotherapy in strictly defined SR/Ph-negative precursor B-ALL patients who were treated with a standard protocol using quantitative real-time PCR with the rearranged immunoglobulin heavy chain gene as a molecular marker
Results: The cytologic complete response (CR) rate was 92.3% after induction. At this time point the molecular CR rate was 73.9%. Patients with molecular CR (MolCR) after induction had a significantly higher probability of disease-free survival (DFS; 78.8% *vs* 30.8%; p=.001) and of overall survival (OS; 82.4% *vs* 41.7%; *P*<.0001) compared with patients with molecular failure (MolFail). MRD at end consolidation had the same significance. Quantitative MRD assessment identified patients with MolFail after induction and/or consolidation as a high-risk group, with 3-year DFS and OS rates of 28.6% and 35.7%, respectively. Patients with MolCR after induction and consolidation were classified as low-risk and had 3-year DFS rate of 89.7% and OS rate of 93.3%.
Summary and Conclusions: MRD quantification during treatment identified prognostic subgroups within the otherwise homogeneous SR/Ph-negative precursor B-ALL population who may benefit from individualized treatment.

PB1409

IMPACT OF MYELOID ANTIGEN EXPRESSION ON OUTCOMES OF PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Several factors have been implicated in risk assessment of adult patients with T-cell acute lymphoblastic leukemia (T-ALL). These include age, sex, white blood cell (WBC) count, mediastinal mass, central nervous system (CNS) involvement as well as cytogenetic, molecular and immunophenotyic characteristics. Yet, their value in predicting outcome is not well established as compared to B-ALL subtype.
Aims: The objective of this study was to evaluate the impact of myeloid antigen expression on complete remission (CR), event-free survival (EFS) and overall survival (OS) in patients with T-ALL treated with intensive chemotherapy.
Methods: We retrospectively reviewed the charts of 39 consecutive patients with T-ALL diagnosed and treated in two tertiary centers. The diagnosis of T-ALL was established by French-American-British classification or WHO criteria. Patients were considered having myeloid antigen expression if they expressed CD13, CD33 or both.
Results: Median follow-up was 12 months for all patients and 14 months for those still alive. Of the 32 patients assessable for response to induction therapy, 29 (91%) achieved CR with one or two courses of chemotherapy. The difference between those with and without myeloid antigen expression was not statistically significant (p=0.88). Twenty five percent (5/20) of patients with no myeloid markers required two courses of induction chemotherapy to achieve CR, whereas 58% (7/12) of the other group required two induction courses and this difference was statistically significant (p=0.04). In multivariable analysis, there was no statistically significant predictor of CR using age, gender, initial WBC count, CNS disease or co-expression of myeloid antigens. There was no significant difference in the EFS and OS between the two groups in the univariable and multivariable models.
Summary and Conclusions: Our analysis suggests that patients with T-ALL and positive myeloid antigen expression required more than one cycle of induction chemotherapy to achieve CR. No significant impact on EFS or OS was seen. However, our study is limited by small sample size and short follow-up period. Thus, larger prospective trials are required to confirm these findings.

PB1410

INFECTIOUS EVENTS DURING INTENSIVE TREATMENT IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Many children with acute lymphoblastic leukemia (ALL) experience one or more infectious complications during treatment. Infections are important to study in children with ALL because they continue to contribute to morbidity and mortality, affect quality of life for children and their families and require considerable health resources to prevent and treat.
Aims: To analyze the characteristics of infective episodes (*I.E.*) during intensive treatment (Protocol I, M and II) in children with ALL.
Methods: Objective of this study ware 55 patients with ALL who were treated according to ALL-BFM 90 and ALL-BFM 95 Protocol between January 2000 and December 2007 at the University Children's Hospital in Skopje. We explored the characteristics of *I.E.*, together with the causative pathogens, the episodes of febrile neutropenia (FN), the length of antibiotic treatments and the treatments with G-CSF during intensive phases of treatment (Protocol I, M and II).
Results: From 55 analyzed records 24 (43.64%) were male and 31 (56.36%) were female. Mean age at diagnosis was 6.0 years (1.1-15.0). Majority of the patients 43 (78%) were under 10 years and 12 (22%) were over 10 years. All of them experienced 132, 52 and 73 *I.E.* with 2.4, 0.9, and 1.3 infections per patient during Protocol I, M and II respectively. Regarding to the pathogens 184 (71.5%) were bacterial (102, 30 and 52 in Protocol I, M and II), 45 (17.5%) were viral (20, 14 and 11 in Protocol I, M and II) and 28 (10.8%) were fungal (10, 8, 10 in the three intensive phases respectively). There was a slight predominance of gram positive bacteria in Protocol I [Gram positive 42 (51.85%) *versus* gram negative 34 (41.97%)], and a very slight predominance of gram negative bacteria in Protocol II [Gram positive 16 (45.71% *versus* Gram negative 18 (51.42%)]. The infections were treated with antibiotic treatment in average of 23.69, 11 and 15.05 days and the number of treatments with G-CSF were in average 7.22, 2.44 and 9.20 per patient respectively in Protocol I, M and II. The number of episodes of FN in these three phases was 16.4 (29.1%), 4 (7.3%) and 22 (40%).
Summary and Conclusions: Evaluation of the characteristics of *I.E.* presented that the majority of infectious events were observed in Protocol I and also the length of antibiotic treatment was longer in this phase. But the episodes of FN together with the treatments with G-CSF were higher in Protocol II possible due to the cumulative effect of chemotherapy.

PB1411

SAFE AND EFFECTIVE ADMINISTRATION OF BORTEZOMIB IN ASSOCIATION WITH RITUXIMAB IN A CHILD WITH RELAPSED/RESISTANT B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA (BCP-ALL)

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Background: Childhood B-lineage ALL is associated with a very good prognosis. Despite a high cure rate, 20% of children still relapse. In this setting, allogeneic hematopoietic stem cell transplantation (A-HSCT) is effective when a complete remission is obtained. Few cases showed a poor response to second line treatment. To overcome this therapy resistance, alternative or experimental drugs are needed. Recently, Bortezomib, a proteasome inhibitor, was successfully administered to a cohort of children with resistant BCP-ALL (Messinger Y, Blood 2012). Moreover, high risk ALL is characterized by the increasing expression of CD20 (Dworzak MN, Blood 2008).
Aims: We here report on a child who was given a course of treatment including Bortezomib and Rituximab, prior to receive an A-HSCT from an HLA-identical familiar donor.
Results: We report on a 6 year-old boy with a pre-B ALL, which was firstly diagnosed in October 2006, at the age of 2 years, and enrolled in AIEOP ALL R-2006 protocol. He showed a prednisone good-response (PGR) at day 8, resulting as a standard risk (SR) based on the detection of minimal residual disease (MRD) at the end of Induction. He went off-therapy on October 2008. Eight months later, he presented an isolated bone marrow (BM) relapse, with a different clone, backtracked to diagnosis. He was enrolled in protocol AIEOP-REC-2003, risk S2. After the first two blocks (F1+F2) he still presented with 15% of blasts at BM. We addressed him to a more intensive treatment including FLA-DNX (Fludarabin-Aracytin-DaunoXome), followed by BFM-like blocks 1, 2 and 3. Then, he showed an increasing signal of MRD, which became a hematological relapse. We administered a CLOVE (Clofarabine-VP16-Ciclophosphamide) schema. The child recovered in the BM with a 25% of blasts presenting with a complex karyotype [46,XY[27]/45,XY,del(6)(p21).-7,-17,+mar[3]/46,XY,del(6)(p21),-7,-17,+mar,+mar[3]/46,XY,-7,-17,+mar,+mar[2]. As salvage therapy, we decided to administer the following schedule: Bortezomib [1,3 mg/mq intravenously (i.v.) days +8, +11, +15 and +18]; Dexametazone (10 mg/mq/day i.v. for 14 days); Erwinase (20.000 UI/mq i.v. days +1, +3, +5, +8, +10, +12, +15); Vincristine (1,5 mg/mq i.v. in 1 hour-infusion, days +8, +15). The child presented a grade II peripheral neurological toxicity, as adverse event. BM aspirate (BMA) at the end of cycle showed a morphological remission, but not at molecular level. For this reason, we decided to add on top of the previous cycle, the administration of Rituximab (375 mg/mq i.v. days 0, +3, +10, +17, +25), based on the high expression of CD20 in the leukemic blasts. BMA at the end of this re-induction cycle showed a $1x10^{-3}$ MRD detection. He underwent a TBI-VP16-Cyclophosphamide conditioning regimen followed by a $3x10^6$/kg of $CD34^+$ cell dose, harvested from bone marrow of an HLA-identical familiar donor. He remained in complete remission and full donor chimerism for 8 months, until he presented a bone marrow isolated relapse. He was given a protocol B-modified (with prednisone) and surprisingly achieved a complete molecular remission. For this reason we performed a second A-HSCT, using the same familiar donor and a Busulphan/Melphalan-based regimen. Unfortunately the child presented a severe pulmonary graft-*versus* host disease associated with a recurrence of disease. He died at 6 months from 2nd A-HSCT.
Summary and Conclusions: Our experience strongly demonstrated that Bortezomib and Rituximab could be safely administered with an effective result in children with resistant and/or relapsed BCP-ALL. We also suggest that the earlier the better.

Acute myeloid leukemia - Biology

PB1412

TELOMERASE REVERSE TRANSCRIPTASE (TERT) A1062T MUTATION AS A PROGNOSTIC FACTOR IN EGYPTIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: Acute myeloid leukemia (AML) is a clonal malignant disease of haematopoietic tissue that is characterized by the proliferation of abnormal myeloblast cells in marrow and impaired production of normal blood cells. Telomeres are complex structures capping the ends of all eukaryotic cell chromosomes. In vertebrates, telomeres consist of thousands of double-stranded tandem TTAGGG nucleotide repeats shielded by various proteins that seal the DNA structure.
Aims: This study aimed to evaluate the incidence, clinical and prognostic impact of the most common *TERT* mutation A1062T in AML patients treated in Mansoura Oncology Center, EGYPT.
Methods: Mutation screening for *TERT* (A1062T) mutation in exon 15 of the *TERT* gene was performed on diagnostic DNA samples from 153 AML patients, age range from 17-65 years, by using sequence specific primers.
Results: *TERT* (A1062T) mutation was detected in 18 cases out of 153 patients (11.8%). There was no difference between the two groups as regard sex, French-American-British subtypes, cytogenitcs status **(**favorable or intermediate/adverse), hemoglobin concentration and platelet count. On the other hand, there were a statistical differences in age, type of leukemia (de novo or secondary), extramedullary disease and performance status. There was a statistically difference in the rates of complete remission (CR) (16.7% *vs* 53.3%) between *TERT* (A1062T) mutant and wild type patients, respectively. Also there was a statistically difference in the rate of relapse (62.5% *vs* 28.2%) between *TERT* (A1062T) mutant and wild type patients, respectively. As regard the overall survival (OS), the patients with *TERT* (A1062T) mutations had shorter overall survival (OS) than patients with wild type (p=0.001). In a multivariable analysis, *TERT* (A1062T) mutational status is independently worse predictor factor (p=0.007) when controlling for cytogenetic status (p=<0.001), denovo or secondary AML (p=0.001) and bone marrow blast cells (p=0.001).
Summary and Conclusions: TERT A1062T mutation is an independent negative prognostic factor in AML patients. Therefore, molecular testing for *TERT* A1062T mutation in patients with AML is recommended in order to delineate their prognostic status.

PB1413

HEDGEHOG INHIBITOR NVP-LDE225 ENHANCES SENSITIVITY OF CHEMORESISTANT ACUTE MYELOID LEUKEMIA CELLS TO CHEMOTHERAPEUTICS BY DOWN-REGULATING MRP1 VIA A MECHANISM INVOLVING IGF-1R/PI3K/AKT SIGNALING

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Background: Although a great progress has made in the treatment of acute myeloid leukemia(AML), drug resistance and relapse remain a therapeutic challenge. The poor prognosis is mainly due to multidrug resistance (MDR) characterized by disregulated signaling cascades.We have proven that LBH589 combined with Bortezomib had synergistic effects on proliferation, apoptosis and sensitivity to cytotoxic drugs via AKT and NF-κB pathways in chemoresistant HL60/ADR cells and refractory AML primary cells. Using gene chip analysis, we found that among those disregulated pathways, Hedgehog(Hh) signaling pathway was probably the upstream paths which could regulate others.
Aims: The purpose of the study was to investigate the activation of Hh pathway between chemosensitive and chemoresistant AML cell lines and primary cells. Also researches on Hh pathway inhibitor NVP-LDE225 in reversing drug-resistance in multiple drug-resistant HL60/ADR cells and refractory or relapse primary acute myeloid leukemia cells were done.Besides the mechanisms were further investigated.
Methods: Western blot assay were used to determine the protein levels of Ptch, Smo, Gli-1, Shh in HL60 and HL60/ADR cell lines also primary AML cells . HL60/ADR cells and refractory primary cells were treated with signal drug NVP-LDE225(concentration among 0-20 μM).Proliferation were evaluated by 3-(4,5)-dimethylthiahiazo (-z-yl)-3,5-di-phenytetrazoliumromide (MTT) assay and cell apoptosis were analyzed by Annexin V-FITC/PI staining through flow-cytometry(FCM). Intercellular adriamycin accumulation(MFI) were analyzed by

FCM. The changes in protein levels of Gli-1, IGF-1R, p-IGF-1R, Akt, p-Akt, MRP-1 were detected by Western blot.
Results: We found that the chemotherapy-resistant phenotype of myeloid leukemia cells correlated with activation of the Hh pathway, however in chemosensitive cells or non-refractory primary cells, such activation was less pronounced(a). NVP-LDE225, a potent and selective Hh inhibitor, significantly reverted resistance of chemotherapeutics, increased the intracellular adriamycin accumulation, inhibited MRP1 protein suppression both in cell lines and primary cells(b and c). These effects were likely to be mediated via inhibition of IGF-1R/PI3K/Akt pathway(d).
Summary and Conclusions: These findings provided evidence that targeting the Hh pathway might be a therapeutic avenue for overcoming MDR resistance in myeloid leukemia.

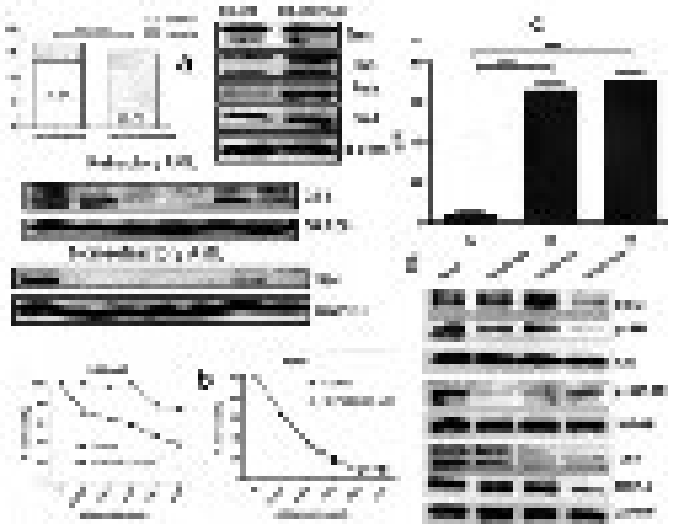

Figure 1.

PB1414

IMPACT OF IMMATURE MARKERS BY FLOW CYTOMETRY IN LEUKEMIC BLAST CELL HETEROGENEITY AML

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease, often characterized by coexistence of more than one subpopulation of blast cells with different immunophenotypes.
Aims: The aim of our study was to evaluate the AML heterogeneity expression in the different blast cell subpopulations detected at diagnosis in a group of 151 adult *de novo* AML patients (pts) (M3 excluded) by multiparametric flow cytometry (MFC).
Methods: We analyzed the prognostic impact of percentage of positive cells (%) and mean fluorescence intensity (MFI) expression of several immaturity cell surface markers (CD34, CD117, CD7, and CD123) on leukemic blasts by MFC treated according to the multicenter CETLAM AML-03 protocol (clinicaltrials.govNCT01723657). We also determined its correlation with other clinical and biological variables and the prognostic significance in terms of overall survival (OS), leukemia-free survival (LFS) and cumulative incidence of relapse (CIR) in the whole AML series, and latter focussing on clinico-biological characteristics of 151 heterogeneously AML cases using plots displaying data in a 3D density to better assignement blast intraleukemia heterogeneity.

Table 1.

Results: CD34$^+$ measured as the percentage (≥2.88%) and MFI (≥284, arbitrary units (a.u.)) expression had prognostic impact in terms of OS (P=0.005, P=0.003), LFS (P=0.011, P<0.001) and CIR (P<0.014, P=0.001). The percentage of CD117$^+$ cells (≥61.29%) was associated with shorter LFS (P=0.043), and CD117 MFI (≥146 a.u.) expression was associated with a shorter OS (P=0.033) and LFS (P=0.028). High CD7 MFI (≥15 a.u.) expression showed prognostic relevance in terms of LFS (P=0.015) and CIR (P<0.001). In the multivariate analysis, high CD34 MFI expression levels retained the independent value as a predictor of LFS and CIR (P=0.012; HR=1.59, 95% CI=1.11-2.228 and P=0.045; HR=1.58, 95% CI=1.01–2.46). High percentage of CD117$^+$ cells and CD117 MFI expression levels were independent predictors of a shorter OS (P=0.010; HR=1.49, 95% CI=1.09-2.01; P=0.038; HR=1.32, 95% CI=1.01–1.71) and LFS (P=0.004; HR=1.66, 95% CI=1.17-2.37; P=0.035; HR=1.40, 95% CI=1.02–1.91). Focussing on immunophenotypically different blast cell heterogeneity patients (n=151) these correspond to 6/151 cases (4 phenotypically distinct subpopulations), 5/151 cases (3 clusters), and in 140/151 cases (2 clusters) were detected and gated.There were significant differences in high heterogeneity expression by gender (P=0.001) and MRC (P=0.004). Regarding the expression profile, except for higher CD34 MFI expression levels (P=0.001),% of CD34$^+$ cells (P=0.003) and CD123 MFI expression levels (P=0.003), no other significant differences were found. No statistically significant differences in heterogeneity AML cases *vs.* homogenously cases were found in terms of OS, LFS and CIR (P=NS). Interestingly, combined antigenic into 5 groups composed based on MFI immature measurements (CD34, CD117, CD7 and/or CD123) showed prognostic impact in AML in terms of OS (P=0.001, P<0.001,P=0.003, P<0.001, P=0.007), LFS (all P<0.001) and CIR (P=0.005, P<0.001, P=0.001, P<0.001, P=0.003).
Summary and Conclusions: Our findings suggest that prognosis information

of the immunophenotypic patterns in AML could be based in a complex analysis as it can be provided by improved quantitative analysis of antigenic surface expression patterns and combined of heterogeneous immature cell subpopulations based on MoAbs combinations.

PB1415

CPI203 (BET INHIBITOR) SHOWS POTENT SINGLE AGENT ACTIVITY AGAINST AML CELL LINES AND SYNERGIZES WITH CYTARABINE AND NUTLIN-3

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Background: There remains a critical requirement for novel therapies for Acute Myeloid Leukemia (AML); the backbone of AML therapy still comprises conventional cytotoxic agents and five-year survival rates remain below 50% at five years. Bromodomain and extra-terminal domain (BET) inhibitors are emerging as exciting therapeutic agents for hematopoietic malignancies. Pharmacological inhibition of bromodomains targets malignant cells by preventing reading of acetylated lysine residues, thus disrupting chromatin-mediated signal transduction, which reduces transcription at oncogene loci, most notably Myc.
Aims: We sought to assess the activity of CPI203 (Constellation Pharmaceuticals), a BET inhibitor, against 4 heterogeneous AML cell lines (AML3, KASUMI-1, THP1 and KG1a) both as a single agent and in combination with standard (cytarabine) and targeted (nutlin-3) therapies.
Methods: Cell viability was assessed using resazurin (Alamar blue dye) and analysed using the Envision Fluorescent Reader. IC50 values were subsequently calculated using GraphPad Prism (version 6.0) and drug combinations were evaluated using Calcusyn (version 2.0). Apoptosis was assessed using flow cytometry staining for Annexin V and propidium iodide (PI). Protein expression was determined using Western blotting. All experiments were performed in triplicate.
Results: Following 24 hours (h) of treatment with CPI203, there was a dose-dependent decrease in MYC in all four cell lines tested and a dose-dependent increase in p53, MDM2 and p21 in AML3 (p53 wild-type) cells treated with nutlin-3 for 24h. IC50 values after 72h treatment with CPI203 for AML3, KASUMI-1, THP1 and KG1a cell lines were: 40.3nM, 45.80nM, 102nM and 128nM respectively. Resazurin analysis revealed synergistic effects of combining CPI203 with cytarabine, most notably at a 1:10 ratio (25nM to 400nM CPI203 with 250nM to 4μM cytarabine). Mean combination index (CI) for AML3 was 0.78, (moderately synergistic), the optimal dose combination was 200nM CPI203 with 2μM cytarabine (CI 0.47 *i.e.* synergistic), which demonstrated a 20% increase in apoptosis compared with single agent treatment. Mean CI for KASUMI1 cells across 1:10 dose ranges was 0.45 (synergistic) with an increase in apoptotic cells from 58% and 44% for single agent 25nM CPI203 and 250nM cytarabine, respectively, to 88% for the combination. THP1 cells also showed enhanced apoptosis with the combination at a 1:10 ratio, increasing from 26% and 23% for THP1 cells treated with 200nM CPI203 or 2μM cytarabine, respectively, to 42% for the combination. KG1a cells (which possess the highest IC50 value of the series for CPI203) were only slightly synergistic at a 1:10 ratio with a mean CI of 0.86. In the AML3 cell line, resazurin analysis demonstrated that combining CPI203 with nutlin-3 was potently synergistic for a 1:12.5 (mean CI=0.07) and 1:25 (mean CI=0.299), and synergistic for a 1:50(mean CI=0.44) and 1:100(mean CI= 0.66) ratios. Apoptosis was enhanced in combination treated cells at ratios of 1:50 and 1:100. For example, apoptosis increased from 19% and 10% for single agent 50nM CPI203 and 5μM nutlin-3 respectively to 51% for the combination and 71% and 29% for single agent 100nM and 10μM nutlin-3 and 90% for the combination.
Summary and Conclusions: The BET inhibitor CPI203 potently induces apoptosis of AML cell lines and acts synergistically with cytarabine and nutlin-3. These combinations are currently being assessed in primary samples.

PB1416

CLONAL EVOLUTION IN PRIMARY RESISTANT AML ASSESSED BY GENETIC AND MOLECULAR PROFILING, SINGLE CELL SIGNALLING PROFILING AND EX VIVO DRUG SENSITIVITY AND RESISTANCE TESTING

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease, with substantial variation in genotype and phenotype often including various clones of malignant myeloid cells evolving within the same individual over time. This clonal diversity represents a serious challenge to both conventional as well as targeted therapy.
Aims: To better understand disease progression mechanisms and drug response for future improved and individualized treatment protocols, we present the course of AML development in a 68-year-old male AML patient in correlation with deep molecular profiling and functional assessment.
Methods: The mononuclear cell fraction from the patient's peripheral blood was obtained at diagnosis, early relapse, and late relapse. Cytogenetic assessment, exome sequencing, copy number analysis, targeted PCR, RNA sequencing and super-SILAC protein analysis were performed. Functional evaluation further included immunophenotyping, single cell profiling of intracellular signalling pathways by phosphospecific flow cytometry and *ex vivo* drug sensitivity and resistance testing (DSRT). Clonal evolution was further characterized by serial xenografting in NSG mice.
Results: At diagnosis (normal karyotype, FLT3-ITD) copy number analysis revealed a heterozygous deletion of 2,1Mb on 21q22.11q22.12 including RUNX1 and on 18q21.2 including TCF4. The former aberration is believed to be a founding event. Identification of clusters of mutations with similar variant allele frequencies in the exome sequencing data demonstrated a diverse clonal architecture already at the time of diagnosis with several genetically distinct subclones. The dominant clones at diagnosis were found to carry mutations in NRAS and ASXL1, both being frequent driver lesions in AML. Targeted PCR showed a FLT3-ITD mutation with a ITD/wild-type ratio of 0,03, indicating that the NRAS and FLT3-ITD mutations were mutually exclusive, and that only a small subpopulation harboured FLT3-ITD. *Ex vivo* DSRT screening showed selective sensitivity to MEK-inhibitors. At relapse the genotype of the AML cells was altered, now comprising complex karyotype 46,XY,del(6)(q14q22), der(7)t(7;8)(q21,q23),t(11;18)(q21;q22)[7]/46,idem,t(3;15)(p?21;q?23)[3]. Targeted PCR demonstrated a FLT3-ITD/wild-type ratio of 0,57 indicating that the FLT3-ITD positive clone at this time point dominated the blast population. This was well reflected in the DSRT data by increased sensitivity to tyrosine kinase inhibitors compared to the diagnostic sample. The NRAS and ASXL1 variant allele frequencies were correspondingly low. At late relapse there was a marked increase in genomic complexity, now indicating that the NRAS and ASXL1 clone again was dominating, but co-occurring with a substantial FLT3-ITD clone. This was supported by the DSRT profile that was very similar to the diagnostic sample. Hydroxyurea (HU) and 6-Mercaptopurine (6MP), both well established cytoreductive agents, failed when administered as monotherapy and in DSRT. However, when provided as combination treatment, HU and 6MP repeatedly resulted in controlled leukocyte counts. Reduction of the FLT3-ITD clone towards late relapse suggests that combination treatment with HU and 6MP selective targets the FLT3-ITD clone.
Summary and Conclusions: Thorough investigation of serial patient samples offers an efficient tool for understanding AML biology, disease evolution and limitations in treatment response. We hypothesize that the combination of HU and 6MP works synergistically in FLT3-ITD positive AML, representing a treatment option for selected AML patients.

PB1417

MONITORING OF CHIMERISM IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION BY QUANTITATIVE ASSESSMENT: THE BEST TOOL FOR MONITORING OF MINIMAL RESIDUAL DISEASE?

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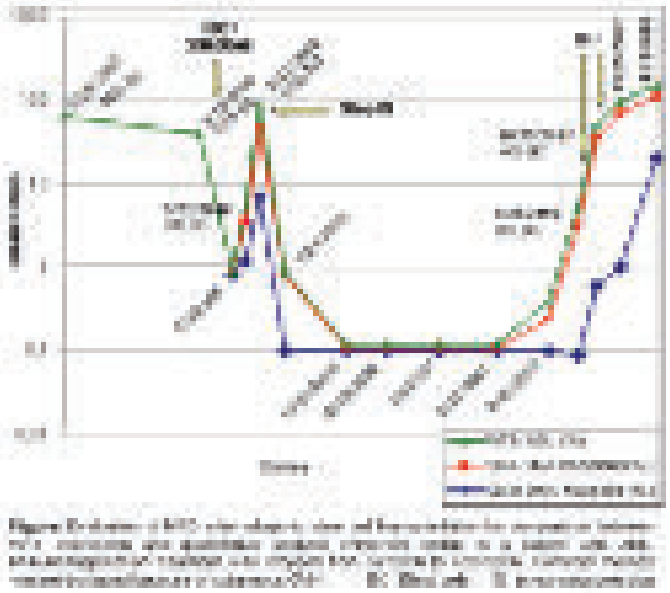
Background: Minimal residual disease detection (MRD) by the analysis of acute myeloid leukemia (AML) specific molecular markers is a critical issue for the monitoring of the disease response to treatment. MRD testing by quantifying fusion genes or gene mutations is not possible for all AML patients. Hematopoietic chimerism study after allogeneic stem cell transplantation enables assessing the engraftment rate and its evolution over time, on both CD3-positive and CD3-negative circulating blood cells through cell sorting/selection.
Aims: The aim of this prospective study was to assess whether quantitative chimerism kinetic was of some use, not only as a marker of engraftment, for detecting disease recurrence in AML patients after hematopoietic stem cell transplantation when compared to the quantification of classic MRD markers such as WT1, mutated NPM1 of fusion genes.



Methods: We prospectively studied 47 AML patients transplanted between 2006 and 2013 who had available ARN samples for specific molecular marker (*i.e.*, fusion gene, NPM1 mutation, WT1 overexpression). The quantitative assessment of the fusion gene, NPM1 mutation and WT1 transcript was performed by real-time quantitative polymerase chain reaction (RQ-PCR). The determination of chimerism was performed by RQ-PCR using TaqMan Technology® of single nucleotide polymorphism (Alizadeh M. *et al*, 2002). Our RQ-PCR assay was performed on DNA extracted from CD3-positive cells and CD3-negative peripheral blood mononuclear cells, separately. We used manual magnetic separation of T cells using magnetic beads directly conjugated with anti-CD3 monoclonal antibody (Dynal®). Since 2011, CD3 cells were isolated using a Robosep® and EasySep® Positive Selection Human CD3 positive Selection Kit (Stemcell technologies®). The CD3-negative fraction was "the waste" of the magnetic selection, allowing an indirect enrichment in AML cells in case of disease recurrence.
Results: For all the patients, we found a good agreement between the monitoring of chimerism analysis and MRD as evaluated either by fusion gene, NPM1 mutation or WT1 expression. Out of 47 patients transplanted, 24 experienced haematological relapse, whereas 23 patients remained in complete remission. The CD3-negative fraction chimerism analysis could be accurately used to predict early AML relapse (Figure 1). The limits of the technique were (i) the detection of donor cell leukemia in patient with complete chimerism, an exceptional event, (ii) the maintenance of mixed chimerism in normal hematopoietic cells. Out of the 47 patients, we have only one patient with stable MC (1%) on CD3-negative fraction and 40% on CD3-positive cells with fusion transcript undetectable (RUNX1-RUNX1T1).
Summary and Conclusions: This study highlights the interest of monitoring chimerism by RQ-PCR assessment as an indicator of early AML relapse, especially in patients without specific molecular marker available. Although our population of patients is relatively small, this quantitative chimerism method appears as at least as sensitive as classical MRD analysis.

PB1418

HAPLOTYPE ANALYSIS OF CYP2B6 CYTOCHROME P450 GENE IN CORRELATION WITH AML SUSCEPTIBILITY

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Background: Acute myeloid leukemia (AML), the most frequent acute leukemia in adults, constitutes a broad range of disorders with marked clinical and biological heterogeneity. The etiology of the disease is currently unknown; however, the interaction between environmental exposure and genetic individual's background has been postulated to be a possible cause for the development of AML. Certain detoxification genes encoding antioxidant enzymes such as cytochrome P450 genes comprise well-known AML risk factors. *CYP2B6* is a highly variable and polymorphic cytochrome P450 gene. The encoding enzyme plays a vital role in the degradation of some genotoxic exogenous and endogenous xenobiotics and harmful compounds. Single-nucleotide polymorphisms (SNPs) of *CYP2B6* gene result in lower CYP2B6 protein activity and some of them have been associated with various types of cancers. In view of the fact that AML may be related to the exposure of exogenous chemicals, the *CYP2B6* SNPs may be a risk factor for the pathogenesis of AML.
Aims: In the present study, we analysed three polymorphisms of the *CYP2B6* gene (516G>T in exon 4, 777C>A and 785A>G in exon 5) in 71 AML patients and 109 healthy donors (case-control study). Furthermore, we investigated the co-existence of the above SNPs in order to determine the *CYP2B6* high-risk haplotype in AML susceptibility.
Methods: Genotyping was performed by Real-Time PCR (Roche) using the LightSNip technology.
Results: Our analysis revealed that 516G>T variant genotypes (*GT* and *TT*) had statistically higher frequency in AML patients compared to the controls (*GT*:43.7% *vs* 29.4% and *TT:15.5*% *vs* 3% respectively, $p<0.001$). Moreover, a higher incidence of the 785A>G heterozygotes was observed in patients compared to healthy donors (57.7% *vs* 21.1% respectively, $p<0.001$), while the polymorphic site 777C>A showed the same allelic and genotype frequency between patients and controls. Haplotype distribution of *CYP2B6* gene was different between patients and controls ($p<0.0001$). Specifically, the frequency of the CYP2B6*1 haplotype, indicating wild type status for all sites, was significantly increased in the control group compared to AML patients (78.9% *vs* 59.9%, respectively, $p<0.001$) suggesting that AML patients have a 2.5-fold increased risk of carrying at least one of the three *CYP2B6* polymorphisms (516G>T, 777C>A and 785A>G). In AML patients' group, 6 haplotypes with mutated alleles were found: 4 have already been characterized as CYP2B6*3 (777C>A), CYP2B6*4 (785A>G), CYP2B6*6 (516G>T and 785A>G) and CYP2B6*9 (516G>T), while 2 new haplotypes were described for the first time in the current study. The first one includes the mutated alleles of 777C>A and 785A>G polymorphic sites and the other one carries all three mutated alleles (516G>T, 777C>A and 785A>G). Interestingly, among AML patients the most frequent haplotype was the new one that simultaneously harbours the three mutated alleles (16.9%), while only 2.3% of control group carried this haplotype. Statistical analysis showed that this new haplotype was strongly correlated with AML patients (10-fold higher than in controls).
Summary and Conclusions: This is the first study of haplotype analysis (516G>T, 777C>A and 785A>G) of *CYP2B6* gene in combination with AML. Our data provide evidence for a simultaneously presence of the mutated alleles of the polymorphic sites of *CYP2B6* gene in AML patients, indicating their positive implication in AML development.

PB1419

ACQUIRED CHEMO-RESISTANCE IN FANCONI ANAEMIA-DERIVED ACUTE MYELOID LEUKAEMIA

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Background: Fanconi anaemia (FA) is caused by a defect in a DNA damage response pathway (FA/BRCA pathway) and is associated with mitomycin C (MMC) sensitivity, chromosomal instability and an extreme predisposition to acute myeloid leukaemia (AML). In addition, heterozygous carrier status of *FANCD1/BRCA2* is associated with familial breast and ovarian cancer. Acquired resistance to chemotherapeutic agents is a common problem in cancers associated with a FA/BRCA pathway defect and sporadic leukaemias. In order to understand phenotype reversion and acquired chemo resistance, we compared FA-derived AML and MMC resistant progeny we have derived.
Aims: Molecular and cellular characterisation of MMC resistance phenomena in FA using specific AML cell lines we have generated.
Methods: From the FA-derived AML cell line SB1690CB with bi-allelic disruption in *FANCD1/BRCA2* caused by the mutations IVS7+2G>T and c.3827delGT the MMC resistant sub clone SBRes was generated under hypoxic conditions and low dose MMC. Conservation of identical genetic background of MMC resistant clone was confirmed by DNA fingerprinting. Differences between chemo-sensitive and resistant cells were determined by systems biology approaches (expression array and proteomics) and cell biological assays.
Results: MMC sensitive cells showed higher levels of spontaneous γH2AX labelling in flow cytometry assays and slower proliferation compared to resistant cells. There were 420 differentially expressed genes in resistant SBRes cells compared to sensitive SB1690CB. VAC values of 113 genes were indicating alternative splicing in sensitive compared to resistant cells. Proteomic analysis identified 131 proteins that were differentially expressed between the 2 cell

populations. Integrated network analysis of gene expression and proteomic datasets suggests impact of acquired chemo resistance on multiple signalling networks linked to the DNA damage response.
Summary and Conclusions: We have generated a model for investigations of acquired chemo resistance in AML that can be used for further investigations into acquired chemo-resistance in AML.

PB1420

THE UTILITY OF MULTIPLEX RT-PCR AND MUTATIONAL ANALYSIS FOR DIAGNOSIS AND RISK STRATIFICATION IN PATIENTS WITH ACUTE LEUKEMIA

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Background: The chromosomal rearrangements and a few mutations of the blasts is a milestone for prognostication in acute leukemia. Recent advances include an introduction of multiplex RT-PCR platform to simultaneous screen for recurrent genetic abnormalities and rapid molecular screen for mutations. These have been expected to be valuable for early risk stratification and refining karyotype-based risk stratification.
Aims: The aims of this study were to report our data on combined molecular platforms composed of multiplex RT-PCR and mutational analysis for the detection of prognostically important genetic changes, and to reveal the clinical utility of this approach.
Methods: A total of 847 consecutive specimens from 756 patients with acute leukemia were investigated for genetic alterations using a commercial multiplex RT-PCR kit (HemaVision, DNA Technology) for the detection of clinically relevant fusion transcripts and, for patients with acute myeloid leukemia (AML) without any abnormal bands in multiplex RT-PCR, by GeneScan analysis followed by direct sequencing for the detection of *NPM1*, *FLT3*-ITD, and *CEBPA* mutations. We then compared the results from molecular testing with cytogenetic findings.
Results: A total of 267 samples (31.5%) were diagnosed as showing recurrent genetic abnormalities. The concordance rate between molecular testing and cytogenetics was 97.8%. Cryptic translocations were detected in 19 samples (2.2%) including ETV6-RUNX1, BCR-ABL1, and MLL rearrangements. Additional information by multiplex RT-PCR included an identification of submicroscopic aberrations such as STIL-TAL1 (N = 3) and SET-NUP214 (N = 5). Furthermore, multiplex RT-PCR revealed very rare rearrangements including CBFB-MYH11 type D (N = 2), FUS-ERG (N = 1), MPM1-MLF1 (N = 1), RUNX1-MDS1 (N = 1), and coexistence of RUNX1-RUNX1T1 and ETV6-MN1 (N = 1). However, multiplex RT-PCR missed translocations of rare breakpoints including t(11;17)(q23;q25) (N = 1) and t(8;21)(q24.3;q22) (N = 1). The frequencies of important mutations among multiplex RT-PCR-negative AML were 26.2% for NPM1, 20.9% for FLT3-ITD, and 7.0% for CEBPA. In particular, 16.2% of multiplex RT-PCR-negative AML was classified into favorable risk group owing to NPM1-positive and FLT3-ITD negative by molecular testing.
Summary and Conclusions: Our results demonstrate that molecular testing performed simultaneously with morphologic evaluation of acute leukemia provides relevant prognostic information, especially for the identification of favorable risk in patients with AML. This approach can enable physicians to make a prompt decision when determining optimal therapeutic modalities at a remission stage. Moreover, additional information including submicroscopic or cryptic translocations can be obtained from molecular testing.

PB1421

DNA METHYLATION INHIBITORS INDUCE TELOMERE DYSFUNCTION AND APOPTOSIS IN HUMAN LEUKEMIA CELLS THAT IS ATTENUATED BY OVER-EXPRESSION OF TELOMERASE REVERSE TRANSCRIPTASE (TERT)

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Background: DNA methyltransferase inhibitors (DNMTi) such as 5-azacytidine (5-AZA) and decitabine have been applied to the treatment of acute myeloid leukemia (AML) and other malignancies. Inhibition of global/gene-specific DNA methylation is widely accepted as the key mechanism behind the anti-tumor activity. It is currently unclear whether other mechanisms are involved in DNMTi's action.
Aims: Because telomerase activation or telomerase reverse transcriptase (TERT) is essential for unlimited proliferation of malignant cells by elongating telomeres, and TERT or telomerase has been shown to confer chemo- or radio-resistance to cancer cells, we determined whether DNMTi affects telomere function and whether TERT interferes with its efficacy against AML cells.
Methods: AML cell lines and primary AML cells were treated with 5-azacytidine (5-AZA). TERT and telomerase activity was assessed. The TERT expression lenti-viral vector was used to make a TERT-over-expressing cell lines. Apoptosis was determined using flow cytometry and telomere dysfunction was detected using Q-FISH combined with immunofluorescence.
Results: 5-AZA induces telomere dysfunction in AML cell lines and primary AML cells by demonstrating the co-localization of 53-BP1 and TRF1 foci. Telomere dysfunction was coupled with substantial apoptosis in 5-AZA-treated cells. TERT over-expression significantly attenuated 5-AZA-mediated telomere dysfunction and apoptosis of AML cells.
Summary and Conclusions: 5-AZA-mediated telomere dysfunction may represent a novel mechanism behind its AML therapeutic efficacy, and targeting both DNA methyltransferases and telomerase or TERT may synergistically kill AML cells.

PB1422

INHIBITION OF LSD1 REACTIVATES THE RESPONSE TO ALL-TRANS-RETINOIC ACID IN AML

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Background: Acute promyelocytic leukemia (APL), a cytogenetically distinct subtype of acute myeloid leukemia (AML), characterized by the t(15;17)-associated *PML-RARA* fusion, has been successfully treated with therapy utilizing all-*trans*-retinoic acid (ATRA) to differentiate leukemic blasts. However, among patients with non-APL AML, ATRA-based treatment has not been effective. We previously reported that expression of RARα isoforms, particularly ATRA-inducible RARα2, are down-regulated in AML (Blood. 2008; 111:2374). Epigenetic analysis of patient samples revealed that relative to normal $CD33^+$ cells, the loss of RARα2 in AML is associated with a diminution in levels of histone histone H3 lysine 4 dimethylation ($H3K4^{me2}$) on the ATRA-responsive *RARA2* promoter (a modification associated with transcriptional activation). Interestingly, the $H3K4^{me1/me2}$ demethylase LSD1/KDM1 (*AOF2*) is highly expressed in AML, as well as other cancers. Additionally, LSD1 has been demonstrated to play a role in stem/progenitor cell self renewal.
Aims: The overall goal of our research is the development of better translational approaches to cancer treatment through elucidation of the molecular mechanisms, in particular epigenetic processes, underlying transcriptional deregulation and pathogenesis of acute myeloid leukemia (AML).
Results: We show that in part through epigenetic reprogramming, inhibitors of LSD1 (LSD1i), including tranylcypromine (a monoamine oxidase used as an antidepressant and anxiolytic agent in the clinical treatment of mood and anxiety disorders, respectively), as well as newly-developed, highly specific compounds, unlocked the ATRA-driven therapeutic response in non-APL AML. LSD1 inhibition did not lead to a large-scale increase in histone 3 Lys4 dimethylation ($H3K4^{me2}$) across the genome, but increased $H3K4^{me2}$ and expression of myeloid-differentiation-associated genes. The combination of ATRA plus LSD1i also led to the downregulation of expression of genes important in AML pathogenesis and treatment response such as *BCL11A*, *BCL2* and *MYC*. Furthermore, our recent studies indicate non-genomic effects of LSD1 inhibition also contribute directly to the therapeutic response.
Notably, treatment with ATRA plus LSD1i markedly diminished the clonogenic capacity of AML cells *in vitro* and engraftment of primary human AML cells *in vivo* in nonobese diabetic (NOD)-severe combined immunodeficient (SCID) mice, suggesting that ATRA in combination with LSD1i may target leukemia-initiating cells. Furthermore, initiation of ATRA plus LSD1i treatment 15 days after engraftment of human AML cells in NOD-SCID gamma (with interleukin-2 (IL-2) receptor gamma chain deficiency) mice also revealed the ATRA plus LSD1i drug combination to have a potent anti-leukemic effect that was superior to treatment with either drug alone.
Summary and Conclusions: These data identify LSD1 as a therapeutic target and strongly suggest that it may contribute to AML pathogenesis by inhibiting the normal pro-differentiative function of ATRA, paving the way for new combinatorial therapies for AML.

PB1423

ALDH STAINING IS CORRELATED TO THE EXPRESSION OF GENES INVOLVED IN STEM CELL MAINTENANCE OR MYELOID COMMITMENT IN AML WITH DIFFERENT GENE REARRANGEMENTS.

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Background: Leukemia Stem Cells (LSCs) are described as being the

responsible for chemotherapy failure and recidive/recurrence due to their self-renewal properties. The fact that cells harbouring identical gene rearrangement and derived from the same tumour can behave in a functionally distinct manner suggests a role for additional mechanisms which confer advantage to specific cell subsets. Aldehyde dehydrogenase (ALDH) is an enzyme that protects stem cells from alkylation. Increased activity of ALDH has proven to be a successful strategy to identify acute myeloid leukemia (AML) LSC among the leukemic bulk in bone marrow samples. Different studies have reported that cells with intermediate ALDH activity have self-renewal properties and clonogenic potencial. In contrast, cells with high ALDH activity were able to generated committed progenitors of different hematopoietic lineages, therefore resembling the normal hematopoietic stem cells (HSCs)

Aims: To compare the expression of genes regulatiing stemness and myeloid commitment in AML cells expressing high or intermediate ($ALDH^{hi}$ and $ALDH^{lo/int}$) ALDH activity.

Methods: We took advantage of Aldefluor reagent to identify LSC among cell lines representative of AML with different prognosis, based on their higher activity of ALDH: Kasumi-1, THP-1, MV-4;11, NB4 and OCI-AML3. Cell subsets presenting high ALDH activity ($ALDH^{hi}$) were identified in Kasumi-1 (13%), THP-1 (25%) and OCI-AML3 (12%). To assess if the ALDH staining was correlated to the expression of genes involved in stem cell maintenance or myeloid commitment, cell lines were further sorted in $ALDH^{hi}$ and $ALDH^{lo/int}$ subsets, and the expression levels of *CEBPA*, *BMI-1*, *NOTCH-1*, *C-MYC*, *HOXA9*, *E2F1*, *NANOG* and *OCT3/4* were evaluated by RQ-PCR, by using the DDCt method.

Results: *CEBPA, BMI-1* and *NOTCH-1* were upregulated (9, 2.3 and 2 fold, respectively) in Kasumi-1 $ALDH^{hi}$ cells in comparison to $ALDH^{lo/int}$, whereas *C-MYC* and *E2F1* were downregulated (0.3 and 0.5 fold, respectively). Some molecular mechanisms responsible for self-renewal, like Bmi-1 and Notch signaling pathways, were found to be shared by both HSC and LSC, suggesting that the higher expression of both in $ALDH^{hi}$ cells would be in accordance with stemness properties. Still, the lower levels of *C-MYC* and *E2F1* expression in $ALDH^{hi}$ cells suggest that they are somehow more quiescent, supporting the idea that LSC underexpress cell cycle genes.

Summary and Conclusions: Our results reveal an important correlation between ALDH activity and the expression of genes associated with stemness properties in identifying stem-like cells in Kasumi-1.

PB1424

Abstract withdrawn

PB1425

EVALUATION OF TNF SUPERFAMILY MOLECULES IN ACUTE MYELOID LEUKEMIA PATIENTS: CORRELATION WITH BIOLOGICAL AND CLINICAL FEATURES

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Background: Two decades ago, the existence of receptors activated by the tumor necrosis factor alpha (TNF-α) has been demonstrated for several hematological diseases including acute myeloid leukemia cells (AML). B-cell activating factor (BAFF), a proliferation-inducing ligand (APRIL) and apoptosis inducing ligand (TRAIL), all members of the tumor necrosis factor family can directly activate the NF – κB pathway, which is constitutively activated in AML blasts. Therefore, the inhibitors of NF-κB have emerged as potential therapies against AML. BAFF, APRIL and TRAIL molecules were detected in monocytes /macrophages, dendrite cells and activated T lymphocytes. Neutrophils, taking part in an early stage of an anti-cancer response, are also a significant source of these molecules.

Aims: The purpose of the present study was to evaluate serum levels of BAFF, APRIL and TRAIL in healthy volunteers and in AML patients to determine whether there was any correlation between ligands and some prognostic biological parameters of AML patients, and to explore their clinical significance in predicting the disease activity of AML.

Methods: 74 patients with newly diagnosed AML were included in the study. Median age of patients at the time of sample collection was 45, and the range was 19-65. Diagnoses were established according to the WHO classification system. Patients were treated with the seven days induction chemotherapy with a standard therapy based on Polish Adult Leukaemia Group: DAC schedule. The control group consisted of 40 healthy volunteers, age- and sex-matched. Quantitative assessments of cytokines were performed by commercially available ELISA assays.

Results: Pre-treatment AML patients had significantly higher serum concentration compared to healthy volunteers: BAFF (6847.7±6071.3pg/ml *vs* 309.2±216.1pg/ml, p<0.001), APRIL (9.14±8.71ng/ml *vs* 1.94±1.51ng/ml, p<0.001) and lower for TRAIL (67.44±21.45pg/ml *vs* 80.28±16.24pg/ml, p=0.04). The analyses did not exhibit marked differences in concentration of cytokines according to FAB classification for all studied cytokines (p > 0.05) and a subgroup of patient with different cytogenetic risk except the TRAIL (80.13±18.11, 64.56±21.45 and 62.64±20.57 pg/ml, p=0.03). Furthermore, the concentrations after the treatment were found to be lower in the subgroup of patients with CR compared to the NR: for BAFF 4025.7±1704.1pg/ml *vs* 6520.1±4972.1pg/ml, p=0.001, APRIL 7.21±5.05ng/ml *vs* 13.26±9.59ng/ml, p=0.04. On the other hand the concentration of TRAIL was found to be higher in a patient with CR compared to NR: 72.3±34.52pg/ml *vs* 53.12±17.68pg/ml, p=0.02. Furthermore, the study demonstrated statistically positive correlations between the BAFF concentration and WBC, neutrophils and monocytes counts (for all, p=0.04) and negative for TRAIL (for all, p<0.001). More importantly the study showed the positive correlation between concentration of APRIL and counts of blastic cells in a bone marrow smear (p=0.001) and negative between TRAIL concentration and blastic cells counts in peripheral blood (p=0.002). In addition, the concentration of BAFF was found to correlate significantly and positively with the concentration of APRIL and negatively with TRAIL (p=0.001). Additionally, we observed that pre-treatment AML patients with serum BAFF values higher than the median (3615.14pg/ml) and with serum TRAIL values lower than the median (68.54 pg/ml) had significantly shorter overall survival (OS) than patients with higher values: for BAFF and TRAIL, p=0.03. There were no statistically significant differences between OS values in the subgroups of AML patients with regard to the median values (5.96 ng/ml) of APRIL, p=0.6. Multivariate Cox proportional showed that only BAFF can be considered as independent risk factor.

Summary and Conclusions: In conclusion, our results have demonstrated that serum concentrations of BAFF and TRAIL (but not APRIL) could be a useful biomarker of AML disease activity and progression. Pre-treatment concentrations of BAFF could also serve as a prognostic factor of OS. Both ligands may therefore be a novel therapeutic target in AML.

PB1426

INVESTIGATION OF MECHANISMS INVOLVED IN APOPTOSIS CAUSED BY A SYNTHETIC NAPHTHYLCHALCONE IN ACUTE LEUKEMIA CELL LINES

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Background: Acute leukemia is a disorder of the hematopoietic system characterized by the expansion of a clonal cell population that may arise at distinct differentiation stages of the lymphoid and myeloid precursors. Despite being the most effective treatment for leukemia, standard chemotherapy is still associated with patient relapse and high morbidity and mortality rates. The killing of tumor cells by these therapies is mediated primarily by apoptosis, a regulated type of cell death. Previous studies reported the cytotoxic effects of chalcone A1, derived from 1-naphthaldehyde, in leukemia cell lines. Chalcone A1 significantly reduced cell viability of K562, Jurkat, Kasumi, U937, CEM and NB4 cells in a concentration and time-dependent manner (IC_{50} between ~1.5 μM and 40 μM) and it was non-cytotoxic to PBL cells. It also caused significant cell cycle arrest and increased the proportion of cells in subG0/G1 phase, which has been confirmed to be apoptosis.

Aims: The main purposes of this study were to investigate the apoptotic mechanisms involved in its cytotoxicity and to test the compound in *ex vivo* assays.

Table 1.

Patient	Age (years)	Diagnosis	Leukocytes /mm^3	Blast Cells (%)	IC50 (μM)
1	48	ALL	142,340	89.9	26,18±1,42
2	17	ALL	61,580	84.5	21,38±1,33
3	87	APL	20,690	24.6	7,19±0,86
4	76	AML	35,500	31.8	42,27±2,04
5	44	AML	14,890	30.0	76,51±1,88
6	18	APL	25,680	62.0	18,45±1,73
7	38	AML	25,370	59.0	16,04±1,20
8	67	AML	5,680	27.0	11,15±1,01

Methods: The mitochondrial membrane potential has been evaluated by MitoView 633 kit (Biotium®, USA). The evaluation of Bax, Bcl-2, FasR, AIF and caspase 3 protein expressions has been conducted by flow cytometry. The expression of survivin and KI67 proteins has been evaluated by immunocytochemistry using the streptavidin-biotin-peroxidase method. For *ex vivo* experiments, eight samples obtained from patients diagnosed with AL

before the first treatment have been included in this study. Patients signed an informed consent according to the ethics committee requirements. Data are presented as mean±standard deviation of at least three independent experiments and the statistical significance level was set at p<0.05.

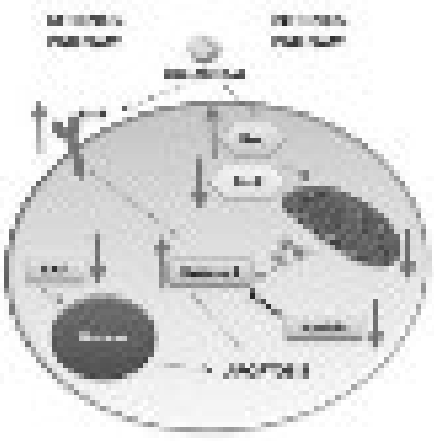

Figure 1.

Results: After treatment with chalcone A1 (12h), only 36.25±1.55% of K562 and 28.00±3.51% of Jurkat cells maintained the integrity of mitochondrial membrane. The compound significantly reduced the expression of anti-apoptotic protein Bcl-2 in K562 cells after 8h and 12h and in Jurkat cells after 12h (reduction of ~ 63%, 65% and 50% respectively). It also increased the expression of pro-apoptotic protein Bax in K562 cells after 8h and 12h and in Jurkat cells after 12h (MFI values of 173.740±16.55%, 207.21±12.43% and 138.45±6.56% respectively). These results indicate the involvement of the intrinsic pathway and a reversal in the ratio of pro-apoptotic to anti-apoptotic factors. Chalcone A1 significantly increased the expression of FasR in Jurkat cells (26%) but not in K562 cells, which suggests that in Jurkat cells both the extrinsic and the intrinsic pathways are involved in cell death, while in K562 cells only the intrinsic pathway is activated. The results also demonstrated an increased expression of effector caspase 3 after a 12h treatment in both K562 and Jurkat lines (MFI of 124.41±3.19% and 130.50±3.09%, respectively) and a decreased expression of IAP protein survivin, consistent to apoptotic cell death. The decreased expression of KI67 protein suggests that the mechanism of action of chalcone A1 also involves a decrease in cell proliferation. No significant changes in the expression of AIF have been observed, which suggests that caspase activation involves the release of other proteins. In *ex vivo* experiments, chalcone A1 reduced the cell viability of blast cells collected from eight patients with different types of acute leukemia, confirming the cytotoxicity results found *in vitro*.
Summary and Conclusions: The results obtained so far are very promising and further tests need to be performed so that compound A1 can become a prototype for the development of new anti-leukemia agents.

PB1427

Abstract withdrawn

PB1428

DYSERYTHROPOIESIS ASSOCIATED WITH GAINS OF THE ERYTHROPOIETIN RECEPTOR GENE REGION

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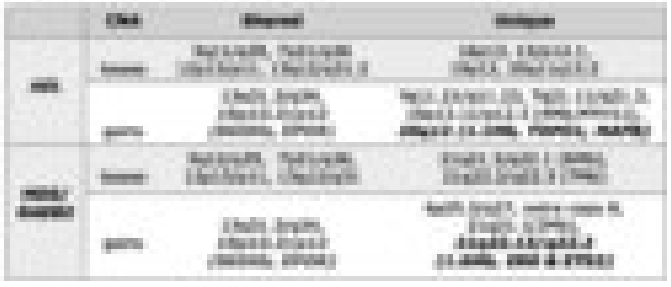
Background: Acute erythroleukemia (AEL) is a rare subtype of acute myeloid leukemia (AML) characterized by the expansion of morphologically abnormal and malignant erythroid cells in the bone marrow (BM), referred to as AML-M6 by the French–American–British (FAB) classification. There are limited reports on the cytogenetics of AEL and common findings include hypodiploidy (47–56%) and complex karyotypes including abnormalities of the long arm regions of chromosomes 5 and 7. While MLL gene rearrangements are common in AEL, genes harbouring mutations specific for AML are rarely seen in AEL (~16%). These include FLT3 mutations reported in cases with normal chromosomes and variant TP53 in samples with highly aberrant karyotypes.
Aims: Our purpose was to obtain comprehensive genome profiles of two cases with marked dyserythropoiesis, A 80 year years old female presented with hypercellular bone marrow, consisting of predominantly (>80%) erythroid cells and blasts accounting for 8% of the total nucleated cells and gross dyserythropoiesis consistentwith AEL. The other patient, a 76 year old male with previous MDS presented with BM aspirate showing evidence for dyserythropoiesis and dysgranulopoiesis with 15% blasts, many of which had cytoplasmic bleeding, indicative of RAEB2
Methods: A range of techniques were applied from classical cytogenetics assays (G-bands and FISH) to whole genome scanning by microarrays (aCGH) and DNA variance analysis of selected genes using targeted Next Generation Sequencing (NGS).
Results: Complex hypodiploid karyotypes with clonal aberrations involving chromosomes 5, 7, 13 and 20 in were found in both cases. FISH confirms the presence of 5q and 7q deletions, while the MLL gene, frequently rearranged in AEL, was intact. NGS analysis on the coding region of 18 genes with an established role in the pathogenesis of AML, identified frameshift mutations in the TP53 gene and variances in DNMT3A gene (MDS/RAEB2) as well as FLT3 in the AEL case. aCGH identified multiple copy number aberrations (CNA) summarised in Table 1. Gain of an 865Kb sequence at chr19:10,748,354-11,613,371 within 19p13.3 gene rich region was seen in both samples, which includes the small (7,138 bp) erythropoietin receptor gene (EpoR). EpoR is a type 1 cytokine receptor lacking intrinsic tyrosine kinase activity with a role in erythropoiesis. An obvious candidate for implication in the dyserythpoiesis shared by both cases, EpoR acts through the EpoR/Jak2/Stat5 signalling axis, regulating proliferation, differentiation, and erythroid cell survival. Although EpoR acts via the kinase Jak2, the contribution of distinct EpoR/Jak2-induced signalling pathways (MAPK, PI-3, and Stat5) to functional erythropoiesis is still unclear. To date amplification of the EpoR gene has been detected in cell lines derived from AEL patients and also mice, but no evidence for such aberrations in patients' samples have been reported until now.
Summary and Conclusions: Here we present the first report for EpoR involvement in the genome profile of AEL offering a potential new diagnostic marker.

Table 1. Common gain at 19p13.3 includes the 7,138pb region of the EPOR gene.



PB1429

DISTINCT FEATURES OF HLA-DR-NEGATIVE/CD34-NEGATIVE ACUTE MYELOID LEUKEMIA: INTERMEDIATE CHARACTERISTICS BETWEEN ACUTE PROMYELOCYTIC LEUKEMIA AND HLA-DR-POSITIVE/CD34-POSITIVE ACUTE MYELOID LEUKEMIA

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Background: Negativity for HLA-DR and CD34 is a critical feature of acute promyelocytic leukemia (APL). This immunophenotype has been also described in patients with acute myeloid leukemia (AML) other than APL. Several studies previously reported that HLA-DR-/CD34- AML had distinct characteristics as compared to APL. However, there were only a few studies on comparison between HLA-DR-/CD34- AML and HLA-DR+/CD34+ AML.
Aims: The objective of this study was to investigate distinguishable features of HLA-DR-/CD34- AML from HLA-DR+/CD34+ AML as well as APL.
Methods: A consecutive series of 422 patients with AML including APL between March 2011 and January 2014 was included in the study. We retrospectively reviewed their immunophenotypes, cellular morphology, cytogenetics, molecular findings, and coagulation profiles.
Results: Of the patients analyzed, 33 were diagnosed as APL, while the remaining 389 AML patients included 33 HLA-DR-/CD34-, 253 HLA-DR+/CD34+, and 103 either HLA-DR+/CD34- or HLA-DR-/CD34+. Statistically significant differences were found between HLA-DR-/CD34- AML and HLA-DR+/CD34+ AML in CD15 negativity (63.6% and 41.1%, respectively, p=0.023) and frequencies of normal karyotype (63.6% and 26.9%, respectively, P<0.0001), *NPM1* mutation (77.8% and 5.2%, respectively, P<0.0001), and

recurrent cytogenetic abnormalities (3.0% and 28.5%, respectively, p=0.003). The frequencies of other mutations including *FLT3* ITD, *CEBPA*, and *MLL* PTD were not different. Regarding the coagulation profile, significant differences were found between HLA-DR-/CD34- AML and HLA-DR+/CD34+ AML in the levels of fibrinogen (median value: 292.5 and 347.0 mg/dL, respectively, p=0.006), FDP (median value: 38.95 and 4.4 µg/mL, respectively, P<0.0001), and D-dimer (median value: 18.085 and 1.185 µg/mL, respectively, P<0.0001). Notably, patients with HLA-DR-/CD34- AML had apparent intermediate characteristics between those with APL and HLA-DR+/CD34+ AML for the levels of fibrinogen, FDP, and D-dimer and frequencies of CD7 positivity and *FLT3*-ITD mutation (P<0.0001, P<0.0001, P<0.0001, p=0.011, and p=0.018, respectively). The medial values of D-dimer, for example, were 26.475 µg/mL in APL, 18.085 µg/mL in HLA-DR-/CD34- AML, 2.13 µg/mL in either HLA-DR-/CD34+ or HLA-DR+/CD34- AML, and 1.185 µg/mL in HLA-DR+/CD34+ AML (Figure 1).

Summary and Conclusions: The present study suggests that HLA-DR-/CD34- AML and HLA-DR+/CD34+ AML can be distinguished by immunophenotypic, molecular and coagulation laboratory characterization. Furthermore, patients with HLA-DR-/CD34- AML show intermediate characteristics between APL and HLA-DR+/CD34+ AML, particularly for the coagulation profiles. In conclusion, HLA-DR-/CD34- AML is biologically separated from HLA-DR+/CD34+ AML and may be an intermediate form between APL and HLA-DR+/CD34+ AML.

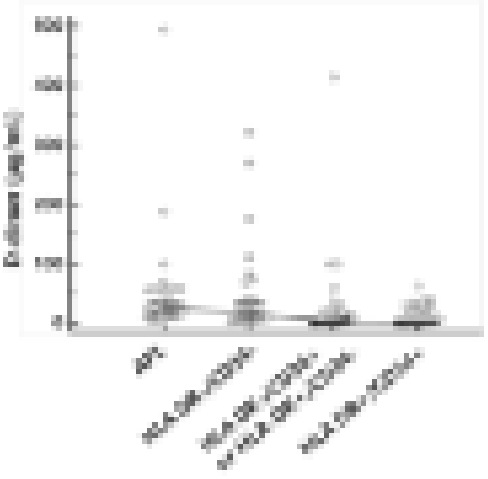

Figure 1.

PB1430

PROTEIN DISULPHIDE INHIBITORS INDUCE DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS

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Background: Arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) are highly effective, differentiation-inducing agents used in the treatment of acute promyelocytic leukemia (APL). Treatment of other acute myeloid leukemia (AML) types continues to be less effective. Thus elucidation of cellular mechanism(s) causing impaired differentiation and identification of new anti-leukemic therapeutic agents is of utmost clinical importance. There is accumulating evidence for the involvement of the unfolded protein response (UPR) in the progression of AML. Surprisingly, induction of key effector proteins of endoplasmic reticulum (ER) stress pathway in clinical AML samples is linked to a favorable patients prognosis (Schardt JA, Clin Cancer Res. 2009). AML patients with activated UPR and increased ER chaperones levels showed decreased CEBPA protein expression (Schardt JA, J Cell Mol Med. 2010). Protein disulfide isomerase (PDI), an enzyme found mainly in ER, is involved in the formation and isomerization of disulfide bonds between cysteine residues of polypeptides as they fold. Recently a unique role of PDI in the regulation of gene expression at the postranscriptional level has been described: PDI binds to the mRNA for CEBPA and inhibits its translation, leading to the blockade of differentiation of AML cells into neutrophils (Haefliger S, Blood, 2011).
Aims: The aim of this study was to investigate the effects of well-known PDI inhibitors: bacitracin and quercetin rutinoside on the survival and differentiation of human AML cells.
Methods: We evaluated the biological effects of PDI inhibitors on proliferation, differentiation, and the transcriptomic changes in human myeloid cell lines: HL60, NB4, KG1 and MOLM14.
Results: Our results suggest that PDI inhibitors reduce cell proliferation and induce differentiation towards monocytic and granulocytic lineages. Accordingly, they up-regulate expression of selected differentiation-related genes.
Summary and Conclusions: We propose a new approach in AML treatment, namely inhibition of protein disulfide isomerase activity leading to the differentiation of the human AML cells.

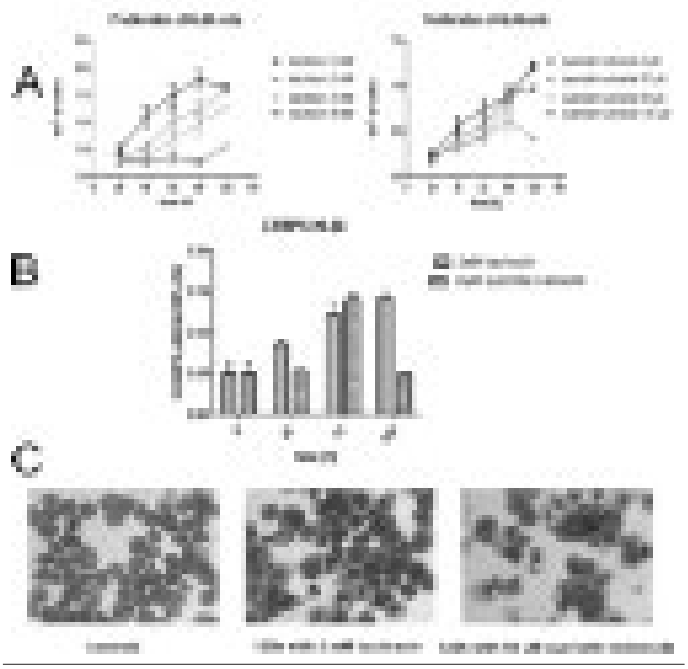

Figure 1. PDI inhibitors induce differentiation of human AML cells. (A) Cytostatic/cytotoxic activity of bacitracin and quercetin rutinoside in HL60 cells evaluated with trypan blue staining; (B) qPCR results presented as mean target-to-reference ratio±SD (left) CEBPA expression in HL60 cells incubated for indicated times with 50 µM quercetin rutnoside or 2 mM bacitracin; (C) evaluation HL60 morphology in May Grünwald-Giemsa staining; asterisks point cells with a more mature morphology.

PB1431

MUTATIONS OF ASXL1 GENE IN PATIENTS WITH AML WITH AN INTERMEDIATE-RISK CYTOGENETIC PROFILE

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Background: ASXL1 is a highly conserved protein which belongs into the ETP (enhancer of trithorax and polycomb) protein family which functions as an epigenetic regulator of gene transcription through the changes in the chromatin structure. *ASXL1* gene is located in the chromosomal region 20q11.21, comprises 12 exons and encodes a nuclear protein of 1541 amino acid residues. Its mutations (particularly found within exon 12) were described in patients with various kinds of myeloid malignancies including AML and MDS.
Aims: We attempted to test the prognostic impact of *ASXL1* mutations in our patient cohort.
Methods: RNA for *ASXL1* mutation analysis was available from 226 patients with AML with an intermediate-risk cytogenetic profile (defined according to Grimwade *et al.*, Blood, 2010) diagnosed at a single institution between years 1998-2011. Median age at diagnosis was 55.1 years (range 18.1-81.7), the initial median WBC count was 23.1 x 10^9/L (range 0.4-483.7). The male/female ratio was 109/117 and the median of follow-up was 13.0 months. The whole exon 12 was amplified using 4 RT-PCR reactions; PCR products were treated by ExoSAP-IT reagent and directly sequenced. Potential polymorphisms were excluded by analyzing either remission samples or DNA isolated from patients' nails.
Results: *ASXL1* mutation was detected in 26 from 226 patients (11.5%). We identified 6 different frameshift changes, 5 nonsense and 4 missense mutations;

in one patient we found two different mutations. Another 5 various missense mutations were found out to be polymorphisms. Only 4/26 (15.4%) patients harboured *ASXL1* mutation together with *FLT3*/ITD, which is the most frequent aberration in AML with intermediate prognosis, while among *ASXL1*-negative cases it was 68 from 200 (34.0%) (P=0.028). Presence of *ASXL1* mutations was not influenced by the occurrence of *DNMT3A* mutations. Patients carrying *ASXL1* mutation had significantly lower WBC counts (26.4 *vs.* 3.5 x 10^9/L; P=0.002) and lower percentage of blasts in bone marrow at diagnosis (44.1% *vs.* 70.0%; P=0.009). Mutations of *ASXL1* slightly decreased the chance to reach complete remission (CR): only 13/25 (52.0%) *ASXL1*-positive cases receiving standard induction treatment achieved CR compared to 125 from 185 (67.6%) patients without this aberration (P=0.062). The initial positivity of *ASXL1* had no impact either on the relapse rate (38.5% with mutated *ASXL1* relapsed similarly as 48.8% patients without it; P=0.239) or on the relapse free survival in patients who reached CR. Presence of *ASXL1* mutation did not influence overall survival (OS) of patients as well (P=0.770).
Summary and Conclusions: We detected *ASXL1* mutation in almost 12% of patients with intermediate-risk cytogenetics, mainly in those lacking *FLT3*/ITD. *ASXL1*-positive patients had lower WBC counts and lower CR rate which is in concordance with results published so far. Although missense mutations are often considered as polymorphisms, we identified 4 missense changes that turned out to be true mutations (disappearing when patients achieved CR and not detectable in nails' DNA). We did not prove any impact of the *ASXL1* positivity on the incidence of relapses as well as on OS. The presence of *ASXL1* mutations did not substantially worsen the prognosis of patients.

PB1432

INCIDENCE OF COLONY STIMULATING FACTOR 3 RECEPTOR MUTATION (CSF3R) IN EGYPTIAN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease that accounts for 20% and 70% of acute leukemia in children and adults, respectively. There are marked differences in survival following intensive chemotherapy based on age, blast cell morphology and cytogenetic abnormalities. Colony-stimulating factor 3 (CSF3), also known as granulocyte-colony stimulating factor (G-CSF) is the main growth factor for neutrophil production. Colony-stimulating factor 3 receptor is under a dynamic balance between signal activation and attenuation to achieve a well-adjusted neutrophil output both under normal and infectious conditions.
Aims: The aim of this study was to evaluate the prevalence, clinical and prognostic impact of the colony stimulating factor 3 receptor mutations in AML patients treated at Mansoura Oncology Center, EGYPT.
Methods: This study were conducted on 179 adult patients (17-65 years old), 89 males and 90 females and *CSF3R* mutation was detected by sequencing technique.
Results: 156 patients were de novo AML and 23 patients were secondary AML on top of sever congenital neutropenia (SCN). *CSF3R* mutation was detected in two out of 156 denovo AML patients (0.012%) and 18 out of 23 secondary AML patients (78.2%). The mutant cases were younger age, have a high WBCs count, high bone marrow blasts, bad performance status, no extramedullary disease and with less induction remission rate.
Summary and Conclusions: The frequency of *CSF3R* mutations was highly prevalent among AML patients secondary to SCN. Finally, molecular testing for *CSF3R* gene mutation in patients with SCN is recommended and to follow up during the course of treatment to clarify the role of G-CSF treatment and leukomogenisis in patient with congenital neutropenia.

PB1433

A PARP INHIBITOR OLAPARIB ENHANCES THE CYTOTOXICITY OF GEMTUZUMAB OZOGAMICIN AGAINST GEMTUZUMAB-OZOGAMICIN RESISTANT LEUKEMIC CELLS IN VITRO

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Background: Gemtuzumab ozogamicin (GO) is a humanized monoclonal antibody directed against the CD33 surface antigen that is conjugated to a derivative of the cytotoxic antibiotic calicheamicin. Because CD33 is specific to leukemic cells, GO is an attractive targeted agent for treating acute myeloid leukemia (AML). However, clinical studies have demonstrated that the therapeutic efficacy of GO is limited. Upon administration, the binding of GO to the CD33 antigen on leukemic cells results in internalization of the drug, followed by the release of calicheamicin that induces DNA strand breaks. Calicheamicin cleaves DNA and produces both double-strand and single-strand breaks. The double-strand break/single-strand break ratio in DNA is reported to be 1:1 to 1:3. If poly (ADP-ribose) polymerase (PARP), a component of the DNA single strand break repair machinery, is inhibited, unrepaired single-strand break lesions are converted into more lethal DNA double-strand breaks during DNA replication. It was therefore hypothesized that the cytotoxicity of GO against leukemic cells would be enhanced by the inhibition of PARP.
Aims: The aim of the present study was to evaluate the cytotoxic effects of GO combined with a PARP inhibitor olaparib on cultured leukemic cells *in vitro*. The cytotoxicity was compared between GO-sensitive and GO-resistant cell lines.
Methods: Human leukemia HL-60 cell line was used. A GO-resistant variant cell line was established by serially incubating HL-60 cells with escalating concentrations of GO. The initial concentration of GO was one-tenth the concentration of the 50%>inhibitory concentration (IC50) of GO. The cells were cloned using the limiting dilution method. Both cell lines were cultured in RPMI1640 media supplemented with 10% fetal calf serum in a 5% CO_2-humidified atmosphere at 37°C. The cell proliferation was determined by the XTT assay. The induction of apoptosis was determined as a sub-G1 cell cycle population and/or caspase 3/9 cleavage. The expression of CD33 was measured by flow cytometry. For comparison, human lymphoblastic cell line CCRF-CEM cells were used.
Results: The XTT assay revealed that the IC50 value was 65 ng/ml for HL-60 cells, while that of GO-resistant variant HL/GO8 cells was 510 ng/ml. This suggested HL/GO8 cells were 8-fold more GO-resistant than HL-60 cells. HL/GO8 cells were also more refractory to GO-induced apoptosis measured as the amount of sub-G1 fraction. The CD33 positivity was reduced in HL/GO8 cells. Olaparib alone did not inhibit the cell growth and did not induce apoptosis in both HL-60 cells and HL/GO8 cells at the concentrations up to 10 μM. When cells were treated with various concentrations of GO in the presence of a non-toxic concentration of olaparib (10 μM), the IC50 for HL-60 was 32 ng/ml while the value for HL/GO8 cells was 210 ng/ml, suggesting that the IC50 values were half reduced in both cell lines regardless of GO sensitivity. The combination index calculated by Chou-Talalay method revealed that the combination between GO and olaparib showed synergism. Olaparib also augmented the induction of apoptosis by GO in both cell lines. CCRF-CEM cells were insensitive to GO and olaparib, because the cell did not express CD33 antigen.
Summary and Conclusions: Olaparib enhanced the cytotoxicity of GO against human myeloid leukemic HL-60 cells and GO-resistant variant HL/GO8 cells *in vitro*. Thus, the combination between GO and olaparib would be promising for treating patients with refractory AML. Moreover, if leukemic cells are deficient for breast cancer susceptibility gene (BRCA)1/2 of DNA double-strand break repair, GO-induced DNA strand breaks and the inhibition of PARP by olaparib may induce the mechanism of synthetic lethality in the cells.

PB1434

STAT5B GENE DYSREGULATION CORRELATES WITH LEF1 GENE EXPRESSION IN ADULT ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Lymphoid enhancer-binding factor 1 (*LEF1*) is a downstream effector of the Wnt/ β-catenin signaling pathway, which controls cell growth and differentiation. Dysregulation of *LEF1* expression may result in several disease patterns, as the Wnt signaling plays a pivotal role in development and cancerogenesis and also controls self-renewal, proliferation and differentiation of many types of stem cells. High *LEF1* expression has been reported as a favorable prognostic marker in cytogenetically normal acute myeloid leukemia whereas it is associated with poor prognosis in adult B precursor acute lymphoblastic leukemia and in chronic lymphocytic leukemia. Moreover, marked downregulation of *LEF1* is associated with disease progression in myelodysplastic syndromes.
Aims: Recently, our group reported LEF1 expression as a prognostic factor in adult acute promyelocytic leukemia (APL). In hematologic malignancies has been reported a link between STATs transcription factor and LEF1; as STAT5b rarely is involved in molecular rearrangement with RARa gene in APL, we investigated whether there was a relationship between LEF1 and STAT5b gene expression.
Methods: *LEF1 and STAT5b* expression was measured by real-time qPCR in 75 APL patients (median age 45 years, range 16 to 88 years). Advanced relative quantification analysis was performed using LightCycler 480 Software 1.5.1, based on the ΔΔCt method. *LEF1* expression was measured using a RealTime intron-spanning ready assay recognizing all 4 major human *LEF1* isoforms (assay ID 103366, Roche); STAT5b quantification was assessed by using specific primer selected according to Primer3 software. The β-glucuronidase (*β-GUS*) gene was employed as housekeeping gene and a pool of cDNA derived from BM cells of 5 healthy individuals was used as calibrator for normalization. APL samples were dichotomized at the median value and divided into two expression groups: low *LEF1* (39 patients) with *LEF1* values below the median

value (LEF1^{low}) and high *LEF1* (36 patients) with *LEF1* values above the median value (LEF1^{high}).
Results: Fifty-one (68%) APL patients showed a STAT5b expression that was higher than that observed in the healthy control group. Patients with *LEF1^{high}* expression had higher amount of STAT5b transcript compared to that detected in the *LEF1^{low}* patients group (2.2 *vs* 1.4 fold change; p=0.04). Moreover, there was a positive correlation between LEF1 and STAT5b gene expression (r = 0.61, p<0.0001); the relation between the two genes expression was more close in APL patients aged<60 years compared to that observed in those with > 60 years (r=0.64, p<0.0001 *vs* r=0.51, p=0.04, respectively).
Summary and Conclusions: Preliminary results from our study suggest that *LEF1* gene expression in APL is linked to STAT5b gene dysregulation. As high *LEF1* expression has recently been reported as a favorable prognostic marker in adult APL the identification of genes involved in LEF1 pathway plays a crucial role for clarifying molecular pathogenesis of APL

PB1435

ANTI-TUMOR ACTIVITY OF FUCOIDAN THROUGH INHIBITION OF AKT AND ERK ACTIVATION IN HUMAN ACUTE PROMYELOCYTIC LEUKEMIA
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Background: Acute myelogenous leukemia (AML) is the leading cause of cancer death among patients younger than 39 years. The main treatment is cytotoxic chemotherapy. However, due to its toxicity and limited effectiveness there is considerable interest in the potential of natural agents with fewer side effects and greater compatibility with human physiology. Fucoidan is a complex sulphated polysaccharide that exists in cell wall matrix of brown seaweeds. This component has become a focus of interest because of its anti-tumor activity and low toxicity. In this study we examined the anti-proliferative activity of fucoidan on human acute myelogenous leukemia cells.
Aims: To determine whether fucoidan inhibits proliferation of malignant hematopoietic cells. To determine the underlying mechanisms of this effect
Methods: Different AML cell lines were treated with various concentrations of fucoidan. The growth-inhibitory effect of fucoidan was measured using cytotoxicity, annexin V/PI apoptosis, DNA fragmentation and cell cycle assays. To determine the underlying mechanisms, expression of apoptosis-related proteins and activation of signalling pathway molecules were analysed using western blotting.
Results: Fucoidan significantly inhibited growth of acute promyelocytic leukemia HL60 cells in a dose and time dependent manner and to a lesser extent inhibited K562 cells growth. In contrast, it failed to inhibit proliferation of KG1a cells. DNA fragmentation and annexin V positive apoptotic cells increased in HL-60 cells demonstrating that fucoidan switches on apoptosis pathway. DNA fragmentation and annexin V were negative in treated KG1a and K562 cells. Cell cycle analysis revealed marked increase in sub G0/G1 population in HL60 cells with no arrest at any stage of cell cycle. In contrast, fucoidan induced G0/G1 arrest in K562 cells.
To determine the underlying mechanisms, we examined activation of various apoptosis-related proteins in HL60 cells and observed that fucoidan decreased pro-caspases 3, 8 and 9 in treated HL60 cells. The cleaved activated caspases therefore increased and activation of caspase 3 was directly proportional to cleavage of PARP (downstream target of activated caspase 3-). The expression of death receptors DR5 and Fas clearly increased but anti-apoptotic protein Bcl-xl expression was not affected. Phosphorylation of ERK and Akt kinases clearly decreased demonstrating strong inactivation of ERK and Akt by fucoidan.
Summary and Conclusions: In this *in vitro* study of the anti-growth effect of fucoidan on AML cells we found that: Fucoidan has a selective inhibitory effect on acute promyelocytic leukaemia HL60 cell line while it does not induce apoptosis in erythroleukemia K562 and minimally differentiated acute myeloblastic leukemia KG1-a cell lines. Fucoidan decreased proliferation of K562 cells while all apoptosis assays were negative. These findings were confirmed by effects of fucoidan on cell cycle whereby it induced G0/G1 arrest in K562 cells. Both caspases 8 and 9 were activated by fucoidan demonstrating it can activate apoptosis through both intrinsic and extrinsic pathways. Fucoidan caused significant inhibition of MAPK signalling pathway molecule ERK and AKT activation. We conclude that fucoidan has potential as a possible agent in the treatment of AML. *In vivo* studies using tumor-bearing animal models are necessary.

PB1436

GENOMIC ALTERATIONS IN MYELOID MALIGNANCIES WITH DOUBLE MINUTE CHROMOSOMES AND OTHERWISE NORMAL KARYOTYPE
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Background: Double minute (dmin) chromosomes are small paired chromatin bodies found in various malignancies. Similar to homogenously staining regions (hsr), they consist of amplified oncogenes and may cause an elevated expression of these genes. Although frequent in cancer, the presence of these gene amplifications by hsr or dmin chromosomes in hematological malignancies is rare; the frequency in acute myeloid leukemia (AML) is estimated around 1%. Within hematological malignancies dmin are generally associated with a poor prognosis. The most common gene amplified in AML as reported in the literature is *MYC* (8q24) (44-76%). The second most commonly amplified gene is *KMT2A* (*MLL*, 11q23) (18-30%). Abnormally high expression of the *MYC* gene is found in a wide variety of human tumors and it is a well described oncogene. However, there is debate in the literature for both loci whether *MYC* and *KMT2A* are the genes of interest in amplification.
Aims: We used Fluorescence In Situ Hybridization (FISH) and Single Nucleotide Polymorphism- (SNP-) array to investigate the constitution of dmin chromosomes and possible other genomic alterations in four patients with myeloid malignancies/AML with presence of dmin chromosomes and an otherwise normal karyotype.
Results: Two patients showed amplification of the 8q24/*MYC* locus. The third patient showed amplification of the 3q26/*MECOM* locus and the fourth patient showed amplification of distal 3p. Surprisingly, next to amplification of genomic regions present in dmin, in all patients additional submicroscopic losses and/or acquired uniparental disomy (aUPD) were seen in regions and genes known to be involved in leukemogenesis or hematopoiesis. In the first patient disruptions of genes in the Wnt-signaling pathway were seen next to disruption of *MNX1 (HLXB9)* and large subclonal loss directly adjacent to 8q24/*MYC*. In the second patient additional loss of 9q21, within the critical region as reported for AML was present, combined with aUPD of 4q. In the third patient loss of the *ETV6*-region was observed in combination with *IKZF1* loss. Finally additional aUPD of 11q was seen in the fourth, suggestive for *CBL* mutations.
Summary and Conclusions: In this small cohort of 4 patients with an age range of 61 to 83 years old, additional genomic alterations to dmin chromosomes were observed in all cases. Furthermore it is known that in older patients the probability for submicroscopic genomic alterations in cytogenetically normal AML is higher compared to younger patients or children. The presence of dmin chromosomes may indicate additional structural rearrangements involved in leukemogenesis. Array based analysis of AML patients with dmin chromosomes may contribute to a better understanding of the consequences of gene amplification in myeloid malignancies and may expand the knowledge of the influence of other involved loci.

PB1437

DISSECTING THE BIOLOGICAL MECHANISMS AND THE CONSEQUENCES OF NUMERICAL CHROMOSOME ABNORMALITIES IN ACUTE MYELOID LEUKEMIA
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Background: Acute Myeloid Leukemia (AML) is a heterogeneous malignancy characterized by the expansion of myeloid precursor cells with limited or abnormal differentiation capacity. A relatively common event in AML is represented by chromosome gain or loss. Numerical chromosome abnormalities, which define the aneuploid condition, have a detrimental effect in primary non-malignant cells, since they dramatically reduce cellular fitness. However evidence suggests that they have a causative role in tumorigenesis and that they are well tolerated in transformed cells belonging to the myeloid lineage.
Aims: Aim of the study is to elucidate the pathogenic mechanisms that sustain and contribute to aneuploidy in AML.
Methods: We have performed gene expression profile analysis of bone marrow cells from 49 AML patients at diagnosis, including 22 aneuploid cases and 27 cases with normal karyotype. All samples contained more than 80% blast cells. The aneuploidy cohort included AML cases carrying one (or more) monosomy, trisomy or a monosomal karyotype. Our analysis covered more than 245,000 and 40,000 coding and non-coding transcripts, respectively (the latter comprising microRNAs), and a significant number of exon-exon junctions, which allow the analysis of multiple splicing isoforms. Quality controls confirmed that the data show comparable signal values.
Results: The gene expression profile of aneuploid cases has been compared with the one obtained from normal karyotype samples. We have identified a set of coding and non-coding transcripts which are differentially expressed between the two groups ($p \leq 0.05$, including more than 20 genes with a fold difference ≥2) and defined a gene signature that allows the discrimination between aneuploid

and euploid samples in our dataset. Moreover we have identified differences between the two AML subgroups and normal bone marrow samples. The analysis of an increased number of cases will confirm the results and allow the sub-stratification of aneuploid samples according to their gene expression profile. Our data will be further validated by comparing them with published gene expression profile datasets and the gene signature will be characterized by pathway analysis.

Summary and Conclusions: By gene expression profile analysis we have identified a signature of aneuploidy in AML. This study provides novel insights into the molecular mechanism that sustain aneuploidy in AML. The biological validation of genes which are commonly and specifically deregulated in aneuploid AML patients will guide the design of future therapeutic strategies targeting key players in the disease.

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PB1438

MOLECULAR ANALYSIS OF IDH2, DNMT3A, EZH2, WT1 AND CBL MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS WITH CBFB GENE REARRANGEMENT

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Background: The development of acute myeloid leukemia (AML) is a multistep process that requires at least two genetic abnormalities including epigenetic modification for the development of the disease. Although the CBFB gene rearrangement is the most common translocation in AML with favorable prognosis, 40–50% of patients relapse, emphasizing the need for risk-adapted treatment approaches. According to current findings for the identification of these gene mutations (*DNMT3A, TET2, IDH1/2, ASXL1, EZH2*, etc.), most of which are frequently found in cytogenetically normal AML, however, so far little is known about the incidence and distribution patterns of genetic and epigenetic mutation in the *CBFB* AML group.

Aims: The aim of this study is to investigate the frequencies and distributions of epigenetic mutations (*IDH2, DNMT3A,* and *EZH2*) and genetic mutations (*WT1* and *CBL*) in the *CBFB* AML group.

Methods: We searched for mutational hot spots of *IDH2, DNMT3A, EZH2, WT1* and *CBL* in 94 AML patients with the CBFB gene rearrangement using the direct DNA sequencing of exon-coding sequences.

Results: In this study, the incidences of mutational hot spots were observed for *IDH2* R140Q (2.1%), *DNMT3A* (5.4%; R882C 1.1%, M880V 4.2%), *EZH2* (3.2%), *WT1* (10.7%), and *CBL* (1.1%) genes, respectively. Compared to the reported frequencies of *IDH2(3.0-8.7%), DNMT3A (22.1%), ASXL1 (10.8%), WT1* (6.8%), and *CBL* (0.6%) in cytogenetically normal AML, the mutations of *WT1* and *CBL* gene are more frequently noted, however, the frequencies of *IDH2* and *DNMT3A* mutations are relatively lower than other studies.

Summary and Conclusions: It is reported that *WT1* mutations are correlated with poor prognosis in cytogenetically normal AML patients, the prognostic significance of the *WT1* mutated *CBFB* AML should be validated prior to clinical implementation. In addition, the low prevalence of epigenetic regulatory genes (*IDH2, DNMT3A,* and *EZH2)* associated mutations in our study highlights the differences in the pathogenesis of *CBFB* AML *versus* cytogenetically normal AML, at the genetic as well as potentially at the epigenetic level. Relatively, the *CBFB* AML specific characteristics of AML underscore the importance of studying the other genetic and epigenetic molecular biology, as the development of novel therapeutics should account for these biologic differences.

PB1439

CYTOGENETICS AND OUTCOME OF 215 CASES OF MYELOID SARCOMA PATIENTS

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Background: Myeloid sarcoma (MS) is a tumorous aggregate of malignant immature granulocytes, monocytes, or both involving any extramedullary site. MS may occur de novo in the absence of any past history or current bone marrow involvement by acute myeloid leukemia.

Aims: To evaluate the frequency of MS in Syrian cohort, diagnostic methods and the impact of induction chemotherapy on survival.

Methods: 215 patients were diagnosed with (MS) between 2003 and 2013 at Al Bairouni university hospital in Damascus (Syria). Biopsies were taken from the primary presentation sites and diagnosed by a pathologist depending on morphology and immunohistochemistry.All patients underwent a bone marrow aspiration and biopsy to support our diagnosis by means of both Karyotype and flowcytometry.

Results: CD68, MPO, CD 117, CD34CD13, CD33, CD117 were positive in 80-90% of cases. Cytogenetic reveals 6 patients with t(8;21), 5 patients with (Inv 16), 4 with t(9;22), 23 with tri 8, 7 with t(2;3), 54 patients with cocktail of anomalies(-2,-3,-5,-7,-11,-12,+12,+8,+17,+13), however remaining 54 had normal Karyotype. The most frequent localizations are: orbit, skin, breast, groin and CNS. All patients started on induction chemotherapy with a median survival of 18 month.

Summary and Conclusions: MS is a relatively rare phenomenon, making it difficult to study its' impact in different AML subgroups. The literature suggests that patients with isolated MS may have a better prognosis compared with AML patients without MS. MS patients treated with AML-type chemotherapy regimens seem to have comparable outcomes to AML patients.

PB1440

DETECTION OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETRIC FLOW CYTOMETRY IS A USEFUL PROGNOSTIC PARAMETER IN ELN NON-FAVORABLE ACUTE MYELOID LEUKEMIA THROUGHOUT THE TREATMENT PROGRAM

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Background: Multiparametric flow cytometry (MFC) may be used to assess the level of minimal residual disease (MRD) in acute myeloid leukemia (AML).

Aims: To evaluate whether MRD can be an additional prognostic factor that helps to improve the risk assessment of AML patients during treatment.

Methods: Eighty-two consecutive, unselected, non-M3, AML patients (median age: 56, range: 18-75; M/F: 48/34), treated according to the NILG protocol (AML BG00) or the GIMEMA protocol (AML17) were enrolled at the Department of Haematology, Spedali Civili of Brescia between June 2010 and February 2014. According to cytogenetic/molecular characteristics patients were classified into the four risk categories defined by the current European LeukemiaNet recommendations. Eight-color MFC was performed on fresh bone marrow at the time of diagnosis in order to potentially identify the leukemia-associated immunophenotypes (LAIPs), and then 6-color MFC was done in the follow-up samples to detect MRD at two time points, post-induction and post-consolidation. The predefined cut-offs used to identify a MRD were 0.1% post-induction and 0.035% post-consolidation according to data from other studies showing their prognostic value (Buccisano et al, 2010; Terwijn et al, 2013). Relapse-free survival differences between MRD^+ and MRD^- patients was compared by Kaplan-Meyer analysis.

Results: LAIPs were found in 72/82 patients (88%): 28 (39%) had a favorable risk, 19 (26%) an intermediate-I risk, 9 (13%) an intermediate-II risk, and 16 (29%) an adverse risk. Complete remission (CR) was achieved in 59/72 patients (82%), with the first cycle in 46 (78%), and after two cycles in 13 (22%). Twenty-seven out of 72 patients (38%) relapsed after a median of 8.5 months (1-20 months), 45 were relapse-free after a median of 19 months (1-44 months). After 24 months one of the relapse-free patients developed a secondary AML, characterized by different immunophenotype and cytogenetic/molecular features. In the 28 patients belonging to the favorable ELN risk group, MRD detection did not add prognostically useful information since the relapse rate of MRD^+ patients was 2/15 (13%), while that of MRD^- patients was 4/13 (31%), with a similar relapse-free survival (hazard ratio: 0.318 [CI 0.062-1.640]; p=0.19). In the other ELN risk groups, 7/19 (37%) patients with intermediate-I risk, 4/9 (44%) with intermediate-II risk and 10/16 (63%) with adverse risk experienced a relapse. At post-induction MRD analysis, relapse occurred in 4/14 (29%) MRD^- patients *vs.* 17/30 (57%) MRD^+ patients. A higher relapse-free survival was demonstrated in MRD^- patients (hazard ratio: 2.444 [CI 1.010-5.914]; p: 0.047). At post-consolidation MRD analysis a relapse was seen in 3/11 (27%) MRD^- patients *vs.* 15/28 (54%) MRD^+ patients. The relapse-free survival did not reach statistical significance (hazard ratio: 2.235 [CI 0.8402-5.943]; p=0.117). However, when the analysis was performed by including only 26 patients younger than 60 years, as suggested by ELN (Mrózek et al, 2012), 10/19 (53%) MRD^+ patients experienced a relapse, whereas this was observed only in 1/7 (14%) MRD^- patients, with a significantly different relapse-free survival (hazard ratio: 3.721 [CI 1.086-12.75]; p=0.028).

Summary and Conclusions: In spite of the relatively small number of patients tested these results confirm that MRD detected by MFC can add significant prognostic information which may be useful to adapt therapeutic strategies during the treatment program of AML patients.

PB1441

SECONDARY ACUTE MYELOID LEUKEMIA PATIENTS DIFFER IN LEVELS OF INTERLEUKIN-7 AND EPIDERMAL GROWTH FACTOR FROM PRIMARY AML

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Background: Acute myeloid leukemia (AML) shows a high degree of heterogeneity due to a variety of mutations and mechanisms involved in leukemogenesis. Cytokines are soluble molecules that take part in intercellular communication, with a specific role in cell proliferation control. Alterations in adhesion molecule network have been shown to impact prognosis of AML patients. We aimed to provide more evidence about baseline changes in cytokine and adhesion molecule profile related to age and AML origin. Further knowledge gained from multiple cytokine and adhesion molecule analysis should allow better diagnosis and disease management.
Aims: Evaluate differences in cytokine and adhesion molecule profile between primary and secondary AML.
Methods: A total of 47 newly diagnosed AML patients, mean age 52.1±13.3, median 54.9 years, 18 males and 29 females, were studied. These patients were divided according to AML origin (primary *vs.* secondary AML, n=35 *vs.* 12). All cases of secondary AML had history of MDS. We evaluated serum levels of the following 22 cytokines and adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML. Probability values (p)<0.05 were considered statistically significant.
Results: The patients with AML of secondary origin had higher levels of IL-7 (8.68±7.07 ng/L *vs.* 3.66±2.02 ng/L; p=0.021) and EGF (22.92±21.90 ng/L *vs.* 8.46±9.03 ng/L; p=0.016) and higher age (63.3±5.6 *vs.* 48.2±14.0 years, p=0.003). Due to higher age in the secondary AML subgroup, we analysed age-related changes in cytokine and adhesion molecule levels in patients without previous history of MDS. The primary AML patients of age 65 years and higher had significantly decreased serum levels of IL-12 (1.41±1.32 ng/L *vs.* 4.51±3.87 ng/L; p=0.034) and IL-13 (2.14±3.19 ng/L *vs.* 5.55±4.02 ng/L; p=0.034) compared to younger individuals, but IL-7 and EGF levels have not been significantly altered. Serum levels of other evaluated cytokines and adhesion molecules were without significant differences.
Summary and Conclusions: Our results indicate that serum levels of IL-7 and EGF are elevated in AML originating from MDS. Elevated levels of EGF trigger upregulation in MAPK/ERK pathway. This alteration might be associated with disease aggressiveness and might be of future therapeutic importance. The role of IL-7 in the pathogenesis is not clear at the moment. To assess their predictive value for patient outcome, further studies in a larger number of patients are necessary. The work was supported by Specific research project "Analysis of defined prognostic factors in acute myeloid leukemia" (FMHS) and by a long-term organisation development plan 1011 (FMHS).

PB1442

SIGNIFICANCE OF OCT1 EXPRESSION IN ACUTE MYELOID LEUKEMIAS

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Background: Acute myeloid leukemias (AMLs) belong to haematological malignancies derived from the early development stages of myeloid cells. Despite polychemotherapy and intensification of doses of cytotoxic drugs, treatment results are still unsatisfactory. Standard protocols allow to obtain complete remission in significant proportion of patients however only in small percentage of patients long-term relapse free survival is observed. Drug resistance is one of the causes leading to failure of chemotherapy. This is multifactor phenomenon which includes different mechanisms such as disturbance in intracellular transport and one of the protein involved in this phenomenon is OCT1 (*organic cation transporter 1)*. The OCT1 belongs to large family of membrane proteins - SLC (*solute carrier family)* and is involved in nutrients, metabolites and drugs transport. In addition, the OCT1 plays a role in mediating of cytostatics influx into neoplastic cells. Association of OCT1 with resistance to chemotherapy was observed in solid tumors. There are limited data on the expression and impact of this protein on the clinical course and treatment response in AML.
Aims: The aim of this study was to evaluate the expression of OCT1 mRNA in patients with de novo AML. These results were compared to healthy controls, the CD34 expression, classic prognostic factors, molecular and cytogenetic indicators and treatment results such as: obtain complete remission (CR) and overall survival (OS).
Methods: 101 non-M3 AML patients and 43 healthy individuals were included in this study. Bone marrow was assessed. The samples of bone marrow were taken before initiation of chemotherapy. The expression of OCT1 mRNA was analyzed by RQ-PCR. Results were evaluated using the STATISTICA 9.0 software.
Results: This study revealed significantly lower expression of OCT1 mRNA in AML patients compared to the control group (0.002 *vs* 0.014, p=0.0000). The OCT1 mRNA expression was correlated with degree of maturation of blast cells and was lower in the $CD34^+$ leukemias than in $CD34^-$ (0.001 *vs* 0.0025, p=0.006). There was no significant association between the OCT1 mRNA expression and cytogenetic and molecular prognostic indicators. Patients with CR after induction therapy had significantly lower OCT1 mRNA expression than in no response individuals (0.001 *vs* 0.003, p=0.036) and a decreased OCT1 mRNA level was correlated with longer OS (p< 0.05).
Summary and Conclusions: OCT1 mRNA expression is significantly decreased in AML patients compared to control group. OCT1 mRNA level correlates with degree of maturation of blast cells and is the lowest in the $CD34^+$ population. Finally, the clinical course of AML patients with lower OCT1 mRNA expression was more favorable with higher probability of CR and longer OS.

PB1443

MINIMAL RESIDUAL DISEASE IN ADULT AML PATIENTS

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Background: The achievement of complete hematologic remission (CR) is used as predictor for treatment response in patients with myeloid leukemia (AML).However <5% blasts in the bone marrow does not reflect the presence of tumor burden precisely. Minimal residual disease (MRD) in the first complete remission (CR1) may play a critical rule in assessment of treatment response and prediction of subsequent relapse.
Aims: to investigate the efficacy of minimal residual disease as method for aml stratification and predicting outcome and collerating with cytogenetics and flt3 mutation.
Methods: Leukemia associated immunophenotyping (LAIP) for 188 patients with denovo AML monitored at diagnosis , day 14 and day28 post-induction by multiparametric flow cytometry (MFC).
Results: CR achieved in 138 patients and 50 patients did not. Among the 138 patients who achieved CR 75 were MRD negative and 63 were MRD positive at day14. Significant association between MRD detection and disease free survival (DFS) using 0.01% cut off value (P=.015). Day 28 post induction show highly significant association between MRD and DFS using 0.01% cut off value (P=0.001). Significant association between MRD detection and overall survival (50 month) at day 14 and day 28 (P=0.02, P=0.001) respectively using cut off value 0.01%. Minimal residual disease was correlated with cytogenetic. It was found that minimal residual disease outweigh cytogenetic in stratifying AML. Patients with favorable cytogenetic having positive MRD eventually relapsed and thus minimal residual disease should be integrated as standard in risk stratification of favorable cytogenetics AML. Intermediate risk and poor cytogenetics AML also had same results and positive minimal residual disease patients showing poor prognosis. Also it was correlated flt3 mutation showing that flt3 had grave prognosis and even minimal residual disease negative with positive flt3 mutation eventually relapsed. it was also found that MRD negative patients intially and have no markers to follow have much better prognosis than those with positive MRD, thus it can be used as an algorthim for stratifying patient post induction to determine treatment plan and predict outcome.
Summary and Conclusions: minimal residual disease should be used as standard for risk stratification since it out weigh cytogenetic used as standard treatment for adult AML patients. Thus we can stratify patients accordingly and those with positive MRD should recieve intensive treatment and consider allogenic BMT at earlier stage.

PB1444

SERUM LEVELS OF MULTIPLE CYTOKINES AND ADHESION MOLECULES IN PATIENTS WITH NORMAL KARYOTYPE - ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) shows a high degree of heterogeneity due to a variety of mutations and mechanisms involved in leukemogenesis. This heterogeneity is often not fully reflected in standard treatment approaches. Cytokines are soluble molecules that take part in intercellular communication, with a specific role in cell proliferation control. Alterations in this interacting functional network may have direct effect on the malignant cells or indirect effect on leukemogenesis through altered functions of bone marrow stromal elements. Further knowledge gained from multiple cytokine and adhesion molecule analysis should allow better diagnosis and disease management.
Aims: The aim of our study was to evaluate baseline serum levels of multiple cytokines and adhesion molecules and changes related to karyotype in patients treated for AML.
Methods: A total of 51 AML patients were studied. Two subgroups comprising 24 normal karyotype (CN-AML) and 27 aberrant karyotype AML patients were studied. These two subgroups did not differ in age (49.8±12.3 *vs.* 55.3±13.4 years), mean leukocyte count (44.8±37.9 *vs.* 21.0±25.7 x 1000/μL) or mean CRP (34.7±37.2 *vs.* 32.4±30.6 mg/L) of studied subjects. Further, in the group of CN-AML, we compared findings of patients having NPM 1 mutated (n=5) to patients with FLT3-ITD and NPM 1 mutated (n=8). Similarly, there was no difference in age (50.1±14.5 *vs.* 49.8±13.6 years), mean leukocyte count (62.46±35.34 *vs.* 73.66±73.36 x 1000/μL) or CRP levels (46.2±34.6 *vs.* 43.0±33.5 mg/L) between these two CN-AML subgroups. We evaluated serum levels of the following 22 cytokines and adhesion molecules: interleukins (IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-23), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), epidermal growth factor (EGF), monocyte chemotactic protein-1 (MCP-1), E-selectin, L-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox). Probability values (p)<0.05 were considered statistically significant.
Results: Comparing serum cytokine and adhesion molecule levels, we found in CN-AML a significant increase in serum E-selectin (34.49±18.23 mcg/L *vs.* 13.98±9.11 mcg/L; p=0.00018), L-selectin (2962.50±706.20 mcg/L *vs.* 2187.12±1060.01 mcg/L; p=0.0092) and VCAM-1 (855.34±386.02 ng/L *vs.* 600.73±180.79 ng/L; p=0.039). On the other hand the serum levels of IL-7 (3.12±1.98 ng/L *vs.* 5.81±3.66 ng/L; p=0.029) and EGF (7.36±7.06 ng/L *vs.* 21.08±23.98 ng/L; p=0.035) were significantly decreased. The presence of FLT3-ITD in CN-AML with NPM 1 mutation was associated with higher IL-1 alpha (0.66±0.16 ng/L *vs.* 0.20±0.27 ng/L; p=0.035), IL-4 (1.69±0.89 ng/L *vs.* 0.34±0.54 ng/L; p=0.046), E-selectin (54.76±16.32 mcg/L *vs.* 25.06±7.61 mcg/L; p=0.011) and P-selectin (206.21±61.44 mcg/L *vs.* 106.72±29.28 mcg/L; p=0.037) levels. Serum levels of other evaluated cytokines and adhesion molecules were without significant differences.
Summary and Conclusions: Our results indicate that serum levels of some cytokines and adhesion molecules (IL-1 alpha, IL-4, IL-7, EGF, E-, P-, L-selectin) are significantly altered in AML patients and may reflect activity of the disease based on cytogenetic and molecular genetic changes. Whether these alterations could serve as a prognostic marker for AML is not known. To assess their predictive value for patient outcome, further studies in a larger number of patients are necessary. The work was supported by Specific research project "Analysis of defined prognostic factors in acute myeloid leukemia" (FMHS) and by a long-term organisation development plan 1011 (FMHS).

PB1445

REPURPOSING PHENOTHIAZINE CHLORPROMAZINE IN THERAPEUTIC TARGETING OF LEUKEMIC STEM CELLS

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Background: Acute myeloid leukaemia (AML) is an aggressive blood cancer of the myeloid linage involving hematopoietic stem cells. AML is clinically and molecularly a heterogeneous group of diseases predominantly affecting the elderly. Although our understanding of the disease has improved in the past decade, overall disease survival does not exceed 50% of patients. Major challenges include relapse and refractoriness to chemotherapy. Considerable evidence suggests that the initiation and sustenance of AML are due to a rare population of cells, namely leukemic stem cells (LSC). Indications that current chemotherapeutics are ineffective against LSC, may explain the frequency of relapse in AML.
Aims: We explored selective targeting of LSC as a novel therapeutic approach. The dopamine antagonist Chlorpromazine (CPZ) is a well-known antipsychotic, which belongs to the class of phenothiazines. Emerging evidence shows that CPZ possesses anti cancer properties by affecting cellular mechanisms involved in genotoxicity and autophagy. Recently it has been shown that dopamine receptors are expressed on LSC and may be important for their survival. In addition blast cells from subgroups of AML patients have been confirmed to express dopamine receptors. We hypothesise that the dopamine antagonism exerted by CPZ has the potential to target LSC in AML.

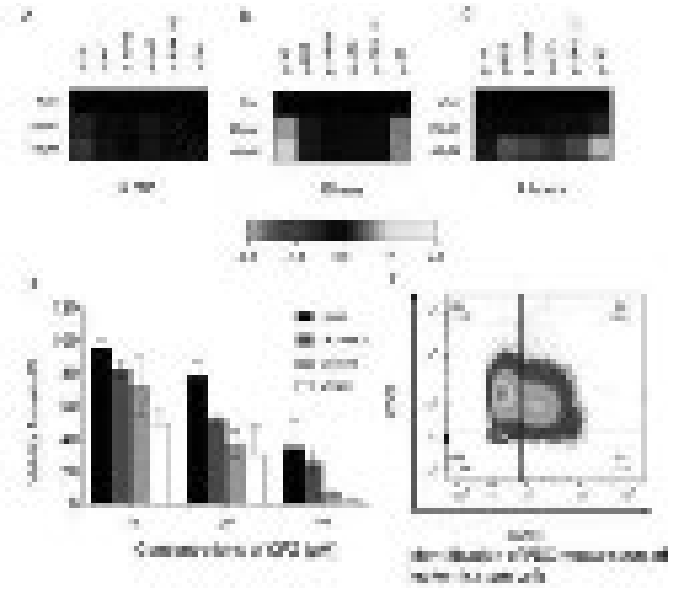

Figure 1.

Results: In order to assess CPZ´s mechanism of action we have used five leukemic cell lines to perform cell death and proliferating assays. Our data confirm the cytotoxic properties of CPZ, as it induces cell death in a dose dependent manner. In the colony-forming assay CPZ treatment resulted in reduced proliferation. Expression of dopamine receptors in the same cell lines was determined. Early CPZ-induced changes in intracellular signalling pathways were examined by single cell signal transduction profiling, employing phosphoprotein specific flow cytometry to investigate. CPZ´s ability to limit the renewability of LSC was screened in a panel of cells isolated from AML patient derived xenografts (PDX).
Summary and Conclusions: Repurposing of approved medicines has the potential to greatly accelerate the clinical application of novel therapies. The dopamine antagonist CPZ is a widely available and established drug that has been in clinical use for decades. If it is possible to utilise CPZ to target leukemic stem cells, CPZ has the potential to be an important therapeutic agent in haematological malignancies.

PB1446

BATIMASTAT, A MATRIX METALLOPROTEINASE INHIBITOR, AS A THERAPEUTIC APROACH IN HEMATOLOGIC NEOPLASIAS

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Background: The bone marrow microenvironment is the main support of normal hematopoiesis, although it is also important in maintenance and development of neoplastic clones, namely in hematological neoplasias. Matrix metalloproteinases (MMPs) are important players in bone marrow microenvironment, since they can degrade the protein components of the extracellular matrix. On account of that, several studies show that MMPs are also involved in cancer development and progression and could be a new therapeutic target. The majority of actual therapeutics for hematological neoplasias fails in some patients leading to relapses. Therefore the use of MMPs inhibitors (MMPIs) may become a new therapeutic approach for these patients.
Aims: The aim of this study was to evaluate the therapeutic potential of Batimastat (BB-94), a matrix metalloproteinases inhibitor, in *in vitro* models of hematological neoplasias.
Methods: For this purpose, we used four hematological neoplasias cell lines, two Acute Promyelocytic Leukemia (APL) cell lines, the NB-4 and the HL-60 cells, the first one with the translocation t(15;17) and the second without this translocation, a Multiple Myeloma cell line, the H929 cells, and a

Myelodysplastic Syndrome cell line, the F-36P cells. All cell lines were cultured in absence and presence of different concentrations of BB-94 ranged from 0,1 μM to 10 μM, in daily or single dose administration. To evaluate the effect of this inhibitor on cell viability and cell density we used the Trypan Blue Assay. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. It was also evaluated the activation of caspases, using the Apostat probe, and cell cycle by FC. In order to understand the role of BB-94 in cell proliferation, we evaluated the expression levels of proteins involved in the MAPK signaling pathway, as ERK 1/2, by Western Blotting.

Results: Our results showed that BB-94 reduces cell viability and proliferation in a time, dose and cell line dependent manner. We found that the half maximal inhibitory concentration (IC50) at 48 hours of exposure was, approximately, 7,5 μM for NB-4 and F36-P, 10 μM for H929, and between 7,5 and 10 μM for HL-60 cells. Besides that, drug daily administration schedule seems to be more effective in the reduction of cell viability and proliferation and with lower doses hen compared to the same doses in single administration, except in H929 cell line. Furthermore, BB-94 induced cell death by apoptosis with activation of caspases, in a dose-dependent manner. The cytotoxic effect of BB-94 seems to be more active in the APL cell line with t(15;17) translocation. The analysis of cell cycle progression also revealed an arrest in G_0/G_1 and S phases, in HL-60 and NB-4 cell lines, respectively. Besides cell cycle arrest, the apoptotic effect induced by BB-94 could be mediated by ERK (extracellular signal-regulated kinase) protein since we observed an increase of phosphorylated ERK expression in the cells treated with BB-94 when compared to untreated cells in every cell lines, except in H929 cells.

Summary and Conclusions: In conclusion, our results suggest that BB-94 could be a new potential therapeutic approach in hematological neoplasias and also support a role for the ERK signalling pathway in BB-94-induced apoptosis. However, therapeutic efficacy may depend on the cell type and genetic characteristics of the neoplasia, as well as the therapeutic schedule used.

This work is supported by Center of Investigation in Environment Genetics and Oncobiology (CIMAGO).

PB1447

ABSTRACT SUBMISSION 3. ACUTE MYELOID LEUKEMIA - BIOLOGY ABSSUB-4562 BERBERINE ACTS AS A PUTATIVE EPIGENETIC MODULATOR BY AFFECTING HISTONE CODE ZHIXIANG WANG1, LIU YUAN2, XUEJIE JIANG1, FANYI MENG* 1

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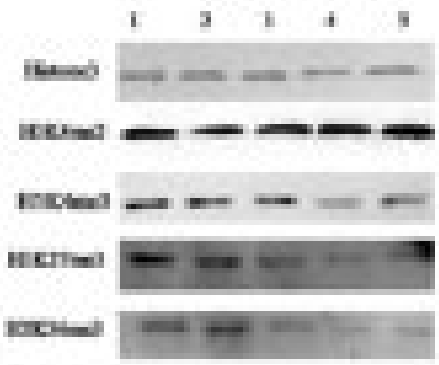
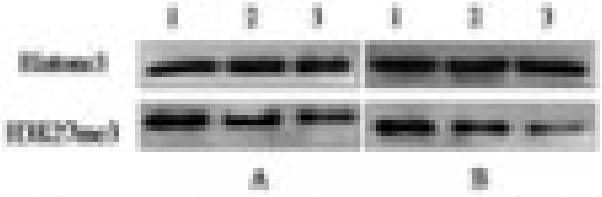
Background: Berberine, an isoquinoline plant alkaloid, has a wide range of biochemical and pharmacological effects. However, the exact mechanism of these bio-activities remains poorly understood.

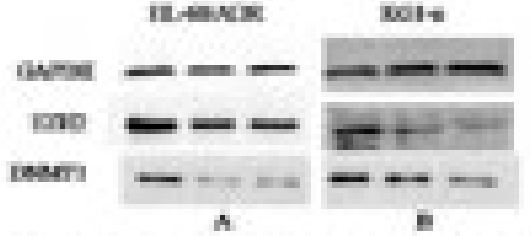
Aims: We conducted this study to explore how these molecular events are regulated by berberine.

Methods: In this study, when analyzing gene expression profile of U266 cells treated by berberine against profiles from external sources, along with PCR array,Real-time PCR (U266,HL-60/ADR and KG1-α) and Western Blot (U266,HL-60/ADR and KG1-α) .

Results: We demonstrated significant similarity between berberine and two epigenetic modulators (CG-1521 and TSA). As compared with gene expression in U266 cells without treatment, 2599 genes were up-regulated and 2155 genes were down-regulated for at least two-fold by berberine in U266 cells. Reverse-docking using berberine as a ligand for possible protein targets identified lysine-N-methyltransferase, among others, as a putative target of berberine. This suggested a possible role of berberine in epigenetic modulation. The results of epigenetic chromatin modification enzymes PCR array experiments support our hypothesis. We quantified the expression of these genes with at least 1.5-fold change in U266 cells with and without berberine. Several key observations were made: (1) Five histone acetylases represented by EP300 and CREBBP were up-regulated whereas at least two histone deacetylases HDAC2 and HDAC8 were seen reduced; (2) Lysine (K)-specific demethylase family was fairly active, among which KDM1 targets H3K9me2/1 and H3K4me2/1, KDM4 demethylates both H3K9me3/2 and H3K36me3/2, HDM5 is specific for H3K4me3/2 and KDM6 targets H3K27me3/2; (3) SET domain-containing histone methyltransferase family was also fairly active. SET1 and SET7 methylate H3K4 whereas SETB methylates H3K9. Both SET2 and SET3 methylate H3K36. SET6 mono-methylates K310 of the rela subunit NF-kB complex. SETD8 mono-methylates H3K20 and SMYD3 di- and tri-methylates H3K4. There is a discrepancy for SMYD3 expression between microarray and PCR array data. After checking by RT-qPCR, we confirmed that SMYD3 was down-, NOT up-, regulated by berberine; (4) DNA methyltransferases, which are responsible for *de novo* methylation (DNMT3) or maintenance of methylated CpG (DNMT1), were down-regulated.







Furthermore, the analysis showed that enzymes involved in histone acetylation and methylation have been impacted predominantly. Up-regulation of histone acetyltransferase CREBBP and EP300, histone deacetylase SIRT3, histone demethylase KDM6A as well as histone methyltransferase SETD7, and down-regulation of histone acetyltransferase HDAC8, histone methyltransferase WHSC1I, WHSC1II and SMYD3. In parallel, the results of western blot revealed that H3K4me3, H3K27me3 and H3K36me3 proteins decreased after berberine treatment. The results were confirmed in acute myelocytic leukemia (AML) cell lines HL-60/ADR and KG1-α. In addition to this, the results showed that berberine also inhibited the expression of EZH2 and DNMT1 in HL-60/ADR and KG1-α cells, both in protein and mRNA.

Summary and Conclusions: The results of this study suggest that berberine might modulate expression of epigenetic regulators importent for many downstream pathways, thereby manifests its various bio-activities.

PB1448

NEONATAL BLOOD SPOT ANALYSIS DEMONSTRATES EARLY EXISTENCE OF A CRYPTIC CBFB-MYH11 GENE FUSION IN A FOUR YEAR OLD AML PATIENT

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Background: Acute myeloid leukemia (AML) with the inv(16) karyotype is commonly referred to as a member of the core binding factor AMLs, and is associated with a favourable prognosis, showing longer periods of complete remission and higher overall survival rates. The inv(16) results in a fusion between *CBFB* (core binding factor beta subunit*)* at 16q22 and *MYH11* (myosin heavy chain 11) at 16p13, leading to a chimeric CBFB/MYH11 protein. Additional studies soon confirmed the expression of *CBFB/MYH11* transcripts in inv(16) and in t(16;16) positive AML-M4Eo, as well as in M4 without eosinophilia.
Aims: To study whether the complex abnormal karyotype with a *CBFB/MYH11* fusion, that was found in a four year old girl, was already present neonatally.
Methods: Morphology, immunophenotyping, and cytogenetic and molecular methods were performed at diagnosis. Blood spots from Gurthrie cards were analysed to track molecular findings at birth.
Results: A four year old girl presented with pallor, a history of viral infections and pancytopenia. Bone marrow aspirate showed 2% blasts and megakaryocytic dysplasia, not indicative of acute leukaemia; serology was negative. On the basis of these results differential diagnosis was a reactive, infectious process. The patient was seen on the outpatient clinic on a regular basis. After a month the full blood count recovered without signs of cytopenia. Surprisingly cytogenetic studies on the bone marrow showed a complex abnormal karyotype of unknown significance: 46,XX,der(15)t(15;16)(q2?2;q?),?iso(16)(p10), add(16)(p?)[6]/46,XX[16]. The next cytological examination of peripheral blood smear showed, however, 12% Sudan Black positive blasts. Immunophenotyping showed 9% myeloblasts (CD34, CD117, CD13, MPO and HLA-DR positive, CD33 negative and TdT weakly positive). Morphology of the bone marrow aspirate showed 21% of blasts and dysplasia. Molecular diagnostics revealed a S/I transcript for *CBFB/MYH11*, leading to a diagnosis of CBF AML. Additional FISH confirmed the presence of a *CBFB/MYH11* fusion on one derivative chromosome 16 and the karyotype was subsequently adjusted to 46,XX,der(15)t(15;16), der(16)inv(16),der(16)t(15;16)[9]/46,XX[1]. The patient was subsequently treated according to the paediatric AML protocol. After the first course of chemotherapy complete remission was achieved with a normal karyotype 46,XX[20]. Nine months after treatment patient is still in complete remission. Since at presentation this girl showed an abnormal karyotype with a *CBFB-MYH11*-fusion but no signs of a leukaemia, we were interested whether the fusion was already present at birth. Therefore, the DNA fusion junction was cloned from diagnostic DNA and the patient-specific sequence used to investigate the neonatal blood spot. Remarkably, the identical *CBFB/MYH11* fusion product was detected in the blood spot.
Summary and Conclusions: In general practice, the detection of a *CBFB/MYH11* fusion as a result of an inv(16) or t(16;16) is indicative of CBF AML, regardless the percentage of blasts in blood or bone marrow. Here we report a patient with a neonatal *CBFB/MYH11* fusion with the leukaemia presenting four years later. This indicates that a pre-leukaemic genetic abnormality may be present for a long period and might support the hit-second hit hypothesis. The genetic abnormality is not constitutional since it disappeared after chemotherapeutic treatment and this finding allows us to perform RQ-PCR for minimal residual disease (MRD) monitoring of *CBFB/MYH11* for this patient.

Acute myeloid leukemia - Clinical

PB1449

EFFICACY OF PSYCHOLOGICAL NURSING ON ACUTE MYELOBLASTIC LEUKEMIA

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Background: Acute myeloblastic leukemia is a common and severe clinical disease.It can cause anxiety.Psychological nursing is needed on the baisis of traditional nursing.Psychological nursing supplies patients with psychological intervention to alleviate anxiety.Patients can have a good therapeutic alliance.
Aims: To analyze the efficacy of psychological nursing on acute myeloblastic leukemia.
Methods: 226 acute myeloblastic leukemia patients were divided randomly into two groups:psychological nursing group(A group) 134 cases and control group(B group) 92 cases.A group accepted routine nursing combinating with psychological nursing.B group accepted only routine nursing.Evaluate and analyze HAMA scores of each group respectively between prior treatment and aftrer 10 days of treatment and compare HAMS scores of two groups prior treatment and aftrer 10 days of treatment.
Results: Prior treatment,HAMA score of A group 20.89±5.82 points and HAMA score of B group 20.46±6.32 points,had no significant differences.After 10 days treatment,HAMA score of A group 14.26±3.45 points and HAMA score of B group 17.39±5.33 points,had signicant differences.A group and B group both had significant differences between prior treatment and posttreatment.
Summary and Conclusions: Psychological nursing can ameliorate anxiety of acute myeloblastic leukemia patients and should be advocated.

PB1450

THE ASSOCIATION OF REDUCTION OF WT1 TRANSCRIPTS IN BONE MARROW AND OUTCOMES IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Evaluation of minimal residual disease (MRD) during chemotherapy is important for the decision on post-remission therapy for acute leukemia, including stem cell transplantation (SCT). Detection of leukemia-specific chimeric genes by real-time quantitative polymerase chain reaction is most sensitive method to quantify MRD among other methods such as karyotype analysis, fluorescence *in situ* hybridization and flow cytometry. However, because over 50% of acute myeloid leukemia (AML) patients lack suitable leukemia-specific chimeric genes for detection of MRD, other MRD makers are necessary as newly prognostic factors for greater proportion of AML patients. WT1 mRNA is overexpressed in most AML blasts. Therefore, WT1 mRNA transcript level in peripheral blood has been reported as a suitable molecular marker of MRD for most AML patients. In contrast, in acute lymphoblastic leukemia patients, it remains to be elucidated whether early reduction of tumor burden make good prognosis in AML patients.
Aims: We focused on the clinical relevance of WT1 mRNA transcript level in bone marrow (BM) as MRD during chemotherapy, and also the association between WT1 mRNA transcript level and outcome in adult AML patients.
Methods: Forty-eight AML patients who received chemotherapy at hospitals in our study group were enrolled in this study. Written informed consent was obtained from each patient before starting induction chemathrapy. We analyzed the transcript levels of WT1 mRNA in BM at diagnosis, after induction therapy and after final consolidation therapy. WT1 mRNA transcript levels were determined using the WT1 mRNA Assay Kit (Otsuka, Japan). The diagnosis and classification of AML were based on criteria according to the WHO classification. Cytogenetic risk group stratification was performed using MRC AML10. All statistical analyses were performed with EZR software (Saitama Medical Center, Jichi Medical University). This protocol was reviewed and approved by an Institutional Review Board at each hospital in our study group.
Results: Thirty-two of 48 patients achieved in complete remission (CR) after induction therapy. Twenty-four of 32 patients who achieved in CR after induction therapy completed consolidation therapies and could be quantified the transcript levels of WT1 mRNA in BM at after final consolidation therapy. Twenty patients were received allogeneic hematopoietic SCT. The median WT1 mRNA expression level at diagnosis was 16,000copies/μgRNA (range 250-400,000). The transcript levels of WT1 mRNA in BM at diagnosis was not associated with overall survival (OS) (0.099) and disease free survival (DFS) (p=0.22). The achievement of 2 log reduction of WT1 mRNA transcript level after induction

therapy in BM was associated with OS (P=0.035), but was not associated with DFS (P=0.074). The achievement of 2 log reduction of WT1 mRNA transcript level after final consolidation therapy in BM was associated with OS (P=0.006) and DFS (P=0.018). Next, we analyzed the impact of log reduction levels of WT1 mRNA transcript level for survival by multivariate analysis. We used Wd/i_log and Wd/c_log to assess the value of reduction of tumor burden. Wd/i_log was difined as a log scale of WT1 mRNA transcript level at diagnosis divided by that after induction therapy. Wd/c_log was difined as a log scale of WT1 mRNA transcript level at diagnosis divided by that after consolidation therapy. Factors were adjustment for SCT and cytogenetic risk. Wd/i_log was associated with DFS and OS. Hazard ratio for DFS and OS were 1.955 (P=0.013) and 2.911 (P=0.021), respectively, by every shallow remission level in log scale. Wd/c_log was associated with DFS, but not OS. Hazard ratio for DFS was 2.0250 (P=0.005) by every shallow remission level in log scale.
Summary and Conclusions: Our results suggest that early and deep reductions may be important for good prognosis in AML patients. In addition, Wd/i_log and Wd/c_log in WT1 mRNA transcript level in BM are useful as predictive indexes for outcome of AML patients.

PB1451

WT-1 FROM PERIPHERAL BLOOD PREDICTS RELAPSE IN AML PATIENTS UNDERGOING STEM CELL TRANSPLANTATION

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Background: Minimal residual disease (MRD) monitoring in acute myeloid leukemia (AML) after allogeneic stem cell transplantation (allo-SCT) is crucial to identify patients at high risk of relapse, who can be cured with early immunosuppressive therapy discontinuation and/or donor lymphocyte infusions. More than 60% of AML lack appropriate markers for MRD. The Wilm's tumor 1 gene (WT1) is over-expressed in more than 90% of cases at diagnosis and it has been proposed as a marker for MRD monitoring.
Aims: This is a retrospective single-center analysis of WT1 expression on peripheral blood (PB) of AML patients at different time-points during treatment and follow up, with the aim to assess a threshold of WT1 copies above which disease recurrence is likely.
Methods: Twenty-three AML patients with overexpression of WT1 at diagnosis, submitted to allo-SCT in our Institution from June 2009 to September 2013 were monitored for WT1 levels by RT-PCR from PB before allo-SCT, at 3rd and 6th month after allo-SCT.

Table 1. Patients' outcome according to peripheral blood WT1 levels (<or>/=5 WT1/ABLx104) evaluated at three deifferent time-points.

Results: The median age of our patients was 53 years (range 24 – 62), the median WBC count at diagnosis was 34.2 x 10^9/L (range 0.7 – 103). Forty-three percent of cases showed a normal karyotype at diagnosis, 35% a complex karyotype and 22% a mixture of the other commonly observed abnormalities. A marker of molecular biology other than WT1 was available in 13/23 cases (26%) and was Flt3-ITD in 6 cases (26%) and NPM mutation type A in 7 cases (30%). The disease status at transplant was morphological first complete remission (CR) in 14 cases (61%). Thirty-five percent of the patients (8 cases) received a transplant from a HLA compatible sibling donor, 56% from a matched unrelated donor (13 cases) and 9% from a matched single cord blood unit (2 cases). Twelve out of 23 cases (52%) received a myeloablative conditioning regimen. The median follow up is 12 months (range 3 – 38). The result of WT1 quantification was available in 22/23 cases (96%), 20/23 (87%) and 16/23 (70%) before allo-SCT, at 3rd month and at 6th month, respectively. As reported in Table 1, considering WT1/ABL x $10^4 \geq 5$ *vs* WT1/ABL x 10^4<5 before transplant, 8/10 patients (80%) and 3/12 patients (25%) relapsed, respectively (p=0.01). Similarly, considering the 3rd month, there was a trend towards a higher relapse rate for patients with a WT1/ABL x $10^4 \geq 5$ *vs* those with WT1/ABL x 10^4<5 [5/8 (62%) *vs* 4/12 (33%); p=0.19]. Finally, 5/6 patients (83%) and 2/10 patients (22%) with WT1/ABL x $10^4 \geq 5$ and WT1/ABL x 10^4<5 at 6th month respectively relapsed and this difference was statistically significant (p=0.01). The 3 years OS was 0% for patients with WT1/ABL x $10^4 \geq 5$ *vs* 54% (95% CI 7.9 – 99.8) for patients with WT1/ABL x 10^4<5 before transplant (p=0.03). When considering the WT1 levels at 3rd month, the 3 years OS was 0% for patients with WT1/ABL x $10^4 \geq 5$ *vs* 52% (95% CI 6.5 – 97.4) for patients with WT1/ABL x 10^4<5 (p=0.002). Similar results were observed for event free survival (EFS).
Summary and Conclusions: Our preliminary data, although derived from a small number of patients, strongly suggest that WT1 monitoring from PB may be a useful tool for identification of patients at high risk of relapse after allo-SCT, with a threshold represented by 5 copies of WT1/ABL x 10^4.

PB1452

ROLE OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTORS AS AN EARLY DETECTOR FOR TREATMENT OUTCOME IN AML

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Background: the urokinase-type plasminogen activator system which consist of a proteinase (the urokinase-type plasminogen activator,uPA), a receptor(the urokinase-type plasminogen activator receptor uPAR or CD87) and inhibitors (PAI-1) are enriched in several types of tumors and involved in proteolysis, cell migration and invasion. high expression of uPA and uPAR are associated with an increased relapse rate and shorter survival in breast cancer and colorectal carcinomas.
Aims: this study shed light on the expression of uPAR in adult acute myeloid leukemia(AML) and its prognostic relevance
Methods: peripheral blood and bone marrow samples are obtained from 54 newly diagnosed AML adult patients ,20 healthy controls stained with anti CD87 and estimated on flow cytometry.
Results: CD87 expression was heterogenous in different FAB subtypes of AML with high expression in monocytic leukemia(M4/M5)(Pvalue0.001). high expression of CD87 was asssociated with shorter survival and poor response to therapy(P=0.028,0.002 respectively). the most discriminating cut off value of CD87expression was 15%, patients with less than 15% expression had 4.6 times better treatment outcome than those with more than or equal to 15%. on the multivariate analysis,CD87 was the most significant single variable that affect treatment outcome compared to total leucocytic count,hemoglobin level,platelets count,cytogentic and FLTexpression.
Summary and Conclusions: high CD87 expression is an independent prognostic parameter associated with poor response and shorter overall survival in adult AML patients.

PB1453

ADRENOMEDULLIN, A HYPOXIA-RELATED PROTEIN, IS ASSOCIATED WITH PROGNOSIS OF ACUTE MYELOID LEUKEMIA

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Background: Previous studies showed that hypoxia condition can promote cancer cells invasion and metastasis in solid tumors. It is known that the bone marrow microenvironment is hypoxia. However, the biologic effect of hypoxia on leukemia has not been clearly documented.
Aims: The purpose of the study was to investigate the roles of hypoxia in acute myeloid leukemia (AML).
Methods: Agilent SurePrint G3 Human GE 8X60K arrays was used to compare the gene-expression profile of the OCI/AML3 cultured under normoxic (21% O_2) and hypoxic (1% O_2) conditions for 8h. The information in Oncomine (version 4.4; http:www.oncomine.org) and PrognoScan (date of analysis: 2011-12-05; http://gibk21.bio.kyutech.ac.jp/PrognoScan/index.html) were recruited for analyses. The former was used to assess differential expression results for a gene of interest across collected data sets, and the latter enables systematic meta-analysis of the prognostic value of a gene in multiple datasets. Human leukemia cell lines U937, OCI/AML3, K562, HL-60 and primary leukemia cells from AML patient were assessed for mRNA expression under normoxic and hypoxic conditions. The study was approved by the institutional review board of National Taiwan University Hospital and written informed consents were obtained from all participants in accordance with the Declaration of Helsinki.
Results: We compared the gene expression stpectrum of OCI/AML3 leukemia cells incubated in hypoxia and in normoxia with Agilent SurePrint G3 Human GE

8X60K microarray coupled with rank consistent lowess. Adrenomedullin was the most significantly up-regulated. Further Q-RT-PCR confirmed this up-regulation of adrenomedullin with 8-200 folds increase in various leukemia cell lines and leukemia cells from patients with AML at diagnosis under hypoxia condition. ONCOMINE, a cancer microarray database and web-based data-mining platform aimed at facilitating discovery from genome-wide expression analyses. According to data from ONCOMINE, there was no significance difference of adrenomedullin expression between normal controls and patients with AML, suggesting adrenomedullin might not be a diagnostic biomarker. The PrognoScan based on the published cancer microarray datasets with clinical annotation provides a powerful platform for assessing the biological relationship between gene expression and prognosis. According to data from PrognoScan, there was the trend that survivals were longer with low adrenomedullin mRNA expression in AML patients than those with high expression (Cox *p*-value = 0.06).
Summary and Conclusions: We have identified a hypoxia-related protein, adrenomedullin, in leukemia cells lines and human leukemia samples. Expression of adrenomedullin is associated with a worse prognosis. The nature and the biologic action of adrenomedullin remain to be elucidated.

PB1454

CLINICAL IMPACT OF SNPS OF P53 GENE PATHWAY ON THE ADULT AML PATIENTS

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Background: Acute myeloid leukemia (AML) is a highly heterogeneous disease, with biologically and prognostically different subtypes. As one of the primary "gatekeepers" of the cell, the p53 tumor suppressor plays a major role in sensing and responding to a variety of stressors to maintain cellular homeostasis. Single nucleotide polymorphisms (SNP) in codon Arg72Pro of P53 results in impairment of the tumor suppressor activity of the gene. A similar effect is caused by a SNP in codon 31 of P21. In contrast, a SNP in position 309 of MDM2 results in increased expression due to substitution of thymine by guanine. All three polymorphisms have been associated with increased risk of tumorigenesis and alter apoptosis.
Aims: To study the impact of p53, p21 and mdm2 genes polymorphisms on clinical outcome of adult AML patients treated at National Cancer Institute (NCI) -Cairo University.
Methods: Molecular genetic analysis involving P53, MDM2 and P21 single nucleotide gene polymorphisms was done using PCR-RFLP coupled analysis for 48 patient. the work was performed accodring to Helsinki declaration, the protocol was approved by the IRB ofNCI, Cairo University and an informed consent was obtained from all subjects. Most of patients have received remission induction chemotherapy with daunorubicin (45mg/m2/day for 3 days) and standard dose cytosine arabinoside (200mg/m2/day for 7days) with or without recombinant human granulocyte colony-stimulating factor (rhGCSF).
Results: Patients with homozygous Arg/arg at codon 72 of P53 had a better median OS of 13.4 months than Arg/Pro (8.4 months) and Pro/Pro (1.5 month) [p=0.045]. patients with both P53 and P21 homozygous polymorphisms are associated with poorer OS (5 months) and P. value 0.037, however the OS for patients with both wild or either variant was 16 and 19.3 months respectively. the presence of P21 wild or MdM2 wild genotype result in abolish the poor effect of P53 Pro/Pro on the Overall survival. patients with both P53 and P21 homozygous genotype are associated with poor DFS (DFS =3.8 & P. value 0.004) however DFS was 14.5 for patients with both P53 and P21 wild genotype. Neither p21or mdm2 polymorphism alone showed an impact on OS and DFS. Complete remission was not affected by any of the three genes polymorphisms.
Summary and Conclusions: p53 pathway gene polymorphisms may affect the overall survival and disease free survival of adult AML patients specially the presence of P53 and P21 combined homozygous variant genotype was associated with a poor clinical outcome.

PB1455

INTENSIVE CONSOLIDATION AFTER STANDARD INDUCTION THERAPY CAN BENEFIT SELECTED ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AND AGE ≥ 75 YEARS: A RETROSPECTIVE ANALYSIS OF THE RETE EMATOLOGICA LOMBARDA

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Background: outcome of AML patients (pts) older than 70 years is poor because of unfavourable disease characteristics and frequent comorbidities. Indeed most pts are excluded from curative therapeutic programs. When standard induction is given to pts ≥ 75 years the complete remission (CR) rate is around 50% but the best consolidation/maintenance strategy after CR is still controversial. Recent guidelines define age > 75 as the limit to consider patients unfit for aggressive therapy. However, nowadays, individualized management based on comorbidity scores and not only on age is encouraged. We collected data from haematologic centers of the regional network Rete Ematologica Lombarda (REL) to get insight into the outcome of fit AML elderly pts when treated with standard anthracyclin-containing induction and intensive consolidation programs in clinical practice.
Aims: retrospective evaluation of data from AML pts ≥ 75 yrs of age who received an intensive treatment program at different centers of the REL network
Methods: period 3/2005 to 5/2013, 33 pts, median age 76 (75-80). Summary of common characteristics of pts in the different centers: PS (ECOG) ≤ 2, renal and hepatic parameters<2 N, no active infections and cardiac ejection fraction > 50%. Diagnosis (WHO): 9 AML with MDS related changes (MDS-AML), 2 AML-M0, 6 AML-M1, 3 AML-M2, 2 AML-M4, 2 AML-M5, 1 AML with inv(16), 4 AML with mutated NPM1, 2 t-AML, 1 biphenotypic. Cytogenetic risk: 22 intermediate (16 normal karyotype), 3 poor, 1 good, 7 not evaluable. WBC at diagnosis: median 37x10e9/l (1,1-250,0).
Results: overall, 42 induction cycles were administered, 30 at "full dose" to 23 pts (3+7, MICE, ICE, FLAGIDA) and 12 at "reduced dose" to 10 pts (1+5). CR rate was 55%, 74% (17 pts) with "full dose" and 10% (1 pt) with "reduced dose" induction. One patient died after a "full dose" cycle. Intensive consolidation (16 pts): 29 cycles with intermediate or high dose cytarabine; 10 pts (62%) received two or more cycles, 3 pts an autologous transplant with myeloablative conditioning. Two pts (12,5%) died during consolidation. After consolidation, 2 pts received maintenance with 5-azacytidine. Twelve pts (675%) relapsed. Median OS from diagnosis was 325 days (5-2023), 345 days (22-2023) for the "full dose" induction pts and 137 days (5-482) for the "reduced dose" induction pts (p=ns). The 18 pts who obtained a CR had a median OS of > 2 years 788 days (range 69-2023) with 20% projected to be alive after 3 yrs. Median DFS was 245 days (27-1871). At last follow up, 5 pts are alive, 4 in CR, all after a "full dose" induction. One of the 2 pts on maintenance with 5-azacytidine is alive in CR 1114 days from diagnosis.
Summary and Conclusions: our analysis suggests that intensive consolidation approaches are feasible in selected older AML pts aged ≥ 75 and may improve survival. Also, standard *vs* reduced-dose induction treatments did not prove more toxic and were more effective. Notably, TRM was very low across the different centers. Strategies to prevent relapse, including new agents with low toxicity profile, should be explored. The criteria to be used in the selection of elderly AML pts for intensive treatment programs need to be fully defined. Age should not be "per se" an exclusion criterion. Guidelines for comprehensive geriatric assessment and the recently published consensus-based definitions of unfitness to intensive and non-intensive therapy in AML could help physicians to select and possibly address more pts with advanced age to potentially curative treatments in prospective clinical trials.

PB1456

MOLECULAR MARKERS IN DE NOVO ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) is a clinically, morphologically and genetically heterogeneous disease, involving one or all myeloid lineages. The heterogeneity of AML is reflected by differences in molecular abnormalities that have been recently discovered as potential prognostic factors in acute myeloid leukemia (AML). A genetic-based classification is essential for accurate diagnosis, prognostic stratification, monitoring of minimal residual disease and developing targeted therapies. Consequently, it is important that any screening panel is large enough to detect relatively rare mutations that might associate with a particular AML class or cytogenetic risk group.
Aims: To determine the frequency of different Gene Mutations in AML among Saudi Nations.
Methods: A variety of AML-specific mutations were screened, *i.e. FLT3, NPM1, IDH1, IDH2, CEPBA, DNMT3A, RUNX- , KIT , WT1, IDH1, IDH2, and PML-RARA* in bone marrow samples of 66 Adult and Pediatric new AML Patients using Sequencing technology during the period of January 2012 to December 2013 at The Molecular Genetics Laboratory, King Faisal Specialist Hospital and Research Centre, Riyadh (General organization), Saudi Arabia.
Results: A total of 66 patients (28 females and 38 males) were investigated for different gene mutations (56 cases at the time of diagnosis, 4 patient in relapse, 5 samples for patients prior to stem cell transplantation and one case post bone marrow transplantation in relapse). The positive cases represented 51% (34 cases of AML/ 28 adults and 6 pediatric patients).
Summary and Conclusions: The frequency of different gene mutation in AML

was similar to previously published studies from different international centers (*e.g.* FLT-3-ITD and NPM1 are 20% and 23% respectively), however, this is the first data presented from Saudi Arabia.

PB1457

Abstract withdrawn

PB1458

PROGNOSTIC FACTORS OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: The incidence of acute leukemia (AML) is increasing substantially for individuals 65 years of age or older. However, no optimal treatment for elderly patients with AML has been established.
Aims: The aim of this study was to assess the outcomes of elderly patients with AML and prognostic factors.
Methods: We retrospectively analyzed 102 AML patients more than 65 years who were newly diagnosed at Tsukuba University Hospital and Hitachi General Hospital from 2008 to 2014. The median age was 73 (65-88). Diagnosis included AML, NOS (n=48), AML with MRC (n=46), therapy related AML (n=2), AML with t(8;21) (n=5), AML with inv(16) (n=1). Karyotypes according to IPSS were good (n=6), intermediate (n=59), and poor (n=37).

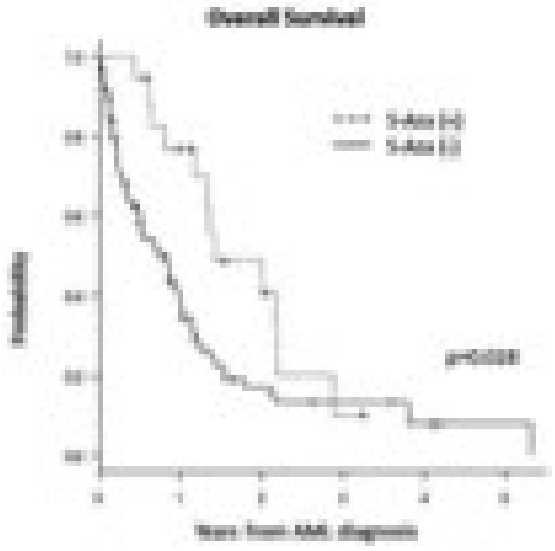

Figure 1.

Results: Intensive remission induction therapy was performed in 38 (37.2%), a less intense chemotherapy such as low-dose cytarabine was performed in 39 (38.2%) and 5-azacitidine (5-Aza) as remission induction was performed in 6 (5.8%). Complete remission (CR) was achieved in 32.3% of all treated patients and the estimated 2-year survival was 23.3%. The median duration of overall survival (OS) was 11 monthes. In univariate analyses, to perform induction therapy, CR and the usage of 5-Aza were found to be associated with good prognosis, on the other hand, poor karyotype significantly shorten the survival. The age, gender, prior to MDS were not significantly related to outcomes. In a multivariate analysis, CR (hazared ratio (HR), 0.091, 95% confidence interval (CI) 0.04-0.18, P<0.01), the usage of 5-Aza (HR 0.20, 95% CI 0.09-0.41, p<0.11) and poor karyotype (HR 1.73, 95% CI 1.06-2.82, P=0.01) were also significant.
Summary and Conclusions: The retrospective cohort analysis suggests that 5-Aza should be considered as a treatment option in elderly patients with AML.

PB1459

CEBPA MUTATIONS IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA: DATA ANALYSIS IN A CHINESE POPULATION

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Background: Although *CEBPA* mutations have been studied for many years in acute myeloid leukemia (AML), there is limited data about its prevalence and prognostic significance in AML patients from Chinese population.
Aims: To explore the clinical characteristics and prognoses of AML patients with *CEBPA* mutations.
Methods: Ninety patients with *de novo* AML were retrospectively analyzed in this study.
Results: *CEBPA* mutations were detected in 23 patients (25.6%), with 19 cases were double mutations, four were single mutation. In those with normal karyotype (NK), 20 cases (34.5%) were detected with *CEBPA* mutations. The following characteristics were observed in *CEBPA*-mutated patients: most (73.9%) of the *CEBPA*-mutated patients were M_1 and M_2; presented with high peripheral white blood cells [34.5 (12.6, 100.2) $\times 10^9$/L *versus* 9.16 (2.87, 48.2) $\times 10^9$/L; u = -3.085, p=0.002] and lower platelet [18.0 (12.0, 49.0) $\times 10^9$/L *versus* 31.0 (17.0, 68.0) $\times 10^9$/L; u = -1.989, p=0.047] than those without mutation; CD7 expression was higher, whereas CD34, CD56, and HLA-DR was lower. Compared with those without mutation, patients with *CEBPA* mutations had better relapse-free survival (p=0.014) and overall survival (p=0.031).
Summary and Conclusions: The frequency of *CEBPA* mutations may be high in Chinese patients with AML, and *CEBPA* mutations were one of these indictors of favorable prognoses.

PB1460

INDUCTION THERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA PRESENTING WITH HYPERLEUKOCYTOSIS.

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Background: Most studies suggest that hyperleukocytosis (HL) is a poor prognostic factor in acute myeloid leukemia (AML). In adult patients HL at the onset of AML and ALL is diagnosed in 5-13% and in 10-30% respectively. HL is more common in AML with monocytic differentiation subtypes (M4eo, M5). During the induction chemotherapy early (first week) mortality in AML, associated with HL reaches 90%. The main reasons are leukostasis and massive tumor lysis syndrome, resulting as respiratory distress syndrome, neurological disorders, disseminated intravascular coagulopathy (DIC) with the development of life-threatening bleedings. In National research Center for Hematology we developed the treatment of AML presenting with HL, aimed for preventing cytolysis, which includes cytoreductive therapy (hydroxyurea, leukapheresis), and we employed plasmapheresis (PF) before the administration of anthracyclines on the 3-5 days of the induction-therapy (7+3), during hydroxyurea and the first days of cytarabine therapy.
Aims: To prevent the massive cytolysis during the induction therapy in patients with AML and HL at the onset of the disease.
Methods: From 2010 till 2014 in our center 75 patients (age ≤ 60) were diagnosed with *de novo* AML. Initial white blood cells (WBC) count ≥100x10^9/l were detected in 13 patients (17,3%) (100-408x10^9/l, med-127x10^9/l). M/F - 6/7. Variants of AML by FAB-classification: M0-2 patients, M1-M2 – 3 patients, M4 – 6 patients and M5 - 2 patients. Age 17-56 (med-38). Two patients had favorable-risk cytogenetics, 11 patients – intermediate-risk cytogenetics. Median of lactate dehydrogenase level was 1477 μ/l (720-6653 μ/l).
Results: In 11/13 patients (84,6%) with initial WBC count ≥100x10^9/l hepatosplenomegaly and generalized lymphadenopathy were found, in 7 /13 (53.8%) - gingival hyperplasia, in 3/13 (23%) - neuroleukemia, 1/13 (7,6%) – had specific skin lesions. All patients had infectious complications at the time of diagnosis of AML. Evidence of leukostasis had more than half of patients: respiratory distress syndrome in 5/13 patients (38,4%), neurological symptoms (headache, blurred consciousness) - in 6/13 patients (46,1%), DIC - in 6/13 patients (46,1%), including 1 - intracranial hemorrhage. Cytoreductive therapy with 1-7 days (med-2 days) hydroxyurea (10 mg/kg/day) was administered to all patients. 1-2 (med–2) LF received 8/13 patients. 1-4 (med-2). PF were performed to all patients before the introduction of Daunorubicin (during hydroxyurea-therapy and the 1st days of cytarabine-therapy). The median of WBC at the 1st day of the induction was 61x10^9/l (29-124x10^9/l). Daunorubicin was administered in 11 patients on 5-7 days, in 1 patient on 4-6 days and in 1 patient on 3-5 days. Massive cytolysis with the development of multiple organ failure were not observed. Only 5/13 patients (38,4%) showed transient signs of volemic overload which was resolved during diuretic therapy and plasmapheresis. Complete remission (CR) was achieved in 8/13 patients (61,5%), a primary refractory (after 1st course of «7+3») was registered in 38,4% (5/13) pts. Early mortality was 0%. Alive 10/13 patients (77,0%) with median follow-up 20 months (4-49 months), CR-duration - 19 months (2-48 months). Allogeneic hematopoietiec stem cell transplantation, as a postremission strategy, was performed in 6/13 patients (46%).
Summary and Conclusions: Our tactic in the induction therapy of AML-patients with HL prevents the development of severe complications such as respiratory distress syndrome, neurological disorders, disseminated

intravascular coagulopathy, that resulted in 0% early mortality without the impairment of induction therapy's results.

PB1461

INTERNAL TANDEM DUPLICATION (ITD) MUTATIONS OF THE FLT3 RECEPTOR AND CXCR4 EXPRESSION IN DE NOVO ADULT ACUTE MYELOID LEUKEMIAS (AML)

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Background: Internal tandem duplication (ITD) mutations of the FLT3 receptor are associated with a high incidence of relapse in acute myeloid leukemia (AML). It is also known that the complete immunophenotypic characterization contributes to define the prognosis of de novo adult AML. Stromal cell-derived factor-1 (SDF-1) is a homeostatic chemokine that is constitutively secreted by marrow stromal cells. SDF-1 signals through CXCR4, which plays an important role in hematopoiesis, development and organization of the immune system. Prognostic impact of CXCR4 expression levels on the neoplastic cells has been demonstrated in breast cancer, renal cell cancer and AML.
Aims: We investigated the expression of the chemokine receptor CXCR4 on bone marrow blast cells in a group of adult de novo AML with FLT3-ITD.
Methods: We have observed 55 young adult de novo AML patients (median age: 44 years, r: 22-62) in the last 5 years, who presented FLT3-ITD-positive AML blasts in bone marrow blood. On the basis of FAB classification the patients were considered LMA-M5 (22 pts.), LMA-M2 (12 pts.), LMA-M4 (11 pts.), LMA-M1 (7 pts.) and LMA-M0 (3 pt.). 15 patients showed complex chromosomal anomalies; 23 patients presented hyperleukocytosis (WBC> 40x10(e)9/L). The clinical outcome was that one of a "high risk" AML; at the present only two patients are still alive in CR (+60 months and +66 months).
Results: We found at the diagnosis, in all cases, an high CXCR4 expression on leukemic blasts, as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5.
Summary and Conclusions: Several studies have shown the prognostic significance of the expression of differentiation myeloid markers at the diagnosis of adult de novo AML. However, specific immunophenotype expression patterns associated with internal tandem duplication (ITD) mutations of the FLT3 receptor are still unknown. Further studies are warranted to confirm the correlation between FLT3-ITD and "CXCR4 over-expression" immunophenotypic pattern.

PB1462

INFUSION OF HLA-MISMATCHED PERIPHERAL BLOOD STEM CELLS DURING TREATMENT AML IN ELDERLY PATIENTS

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Background: The treatment of the elderly patients with AML still remains unsatisfactory. The reasons for the poor prognosis in elderly patients are the unfavorable biology of AML, incidence of relevant comorbidities and increased risk of chemotherapy-related mortality. In 2011 Mei Guo *et al.* published a study showing optimistic outcomes in treatment of the elderly AML patients with chemotherapy followed by infusion of HLA-mismatched peripheral blood stem cells. However, other studies confirming these results are lacking.
Aims: To prove efficacy, feasibility and safety of the treatment published in the study by Mei Guo *et al.*. The preliminary results are presented below.
Methods: Only the patients with de novo and secondary AML aged 60-70 were eligible for the study. Remission induction chemotherapy consisted of Ara-C 100mg/m2 daily for 7 days and daunorubicin 90 or 45mg/m2 daily for 3 days followed by the infusion of G-CSF-mobilized HLA-mismatched donor peripheral-blood stem cells 24 hours after the end of the chemotherapy. Bone marrow assessment was performed at day 15 and after hematological recovery. Patients with CR received 2 consolidations with Ara-C 1g/m2 given every 12 hours on days 1, 3 and 5 followed by the infusion of HLA-mismatched peripheral-blood stem cells 24 hours after the end of the chemotherapy. The donors of 7/8 patients were HLA haploidentical children, in case of the eighth patient, the donor was HLA haploidentical sibling. Donor microchimerism was detected in peripheral leucocytes using a real-time quantitative PCR protocol and was measured 7, 14 and 21 days after the infusion of HLA-mismatched peripheral-blood stem cells.
Results: Between 1/2013-12/2013 eight patients with median age of 65 years (60-70) were treated according to this protocol. The median follow up was 6.8 months. After induction 3/8 patients achieved CR, the other 5/8 did not and were excluded from the study. All 3 patients who achieved CR received both cycles of consolidation chemotherapy. The OS at 6 months and EFS at 6 months were 37% and 37% respectively. Two of three patients with treatment response relapsed during the first year after achieving CR. Thus only one patient is still in long term CR. All patients presented with hematological toxicity gr. 3 or more. Non-hematological toxicities gr. 3 and higher were: febrile neutropenia – 7/8, supraventricular arrhythmia – 2/8, 1/8 patient developed anti HLA antibodies, with refractoriness to platelet transfusion. Only 2 of 8 patients presented after induction with donor microchimerism at day 7 and 0/8 at day 14 and 21. Post-consolidation measurements revealed only autologous recovery in all of the three patients.
Summary and Conclusions: Our (even preliminary) results did not prove the efficacy of previously published approach, while except one, all patients did not responded or early relapsed.

PB1463

THE PROGNOSTIC VALUE OF ADENOSINE TRIPHOSPHATE ATP-BINDING CASSETTE (ABCB5) AND MULTIDRUG RESISTANCE (MDR1) GENES` EXPRESSION IN ACUTE LEUKEMIAS

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Background: The goal of leukemia lines of treatment is to eradicate the leukemic clone and thus reestablish normal hematopoiesis. The resistance of human tumor to multiple chemotherapeutic drugs has been recognized as a major reason for the failure of cancer therapy and is associated with so-called *multidrug resistance (MDR)*.
Aims: The present work aimed at detecting the expression of the MDR1 and ABCB5 genes in acute leukemia patients and correlate with disease outcome.
Methods: To achieve this aim, quantitative assessment of MDR1 & ABCB5 genes expression was performed using quantitative real time polymerase chain reaction (qPCR) for 90 acute leukemia patients and 20 healthy volunteers.
Results: MDR1 gene expression was significantly higher in AML cases compared to healty volunteers (p value = 0.034). Complete remission was more prominent among patients with {MDR1 (low expression), ABCB5 (low expression), NPM1 mut.A (pos.)} (p value =0.003). In ALL, MDR1 gene expression was significantly higher in patients with resistant disease (p value = 0.001). ABCB5 gene expression level was significantly higher in ALL patients compared to the healthy volunteers (p value = 0.04) and in relapsed cases compared to de novo cases (p value = 0.027).
Summary and Conclusions: The results obtained by the current study concerning MDR1 and ABCB5 gene expression in acute leukemia patients provide additional evidence of the role played by these genes as influencing factors on the multidrug resistance status of acute leukemia cells to chemotherapy and hence clinical outcome.

PB1464

FLUCONAZOLE PROPHYLAXIS FOLLOWING INDUCTION CHEMO-THERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA: PATTERN OF FUNGAL INFECTIONS

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Background: Intensive chemotherapy may cause sever and prolonged neutropenia,which may cause potentially fatal fungal infection.Fluconazole is an effective agent for antifungal prophylaxis because of its activity against many candida species,long half life,tolerability and minimal side effects.The result of clinical trials suugest that fluconazole is superior to placebo in preventing fungal infection in neutropenic patients;but its efficacy against systemic fungal infection is not clearly defined.This retrospective review examined the pattern of fungal infections with the use of fluconazole in a dose of 400mg daily as antifungal prophylaxis in patients with acute myeloid leukemia following induction chemotherapy.
Aims: The primary end point was the ocurrance of neutropenic fever and the secondary end points were the site of fungal infection, the rate of development of peumonia and resistant fungal infection.
Methods: Patients (with age >18 years <60 years) who completed induction chemotherapy with AML protocol (between the peroid of Jan 2010 and Jan 2014) were eligible for the study if they recieved antifungal prophylaxis at least 1 day post chemotherapy.Patients were stratified based on antifungal prophylaxis *versus* no prophylaxis and all patients recieved antimicrobial prophylaxis. The patients were excluded from this study if they developed fever during induction chemotherapy or presented by fever.Fever was defined as elevation of oral temperature above 38.3 C or greater once or temperature above 38.0 C over one hour or more than one hour.Neutropenia was defined as ANC< 500/mm^2.The odds ratio with 95% confidence interval was calculated for prolonged neutropenia ,septic shock,fungemia and mortality rate.
Results: See Table 1.

Table 1.

Summary and Conclusions: From this retrospective review,we concluded that fluconazole as prophylaxis in neutropenic patients was effective and help to decrease the rate of superficial fungal infection,invasive tissue infection and with a trend toward reduction in the rate of pneumonia but not reaching a satistical significance.

PB1465

HETEROGENEITY OF ACUTE MYELOID LEUKEMIA WITH TRANSLOCATION T(8;21)(Q22;Q22)

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Background: In spite of good prognosis there are some of the patients with acute myeloid leukemia (AML) and with favorable karyotype who relapsed after a short period of complete remission (CR) or have got resistant variants. These patients as the patients with unfavorable karyotype are potential candidates to allogeneic stem cell transplantation (AlloSCT). For prolongation of overall survival (OS) it is necessarily to stratify the patients with favorable karyotype on groups with different prognosis and select the patients to AlloSCT during diagnosis or early stages of the treatment.
Aims: The aim of this retrospective study was to characterize hematological and clinical variability of AML with t(8;21) and select the signs associated with aggressive history of the disease.
Methods: The data of investigation (44 patients) and treatment (36 patients) were analyzed. Standard karyotyping and PCR methods for studying mutational status of *FLT3, NPM1, NRAS* or *c-Kit* genes were used.
Results: The median age of patients was (11-70). According to FAB classification there were M2 (82%), M1 and M4 morphological variants. One patient was diagnosed with therapy related AML. Additional chromosomal aberrations were found in 24 patients. One of sex chromosomes' loss or damage of chromosome 9 was the most frequent additional aberrations: 34.1% and 16.6%, accordingly. Complex and monosomal karyotype were determined in 6.8% and 9.1%, accordingly. Additional chromosomal aberrations were not correlated with the patients' age. Gene mutations were rare findings. The second case of c-Kit mutation was determined during AML relapse and was associated with the appearance of plural chromosomal aberrations. Three patients died after standard induction chemotherapy (IC). Complete remission (CR) was registered in 23 patients after the first course of IC, in 8 after the second IC and in 1 after the third IC. And that makes the CR rate is 96.9% (32/33 patients). Relapse was diagnosed in 8 (25.0%) patients through 2-18 months period after CR registration. These patients were characterized with the first IC course ineffectiveness (5 patients), 9 chromosome aberration (3 patients), absence of consolidation with high dose cytarabine (2 patients) or *c-Kit* mutation (2 patients). The durability of follow-up period was 5-245 months. The median OS of 32 patients with CR was not reached and the rate of 5-years OS was 57.8%. The median OS of patients with early relapse was 10 months. OS of patients with or without additional cytogenetic aberration was not significant different. OS of patients with additional chromosome 9 aberrations was significant worse: 11.5 months *versus* not reached in patients without these aberrations; p=0.003.
Summary and Conclusions: AML with t(8;21) is a heterogeneous group of patients with different age, morphological nature of blasts, variants (de novo or therapy related) and/or molecular aberrations. Patients with the ineffectiveness of the first CI course or additional chromosome 9 aberrations are the potential candidates to AlloSCT.

PB1466

PHARMACODYNAMICS OF CYTARABINE-INDUCED LEUKOPENIA: A RETROSPECTIVE COHORT STUDY

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Background: Cytarabine is a pyrimidine analogue used to treat a variety of hematological malignancies, most notably acute myeloid leukemia (AML). Several databases such as Micromedex, BC cancer and Pfizers product monograph cite a biphasic pattern of white blood cells (WBC) count decline following cytarabine administration. This pattern was described after the administration of low and intermediate doses of cytarabine for patients with a variety of malignancies, most of them non-hematological, with a nadir on days 7-9, a brief recovery and a second nadir between days 15-24 following therapy. The pathophysiology of this phenomenon is unknown.
Aims: Our purpose was to establish whether this biphasic pattern is reproducible in AML patients treated with cytarabine using more intensive modern protocols.
Methods: We conducted a retrospective cohort study. Our study included 56 consecutive patients with AML in complete remission who received 89 cycles of intermediate or high dose cytarabine. Daily total white blood cells and neutrophils were collected during the first 15 days after the initiation of cytarabine administration and the courses were analyzed. We performed further analysis to assess possible associations of changes in blood counts with cytarabine protocols (IDAC *vs*. HiDAC) and possible confounding factors such as steroid or granulocyte colony stimulating factor (G-CSF) treatment, renal failure or active infections.
Results: Analysis of blood counts failed to reveal a biphasic pattern of descent for WBC, neutrophils, monocytes or lymphocytes, as previously described. High dose cytarabine (HiDAC) was associated with a significantly faster descent of WBC compared to intermediate dose cytarabine (IDAC). No effect of confounders was detected.
Summary and Conclusions: We conclude that in our cohort we could not verify the existence of a biphasic pattern for the decline of WBC post cytarabine therapy and that WBC decline post cytarabine was steeper for patients receiving HIDAC than for those receiving IDAC.

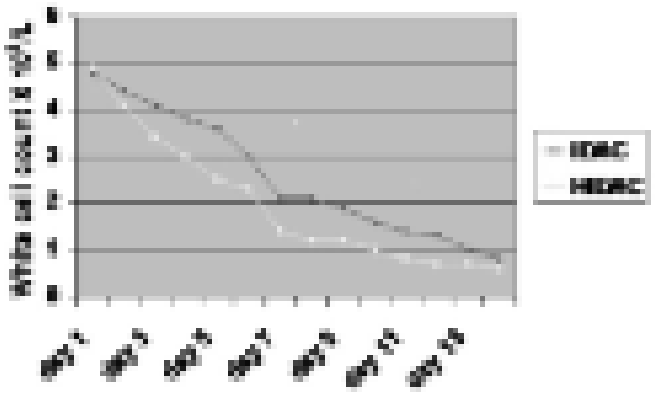

Figure 1.

PB1467

THERAPEUTIC MANAGEMENT OF CHILDHOOD AML AT MPCTRC: A SINGLE CENTER STUDY

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Background: Mahak's Pediatric Cancer Treatment and Research Center (MPCTRC) has been started from April 2007. During these years 1300 new cases have been both diagnosed and treated at this charity center. The most prevalent diagnosis in this center is acute leukemia (n=338, 27.7%) that imply 68 (20%) childhood Acute Myeloid Leukemia (AML). Immunophenotyping by flow-cytometry pattern is the powerful method for recognizing type of AML in considered patients. During the diagnosis, screening the molecular genetic abnormalities can be a good marker for prognosis determination.
Aims: The main purpose of this study is to evaluate some basic parameters in

patients with AML who referred to MPCTRC for diagnosis and treatment in Tehran (Capital City of Iran).
Methods: This was a simple sampling study which consisted children less than 15 years old with AML who have been referred to MPCTRC since April 2007 to April 2013. There was a unique checklist for enrolled patients that implied epidemiological information (age, sex, molecular genetic abnormalities, CD markers and patient's last status) and filled for all of patients. Treatment for patients with AML at MPCTRC is according to BFM protocol that for type of M3 ATRA will be added too. Data analyzed by SPSS software version 22. Chi-square test (P-value <0.05) have been used for variables.
Results: According to inclusion criteria of this study, there was 52 cases with AML that consisted of 33 (63.5%) boys. The mean age of patients was 7.09±0.64 years (range: 6months-15years old). There was not any significant difference in the mean age of patients between boys and girls. The flow-cytometry results showed that type of AML was as: M1 (n=7, 13.4%); M2 (n=9, 17.3%); M3 (n=12, 23.1%); M4 (n=10, 19.2%); M5 (n=5, 9.6%); M6 (n=4, 7.7%); and M7 (n=5, 9.6%). Cytogenetic and molecular abnormalities was shown in 19 patients (36.5%). These abnormalities was as 12 (63.15%) translocations (t(15;17):n=6, 50% ; t(8;21):n=3, 25%; t(10;11), t(12;18) and t(14;2):n=1, (8.3%), 5 (26.31%) deletions and 2 (10.5%) trisomies. Chi-square test showed the significant relation (P-value=0.04) between genetic abnormalities and last status of the patients. Analysis revealed that 16 patients (30.76%) had relapse during their treatment that out of them, 8 cases (50%) died. 11 (68%) patients with early relapse had normal molecular genetic abnormality and the most common abnormality in the relapse patients was deletion (n=3, 18%) and all the early relapse patients with deletions has been died. Patients with subtype of M3 and t(15;17) had no early relapse. Last call of patients determined that out of enrolled patients, there were 17 (32.7%) cases with end of treatment, 8 (15.4%) cases during treatment, 17 (32.7%) cases died and 10 (19.2%) cases were loss of follow up.
Summary and Conclusions: AML is a rare malignancy in childhood leukemia in proportion to ALL. To have an appropriate criteria for the prognosis of these patients, survival rate analysis is also demanded but according to 7-years history of our center, we can have 5-years of survival rate 3 years later. Genetic mutations and flow-cytometry can be also a good marker to evaluate the risk group of childhood AML and treatment protocol selection.

PB1468

CYTOGENETIC AND MOLECULAR GENETIC ABNORMALITIES EVALUATION IN PEDIATRIC ACUTE MYELOBLASTIC LEUKEMIA

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Background: Genetic abberations underlying Acute Myeloblastic Leukemia (AML), is the importance landmark for better managing the pathogenesis of disease. The classical way for investigation of chromosomal abberations is conventional karyotyping. For considering different translocations, the Real-Time Polymerase Chain Reaction will be used. Two main referral centers for childhood malignancies in Tehran (capital city of Iran) are MAHAK Pediatric Cancer Treatment and Research Center (MPCTRC) and Children Center Hospital. Children less than 15 years old with all kind of childhood malignancies will admitted in these two centers for diagnosis and therapy. Patients from all parts of Iran can refer there.
Aims: The main goal of this study was to evaluate genetical abnormalities in patients with AML for better managing this disease in future.
Methods: Enrolled Patients have been referred from all parts of Iran to two referral childhood malignancy centers in Tehran, Iran (MPCTRC, Children Center Hospital) during April 2007 to April 2013. There was a unique check list for all patients than implied basic data about sex, age, date of dead,,,. Bone marrow aspirates of 104 pediatric AML cases were analyzed by G-banding technique, karyotyping and Real Time-PCR for translocations. Finally data analysed by SPSS version 19.
Results: There were 57 boys (54.81%) out of 104 enrolled patients. The mean age of patients were 6.9±0.43 years. Immunophenotyping results showed the M4 (n=20, 19.2%) and non-M3 (n=19, 18.3%) groups as the majority phenotypes respectively. Twenty out of 104 patients (19.2%) had genetic abnormalities; t(15;17) (n=6, 30%), inversion (n=5, 25%), mosaicism with deletion (n=2, 10%), t(8;21), t(6;11), hyperdiploidy, mosaicism with translocation, mosaicism with monosomy, trisomy 8 and gene deletion (n=1, 5%). Fourthy-four patients died (42.3%) during this study. The three-years survival rate was 88%.
Summary and Conclusions: Analysis and literature reviews revealed that t(15;17) was the most prevalence abnormalities. Authors suggestion is comprehensive studies with larger patients series to confirm these evaluation. Focusing on cytogenetic abnormalities will consider prognostic significances of patients with AML that can affect treatment planning.

PB1469

PROGNOSTIC FACTORS AND TREATMENT OUTCOME OF CHILDREN WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA.

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease that accounts for approximately 20% of acute leukemias in children and adolescents. Despite the lack of targeted therapy for most subtypes and a dearth of new agents, survival rates have reached approximately 60% for children treated on clinical trials in developed countries. Improvements on the clinical and biological stratification of AML cases allow provide background for risk-adapted treatment strategies, particularly after complete remission (CR) achievement.
Aims: The aim of this study is to evaluate the response and survival of pediatric patients with AML and factors impacting on these.
Methods: This retrospective analysis included 36 patients with newly diagnosed AML who were treated by the French protocol ELAM02 between 2002 and 2011. Clinical-biological characteristics at presentation were registered. Association between remission after one course of induction, relapse and factors at presentation such as age (< or≥ 10 years), sex, the white blood cell count (WBC) (< or ≥ 50 x10^9/L), the hemoglobin count (Hb) (< or ≥8g/dl), the platelet count (< or ≥ 50 x10^9/L), the cytologic classification FAB, the prognostic cytogenetic groups (favorable, intermediate and high risk) were evaluated by X^2 test. Event free survival (EFS) and overall survival (OS) analysis was carried out with the Kaplan-Meier method. The log-rank test and Cox regression were applied for univariate and multivariate analysis, respectively. A p value below 0,05 was considered as being statistically significant.

Table 1	Percentage
Median age (range)	9 years(1-16)
Male/female	50%/50%
Hb (g/dl):	
<8	47%
≥8	53%
WBC count(1^0 /l)	
<50	86%
≥ 50	14%
Platelet count (x10^9/l)	
<50	55.5%
≥ 50	44.5%
ClassificationFAB:	
AML0	5.5%
AML1	28 %
AML2	39 %
AML4	11%
AML5	5.5%
AML6	8%
AML7	3%
Prognostic cytogenetic groups:	
Favorable	30.5%
Intermediate	44.5%
High risk	25%

Results: Patients´ characteristics at presentation are shown in Table 1. The CR rate after one course of induction therapy was 58% (21 patients) and 80.5% after two courses (29 patients). The rate of early death was 3% and 15% after one course and two courses of induction therapy respectively. Three factors associated to CR after one course: sex (p=0.027), WBC (p= 0.02) and platelet count (p=0.046). The hematopoietic stem cell transplantation was done only in 4 patients in first remission among the 19 patients in transplant indication. The relapse rate was 55%. Two factors associated to relapse: FAB cytologic classification (p=0.04) and cytogenetic groups (p=0.03). The OS and the EFS at 24 months were 54% and 40.6%, respectively. A statistically significant correlation between the cytogenetic groups and EFS was observed (p 0.03).
Summary and Conclusions: Our results are lower than the literature. AML is a biologically and pronostically heterogeneous disease subsidiary to be approached with risk adapted strategies. Currently it is feasible to identify a substantial proportion of patients that may be cured with high-dose cytarabine consolidation or ASCT. In contrast, there are patients with adverse genetic features that need an allogeneic procedure, investigational agents or both.

PB1470

TRANSFORMATION OF CML TO ACUTE LEUKEMIA,OUR EXPERIENCE FROM A TERTIARY CARE CANCER HOSPITAL FROM PAKISTAN

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Background: Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease with a relatively stable clinical course and good prognosis after treatment. Its conversion into acute leukemia heralds an accelerated clinical course that is less responsive to treatment and has much higher mortality.
Aims: To retrospectively assess the proportion of CML patients who underwent progression to acute leukemia at a tertiary-care specialist cancer center in Lahore, Pakistan and to describe the clinical features and mortality associated with conversion.
Methods: Data was analyzed by looking at frequencies and applying t-test to continuous data and chi-square test to categorical data. Factors associated with mortality were assessed using bivariate and multivariate logistic regression analyses.
Results: A total of 240 patients were accepted for treatment between Jan 1, 2012 and December 31, 2013. Out of these patients 21 (76% males; Mean age: 37.5 years; Range: 24 to 58 years; 20 patients positive for t (9;22)(q34;q11) translocation) progressed to acute leukemia during this period. The mean blood cell count at the time of admission was 200.5 (range: 33 to 526). 76% patients received imatinib as initial treatment. The average duration between diagnosis and transformation to acute leukemia was 195 days (range: 24 to 324 days). After transformation, 12 patients were treated with chemotherapy HCVAD, 6 received DA 3+10 protocols, and 1 was offered DA 3+7 chemotherapy. 2 patients did not receive any treatment. During the study period, 11 patients died (average survival after transformation: 40.6 days; range: 2 to 172 days). Mortality was not significantly associated with the duration between diagnosis and transformation to AML or the type of treatment received.
Summary and Conclusions: Nine percent of patients diagnosed with CML progressed to acute leukemia over a period of two years. Among patients who transform to acute leukemia, the mortality is high and average life expectancy after transformation is between 1 and 2 months.

PB1471

TWENTY-FOUR KOREAN MLL LEUKEMIAS AND THEIR PARTNER GENES: A COMPARATIVE STUDY

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Background: Rearrangements involving the *MLL* gene located at 11q23, is a dismal prognostic factor found in about 5-10% of acute leukemia. Numerous *MLL* partner genes are identified, over hundred at chromosome levels and over 70 at gene levels. While the main focus lies on the frequent fusion partner genes of their mechanisms in leukemogenesis and their potential as a molecular therapeutic target, ethnic differences in the frequency of fusion partner genes have not been studied well.
Aims: In order to gain further insights to the ethnic difference of distribution of *MLL* fusion partner genes, here we report the frequency and distribution of *MLL* partner genes from Korean patients, and compare the differences with two other groups of different ethnicity.
Methods: The *MLL* partner genes from 24 Korean *MLL* leukemia patients were analysed using long distance inverse-polymerase chain reaction (LDI-PCR) according to methods previously described (Leukemia. 2009;23:1490-9). Bone marrow specimens of all patients were analyzed by conventional karyotyping and fluorescence *in situ* hybridization (FISH). using the *MLL* LSI dual color, break-apart probes. The results were compared with two other previously published European studies. Statistical analyses were made using statistical software SPSS (SPSS Inc., Chicago, IL, USA) determining statistical significance at $P < 0.05$.
Results: Frequent partner genes of the *MLL* rearrangement detected from Korean population were *AF9* (5 cases), *ENL* (5 cases), *ELL* (3 cases) and *AF4* (3 cases). Rare *MLL* partner genes were also found, which included *CASP8AP2* (novel type), *AF17*, *SEPT9, TET1, MLLT11*, *AF10, ACTN4* and *CASC5* (total of 8 cases). It was also interesting to find three-way and four-way translocations of t(2;19;11) and t(4;11;22;10). Comparison with two other studies is summarized in table 1. While the distribution of individual partner genes were different between groups, it was notable that the six translocation partner genes of *AF4, AF9, ENL* and *ELL* were the most frequently detected partner genes in all three groups, accounting for 56.6%, 66.6% and 66.6% of all cases, respectively. Regarding the infrequent translocation partner genes, the only common genes were the *MLLT11* gene from all three groups and *SEPT9* gene detected from Korean and Portuguese groups. Other infrequent partner genes were only detected once or twice from each study groups.
Summary and Conclusions: Between the three patient groups including Korean *MLL* leukemia patient group, distribution of the frequent translocation partner genes were rather similar. The ethnic difference as a cause of the difference in distribution of infrequent partner genes has not been validated, and further studies in larger scale have to be conducted to determine the presence of an ethnical difference. It is also another limitation of this study, not being able to analyze adult and pediatric patients separately, due to small number of patients included in each study. Currently, state-of-the-art molecular techniques such as the next-generation sequencing (NGS) are being actively utilized in the field of leukemia research. Regarding *MLL* leukemia however, LDI-PCR has been the most cost-effective and precise diagnostic method employed over the past 10 years for identifying partner genes and detecting minimal residual diseases.Continuous investigation of *MLL* leukemia at its molecular level will not only benefit in determining treatment response and prediction of prognosis, but also opening the possibility of molecular targeted therapy of *MLL* leukemia, in which development of tyrosine kinase inhibitors for chronic myelogenous leukemia (CML) would be a good example.

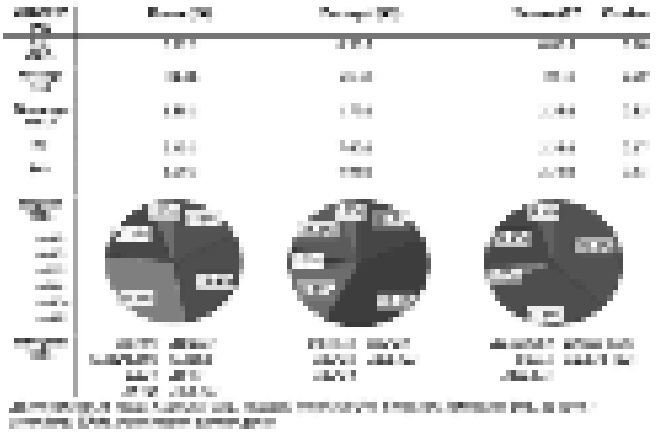

Figure 1.

PB1472

AZACITIDINE IN THE TREATMENT OF OLDER PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA: A REPORT BY THE RETE EMATOLOGICA PUGLIESE (REP)

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Background: The optimal treatment of older patients (>60 years) with Acute Myeloid Leukemia (AML) remains challenging in daily clinical practice; a choice has to be made between intensive chemotherapy and best supportive care. To guide physicians in their decisions, several prognostic factors have been identified and risk scores have been developed. Recently, the DNA methyltransferase inhibitor azacitidine has become available for MDS and AML patients with up to 30% bone marrow blasts. However, limited data are available on the setting of older unselected AML patients treated with azacitidine, regardless of their bone marrow blast count.
Aims: To study the impact of azacitidine treatment in older patients with AML ineligible for intensive chemotherapy, we retrospectively analysed the outcome of 110 newly diagnosed AML older patients in 9 Institutions from the Apulia Region (REP).
Methods: The study included 110 AML patients (59 males and 51 females, median age 75 years, range 61 to 90 yrs); FAB criteria subtypes were: 27 M0, 32 M1, 26 M2, 10 M4, 12 M5, 3 M6. M3 subtypes were excluded from the analysis. Cytogenetic evaluation was performed in 78 cases (71%): 44 (56%) patients were intermediate risk, 34 (44%) adverse, while no patients had a favorable prognosis. Median values of white blood cells and hemoglobin were 2.6x10e3/mmc (r: 0.6 – 72,4), 8.5 gr/dl (r: 5.4 – 12.9), respectively. The median blast count in bone marrow and peripheral blood was 33% (r 20 – 90) and 10% (r 0 – 80), respectively.
Results: After a median of 9 treatment cycles (r: 1 – 36), median OS was 18 months. Azacitidine treatment failure was documented in 46 patients (42%), while the overall response rate after a median time of 4 months treatment was 58% (36% (CR and Cri), 22% PR). Responder patients showed a better median overall survival than non responders (23 *vs* 6 months, p<.001, figure 1), while neither primary treatment failure nor bone marrow blast count (higher than

30%) were correlated with a worse overall survival (29 *vs* 22 months, p= .99; 16 *vs* 19 months, p=.96), respectively.
Summary and Conclusions: The results of our retrospective analysis seem to confirm the benefit of treatment with azacitidine for older AML patients in terms of OS, regardless of the bone marrow blast count, while primary treatment failure should not affect the continuation of treatment.

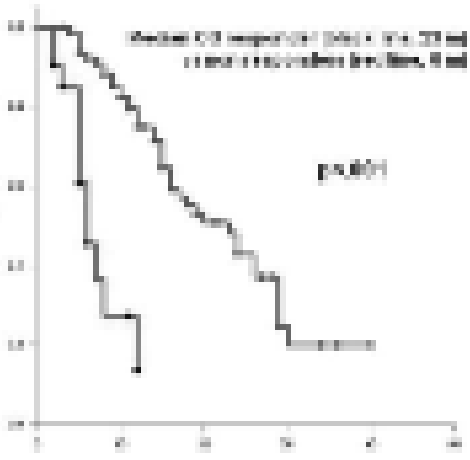

Figure 1.

PB1473

ACUTE MYELOID LEUKEMIA WITH SOLE TRISOMY 6: CASE REPORTS AND LITERATURE REVIEW

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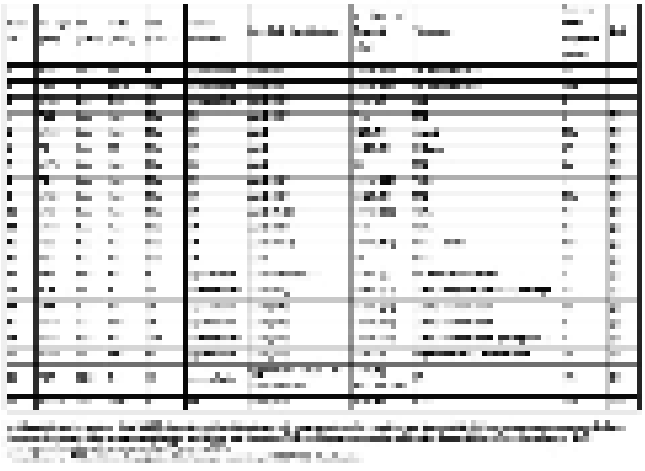
Background: Autosomal trisomies as sole cytogenetic changes have been described in several hematologic malignancies, but associations between specific morphologies with individual structural cytogenetic abnormalities have been reported less frequently. There have been several reports of trisomy 6 as the sole karyotypic abnormality in patients diagnosed with MDS, AML or MPN and has also been reported in association with bone marrow hypoplasia such as aplastic anemia (AA).
Aims: We describe three patients with AML, demonstrate that trisomy 6 is the sole clonal cytogenetic bone marrow abnormality, and review the literature on AML with sole trisomy 6 to establish a relationship between the clonality of AML with a single trisomy 6 abnormality.
Methods: We examined three patients who were diagnosed with AML having trisomy 6 about laboratory findings and clinical features. To identify reported cases of AML having trisomy 6 as the sole clonal aberration, we searched PubMed (http://www.ncbi.nlm.nih.gov/pubmed) with combinations of the search terms “AML” and “trisomy 6”.

Table 1. The laboratory findings and clinical features in AML with sole trisomy 6.



Results: We identified ten reports describing 18 cases of AML presenting with trisomy 6 as the sole karyotypic abnormality. Including those of the three cases we report, there were ten female and 11 male patients, including three children and one infant. The laboratory findings and clinical features are summarized in Table 1.
Summary and Conclusions: By reviewing the literature, we suggest that trisomy 6 is a nonrandom clonal cytogenetic marker of AML and may sometimes be the result of transformation from other myeloid disorders. In addition, further studies to establish the clonality of AML with sole trisomy 6 are needed.

PB1474

MORTALITY AND RELATED FACTORS IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Older patients with acute myeloid leukemia (AML) have significant comorbidities, a poorer performance status (PS), more unfavorable cytogenetic abnormalities, and a higher incidence of secondary AML than younger patients. Due to their age related unfavourable features, most of the patients are regardd as uneligible for intensive treatment.
Aims: The aim of our study is to assess the clinical and laboratory features of our patients with AML and over 65 years of age. With their Charlson performance score, cytogenetical analysis and the treatment given (intensive, hydroxyurea, low dose cytarabine, hypomethylating agent or no treatment), we aimed to evaluate survival and mortality rates compared with these variables.
Methods: Data of 125 patients with AML, over 65 years of age were recruited from our archives. Age, gender, Charlson performance scores, gven treatment and remission durations (months) were recorded. Descriptive and survival analysis were performed.

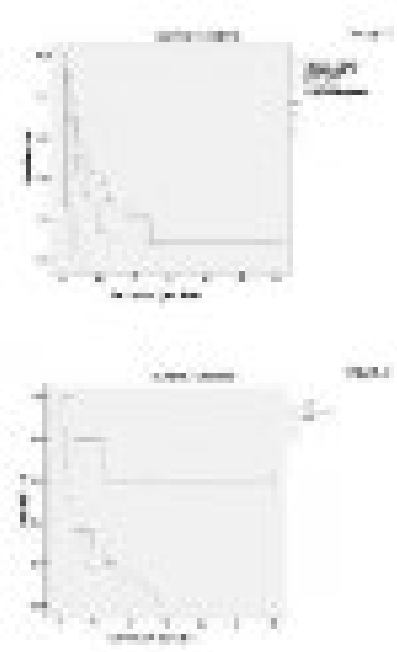

Figure 1.

Results: 50 patients were female (40%) and 75 were male (60%). Mean age 71.36 years (65-87). Charlson comorbidity scores were divided into two groups as score 5 or less (40 patients, 32%) and 6 or more(85 patients, 68%). Regarding the treatment, 45 patients received intensive treatment (36%), 22 received low dose cytarabine (ara-c) (17.6%), 38 received hydroxyurea (30.4%), 16 received hypomethylating agent (12,8%) and 4 patients did not receive any treatment (3.2%). Regarding cytogenetic profiles, 5 patients (4%) showed good, 45 patients (36%) showed intermediate and 18 patients (14.4%) showed poor cytogenetic profile. Mean survival was 7.893 months ±1.197 (95% CI 5.547 – 10.240). Survival was not related with gender or Charlson performance score but significantly related with the given treatment (Figure 11) and cytogenetic profile (Figure 2). Intensive treatment and good cytogenetic profile was observed to be related with longer survival.
Summary and Conclusions: Elder patients with AML are generally regarded as ineligible for intensive treatment because of performance status and comorbidities. More comprehensive and elaborate performance scoring systems are needed to assess these patients and to give better treatment

decisions. Intensive treatment is still related with better survival and hypomethylating agents give promising results.

PB1475

PROGNOSTIC IMPACT OF BODY MASS INDEX ON ACUTE MYELOID LEUKEMIA OUTCOME

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Background: Body mass index (BMI) has been associated with an increased incidence of various malignancies, including acute myeloid leukemia (AML). In addition, a few studies suggested that BMI might be a risk factor for outcome in AML patients.
Aims: To determine the impact of BMI at AML diagnosis on overall survival (OS), complete remission (CR) rate, early death (ED) rate disease-free survival (DFS) in patients (pts) with AML.
Methods: This single-center study involved 204 adult pts with non-promyelocytic *de novo* AML pts managed at the Clinic of Hematology during the period from 2009 to 2013. Patients were treated according to Medical Research Council (MRC) 10 protocol, with 50% reduction of the anthracycline dose for subjects aged > 60 years. According to their BMI value, the patients were categorized as: underweight (<18.5 kg/m^2), with normal weight (18.5 to<25 kg/ m^2), overweight (25 to<30 kg/ m^2) and obese (≥ 30 kg/ m^2).
Results: The mean age of the pts was 52 (19-69) years; 52% were men. Median BMI was 25.23 (range 16.10-38.8). There were 14 (6.9%) underweight pts, 79 (38.7%) with normal weight, while 68 (33.3%) were overweight and 43 (21.1%) obese. Median OS was 3 month; 6 months in pts with normal BMI, 3 months in overweight pts, 2 months in obese, and 1 month in underweight pts. The patient with normal weight showed significantly longer OS compared to underweight pts (p=0.013), overweight pts (p=0.037) and obese pts (p= 0.042). Also, normally weighted pts achieved higher CR rate compared to underweight pts (88.7% *vs* 37.5%; p= 0.023), overweight pts (88.7% *vs* 63.4%; p=0.048) and obese pts (88.7% *vs* 46.4%; p=0.036). Multivariate logistic regression analysis indicated that BMI< 18.5 kg/m^2 was the most significant factor for poor OS (p=0.004 HR1.593 95%CI: 1.162-2.185). In addition, multivariate analysis identified BMI<18.5 kg/m^2 as the most significant factor for both lower CR rate (p=0.031 HR 1.474 95%CI: 1.036-2.099) and shorter DFS (p=0.040 HR 1.445 95%CI: 1.017-2.053). Multivariate analysis identified BMI<18.5 kg/m^2 as the most significant parameter for ED (p=0.005 HR 2.617 95%CI: 1.347-5.083).
Summary and Conclusions: Our study has shown that BMI at presentation is significantly associated with clinical outcome among the non-promyelocytic AML pts.

PB1476

THE GENOMIC LANDSCAPE OF THE LEUKEMIA FROM A WOMAN PRESENTING WITH AN ATYPICAL MYELOPROLIFERATIVE DISEASE

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Background: Recent advancements within genomics have made it possible to detect the full complement of somatic genetic lesions occurring within a cancer. We used whole exome sequencing (WES) and high-resolution single nucleotide polymorphism (SNP) arrays to characterize the leukemic sample from a 41-year old woman initially presenting with an atypical myeloproliferative disease (MPD). At diagnosis she had pronounced eosinophilia, lymphadenopathy, and a soft tissue mass in the submandibular gland. Clinical genetic diagnostics revealed a normal female karyotype, lack of *BCR-ABL1*, *FIPILI-PDGFRA* and *JAK2* mutations (V617 and exon 12). Two months after diagnosis, she developed acute myeloid leukemia (AML), received standard AML treatment and subsequently underwent allogeneic bone marrow transplantation in 1st complete remission (CR). 21 months after initial presentation, the patient relapsed with a local tumor mass in her right breast and underwent local radiation therapy. Three months after radiation therapy she is still in remission but has a small population (0.2%) of atypical monocytes in the bone marrow. Here the results of the extended genetic analysis can be used to confirm and monitor a possible bone marrow relapse, which would be helpful to make a decision on the need of systemic therapy.
Aims: To characterize the full complement of genetic lesions in a leukemic sample from a woman presenting with an atypical disease.
Methods: High resolution SNP array profiling and WES (Illumina platform) was performed on the AML sample and on cultured cells from a skin biopsy at CR (normal sample). Somatic mutations, including single nucleotide variations (SNVs) and insertion deletion mutations (indels), were identified and validation of the putative non-silent mutations and indels was performed by PCR amplification of the initial MPD, the AML and normal sample, followed by deep sequencing.
Results: The SNP array analysis of the AML sample showed a partial tandem duplication (PTD) of the *MLL* gene (*MLL*-PTD) as the only detectable imbalance. Verification of the rearrangement revealed two alternative splice variants corresponding to duplication of *MLL* exons 2-8 and of exons 5-8. Our first round of validation confirmed 11 somatic non-silent mutations (9 missense, 1 nonsense, 1 indel), including the known AML associated NRAS G13D and DNMT3A R882H. All of the validated mutations as well as the *MLL*-PTD were present at initial diagnosis. The other nine somatic mutations (SRPX2 R110H, COL19A1 F1015S, RNF123 S432F, CNTN3 R206X, POTEE R795H, BUD13 R132C, POLR3A P607S, C19orf80 R85W, and TTC39A Q524fs) are not reported in COSMIC, but mutations at other sites in these genes have previously been reported in cancer and mutations in *COL19A1* and *TTC39A* have been observed in AML.
Summary and Conclusions: Genomic characterization using high resolution SNP array profiling and whole exome sequencing of the leukemia from a woman presenting with an atypical MPD, revealed the presence of at least twelve genetic lesions including a partial tandem duplication of *MLL* and missense mutations in *NRAS* and *DNMT3A*. In AML the presence of a *MLL*-PTD or a mutation in *DNMT3A* are known poor prognostic factors. In addition, the presence of a *DNMT3A* mutation in patients with *MLL*-PTD confers an even worse prognosis. Our patient is in remission after extramedullary relapse, but her disease course together with her high-risk genetic lesions suggests a dismal prognosis and that she may benefit from alternative treatment modalities, potentially with epigenetic-targeting therapy.

PB1477

LOW FREQUENCY OF TUBERCULOSIS DISEASE IN ADULT PATIENTS WITH ACUTE LEUKEMIA

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Background: The major cause of mortality and morbidity in patients with acute leukemia is their predisposition to infection. During the neutropenic periods, the patient is prone to develop mainly bacterial and fungal infections; occasionally mycobacterial organisms may also cause infections. These tuberculosis infections (TB) have not been a common infection in most series.
Aims: We assess TB frequency and impact of antituberculous treatment (ATT) in the management of acute leukemia patients.
Methods: A retrospective study was conducted between May 2003 and October 2013. All patients treated for acute leukemia diagnosed on the basis of clinical features and peripheral blood film examination who developed tuberculosis on the basis of clinical features, sputum examination, and microbiologically-/ or histologically confirmed were included in our study. All patients with suspected tuberculosis in the absence of bacteriological or histological proof, or patients with good response to empiric antituberculosis treatment (ATT) started without confirmed diagnosis of tuberculosis were excluded. The medical records, microbiological, histological and radiological data of patients were reviewed. The anti-TB agents included isoniazid (H), rifampin (R), ethambutol (E) and pyrazinamide (Z) in combined form. The treatment regimens were 2 months of combined RHZE and 4 months of RH.
Results: During the study period (10 years), a total of 1,360 leukemia cases of whom 886 were acute myeloid leukemia (AML) and 474 acute lymphoid leukemia (ALL) were admitted in our unit. Then 9 patients received a confirmed diagnosis of TB. Seven patients were AML and 2 patients ALL. This corresponds to a TB frequency of 0.79% for AML and 0.42% for ALL. The median age is 30 years (with extremes ranging from 19 to 42 years). Seven were males and two were females with a male: female ratio of 1.25. The median time from diagnosis of leukemia and TB was 6 months (range 1-24). TB occurred in post-induction phase, simultaneously and before of leukemia diagnosis. TB diagnosis was made in maintenance, consolidation therapy and post-mortem. Pulmonary and extrapulmonary TB was found. Eight patients shown resolution of TB-related symptoms post ATT. Five patients achieved complete remission of leukemia. Two patients died, one in leukemia relapse and another before confirmation of TB diagnosis. One patient was included in palliative care and lost in follow up.
Summary and Conclusions: There was a low rate of TB disease in our population of patients. No delay chemotherapy or worsening of leukemia management was found with TB treatment.

PB1478

SIGNIFICANCE OF FEBRILE NEUTROPENIA RISK SCORE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Chemotherapy induced febrile neutropenia (FN) in patients with acute myeloid leukemia (AML) is a clinicaly important adverse event due to the impact on the course of disease and survival by causing the delay or dosage adjustment of chemotherapy. Although the myelotoxicity of the chemotherapy is the key determinant of risk for developement of the FN, it is now recognised that patient characteristics may increase this risk further.
Aims: To validate the FN mortality risk score in a population of adult AML patients undergoing intensive induction chemotherapy.
Methods: We analysed retrospectively a cohort of 118 patients (pts) with newly diagnosed AML, treated in the Clinic of Hematology during period 2011-2013. All of the pts had febrile neutropenia with absolute neutrophile count (ANC) <0,5x10^9/l after intensive induction therapy according to the …3+7" regimen. A risk score for mortality was estimated for each patient representing summary of all significant variables in a logistic regression model, including: age, sex, hemoglobin level (Hb); white blood cell count (WBC), plateled count (Plt); serum lactate dehidrogenase (LDH) level; Performance Status (PS) by Eastern Cooperative Oncology Group- Performance Status (PS); Karyotype risk group by European LeukemiaNet (ELN) recommendation; Hematopoietic Cell Transplantation Comorbidity Index (HTC-CI); duration of neutropenia; and complications (gram + /gram- sepsis, fungal infection, pneumonia, hypotension, hypoalbuminema, ARDS (acute respiratore distress sy), elevation of serum creatinine, bilirubin and transaminases level).
Results: The average age of the pts was 52 years (19-72). Female/male ratio was 68/50. Median folow up was 34 months. Favorable cytogenetic risk was found in 10 pts (8,5%); intermediate in 86 prs (73,5%) and adverse in 21 pts (17,9%). The median duration of neutropenia was 17 days (2-42). Observed complications during FN in our pts were: elevation of serum creatinine in 9 pts (7,8%); elevation of serum bilirubin level in 32 pts (27,8%); and elevated serum transaminases in 18 pts (15,7%). Furthermore, hypoalbuminemia had 62 pts (53%); pneumonia 49 pts (41,5%); fungal infection (probable/proven) was found in 41 pts (34,7%); gram + sepsis in 4 pts (3,4%); gram – sepsis in 11 pts (9,3%); hypotension in 23 pts (19,5%), and ARDS in 19 pts (16,2%). Complete remission was achived in 61 pts (53,5%). Early death (ED) rate was 33 pts (28%). There was signifficant association of FN duration with following factors: Presence of fungal infection (p=0,005); Occurance of ARDS (p=0,002); ELN Cytogenetic risk group (p=0,007); elevated serum creatinine level (p=0,017) and ECOG PS (p=0,048). Applying binary logistic regression model, we classified risk score for FN developement of into three groups: low risk (0-3); intermediate (4-7) and high (8-12). In our analysed group 19 pts (17,9%) were of low risk for FN, with mortality of 0%; 51 pts (48,1%) had intermediate risk, with mortality of 27,5% and 36 pts (34%) was in high risk, with mortality of 44,4%.
Summary and Conclusions: This validated risk model help clinicians in early identification high risk patients for more intensive supportive care measures.

PB1479

ADVERSE PROGNOSTIC SIGNIFICANCE OF WILMS TUMOR GENE 1 OVEREXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) is characterized by the highest Wilms tumor gene 1 (WT1) expression levels in comparison to other types of acute myeloid leukemia. However, data on prognostic significance of WT1 gene overexpression in APL are insufficient.
Aims: To evaluate prognostic value of WT1 overexpression in APL patients.
Methods: Data on WT1 expression in 28 *de novo* PML-RARA positive APL patients (median age 43.5 years; female/male ratio 15/13; median follow-up 27 months) were analyzed prospectively. The patients were managed in the Clinic of Hematology from 2008 to 2013 with all-trans retinoic acid combined with anthracyclines. Fresh bone marrow samples were collected at diagnosis (28 patients), after the first consolidation and after that every six months (20 patients). WT1 expression levels were quantified by RQ-PCR method, using TaqMan chemistry. Abl served as a housekeeping gene. Relative quantification analysis was performed using comparative Ct method (2-ddCt), where ddCt = dCt(sample) -dCt(healthy(median)). Using mean WT1 expression level + one SD as a cut-off-value, patients were classified into two groups: with high or low expression.
Results: Pretreatment patients' characteristics were as follows: median WBC count 1.5x10^9/L (range: 0.4-88); median platelet count 25x10^9/L (range: 4-101); Sanz' s risk stratification: high 7/28 (25%), intermediate 11/28 (39%), low 10/28 (36%); hypergranular form 27/28 (96%); additional cytogenetic abnormalities (ACA) 2/28 (7%), PML-RARA type stratification: bcr1 14/28 (50%), bcr2 2/28 (7%), bcr3 12/28 (43%); FLT3/ITD positive 6/28 (21%) and FLT/D835 positive 3/28 (10.7%). Early death (ED) rate and relapse rate were 6/28 (21.4%) and 4/22 (18.2%), respectively. WT1 expression was significantly higher in APL patients, compared to healthy controls (mean 4123.57 ±725.91, range 13.64-14164.5813.64-14164.58 *vs.* 1.95±1.18, range 0.06-8.77, p<0,001). Nine patients (32.14%) were categorized into high expression group. High WT1 expression levels were significantly associated with high WBC counts (16.34x10^9/L *vs.* 4.94x10^9/L, p=0,003), higher relapse-risk score (5/9 (55.6%) *vs.* 2/19 (10.5%), p=0,001), bcr3 transcript variant (6/9 (66.7%) *vs.* 4/19 (21.15), p=0,05), FLT3/ITD mutation (4/9 (44.4%) *vs.* 2/19 (10.5%), p=0,03), higher ED rate (4/9 (44.4%) *vs.* 2/19 (10.5%), p=0,05) and shorter overall survival (12.5 *vs.* 24.1 months, p=0,05) compared to low WT1 expression levels. However, no correlation was found with age, platelet count, percentage of peripheral blood blasts and promyelocytes, microgranular subtype, expression of the surface markers, ACA and incidence of differentiation syndrome. In the majority of the patients (N=14) >2 log reduction in WT1 expression was detected after the first consolidation with mean WT1 expression level of 24.34 ±2.52, range 0.95-72. The increase in WT1 expression of at least 1,5 log was observed in samples during relapse, at the same time or even before PML-RARA positivity.
Summary and Conclusions: Our study speaks in favor of negative prognostic value of high WT1 expression levels in APL. WT1 expression levels represent a sensitive marker for MRD monitoring, too. Further prospective trials should evaluate whether this parameter should be included in risk stratification.

PB1480

AGE OVER 40 YEARS AND RENAL FAILURE ARE PREDICTIVE OF ATRA RELATED COMPLICATIONS IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Background: ATRA is considered a major advance in the front line treatment of APL.In combination with anthracycline this drug improved rate of remission and survival but remains associated with several side effects with DS being the most serious one.
Aims: The aim of this study was to identify predictors of ATRA related side effects.
Methods: 71 consecutive genetically confirmed APL were treated in the hematology department of Aziza Othmana Hospital with an AIDA based regimen.
Results: According to Sanz score 25 (35%) ,41 (58%) and 5 pts (7%) were high, intermediate and low risk respectively.61 pts achieved complete remission (87.1%),1 pt died before treatment and 9 during induction phase from severe bleeding (3 pts), DS (4 pts), Myocardial infarct (1 pt), and Septic Shock (1 pt). Prothrombin time<50% (P=0.008) , Renal failure (P=0.021)and High risk Sanz group(P=0.041) were predictive of induction death. 24 pts developed DS which was severe in 16 pts. 2 of 18 children *vs* 22 of 52 adults developed DS (P=0.016) .Other ATRA related complications were scrotal ulcer (4 pts), oesophageal ulcer (1 pt), splenic infarct (1 pt), Sweet syndrome (3 pts) ,headache (17 pts) , myocarditis (2 pts) and myocardial infarct (1 pt). 6 pts relapsed and 2 died in CR from cardiac failure and septic shock leading to 5 years EFS and OS of 72.9 and 74.3% respectively.
Multivariate analysis revealed that high BMI >30 (P=0.031, OR=4.9), high WBC (P=0.013, OR=5.7) and renal failure (P=0.004, OR=10.5) are predictive of DS. Age over 40 years (P=0.029) and renal failure (P<0.0001) are predictive of ATRA related complications.
Summary and Conclusions: Our study suggest that probably reducing ATRA dose to 25 mg/m^2 in adults can be effective as in children but less toxic .

PB1481

PML-RARA FUSION GENE TRANSCRIPTS AND BIOLOGICAL FEATURES IN A POPULATION WITH ACUTE PROMYELOCYTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background: Acute promyelocytic leukaemia (APL) is characterized by the t(15;17), which produces the *PML-RAR*α fusion gene. It´s a distinct subset of Acute Myeloid Leukemia, known by an excellent response to all-trans-retinoic acid (ATRA) treatment, in spite of the high prevalence of early haemorragic complications. Depending of the PML's gene breakpoint in chromosome 15, the

transcript subtypes bcr1 (long), bcr2 (variant) and bcr3 (short) can be formed. The correlation between these transcript subtypes and clinical parameters is controversial. Some studies showed that the s-bcr3 type can be related with increased white blood cell counts and shorter survival; however, other groups found different results.

Aims: To identify PML-RARα isoform frequencies and subtypes in a population of patients, to study its haematological characteristics and to analyze the correlation between them.

Methods: Data from patients with APL diagnosed between 2005 and 2013 were reviewed. Only patients with molecular identification of the PML-RARα subtype were included. Clinical features and haematological parameters were analyzed at presentation, patients were stratified by Sanz risk score and statistical analysis was made using STATA v12.0.

Results: In this study 46 patients were included, 50% men, median age of 43 years (range 14-78 years). The l-bcr1 subtype was found in 44% (20) patients, the v-bcr2 in 15% (7) and the s-bcr3 in 41% (19) patients.

Seventeen (37%) patients were in the high risk category, 21 (46%) in intermediate risk and 8 (17%) patients had a low risk APL. The mean value for haemoglobin was 9.5 g/dl, for leucocytes was $22x10^9$/L and for platelets was $35x10^9$/L. No significant differences were found between these values for the 3 isoform subtypes. As usual in APL, most patients had coagulopathy (72%), with low fibrinogen (mean value 1.7 g/L) and high d-dimers (mean value 20.09 µg/ml). No correlation was found between the coagulation values and molecular subtypes of PML-RARα. The mortality rate was 30% (14 patients). Of these, 8 were early deaths - 4 patients died of haemorragic complications, 2 of differentiation syndrome secondary to ATRA therapy, and 2 because of infectious complications. Relapse was observed in 4 patients and 2 of these had 2 consecutive relapses. One of these 4 patients is still alive and in complete molecular remission. Long-term overall survival (OS) of this population is high with 3 year OS of 77%. In this study, there were no significant differences between the OS of the 3 isoform subtype groups.

Summary and Conclusions: The most frequent PML-RARα isoforms found in this population were l-bcr1 and s-bcr3, in concordance with previous published data. The OS of this population was high as expected, in spite of the high prevalence of coagulopathy and an increased proportion of high risk patients.

However, no significant correlation was found between the haematological parameters, age, sex and OS of the 3 PML-RARα isoforms, wich can be related to the small dimension of the population analysed.

This leads us to suggest that larger, multicentric studies are needed to clarify the hypothesis presented by some groups of different clinical and survival results according to the identified molecular subtype.

PB1482

HIGH INTENSITY INDUCTION CHEMOTHERAPY IS FEASIBLE FOR ACUTE MYELOID LEUKEMIA PATIENTS OLDER THAN 60 YEARS – A SINGLE CENTER EXPERIENCE

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Background: Acute myeloid leukemia (AML) is most commonly diagnosed in elderly patients. Despite a progress in the treatment of young AML patients, treatment decisions in the elderly patients are still controversial. Few data are available on the outcomes of intensive chemotherapy among elderly patients with AML.

Aims: To describe the outcomes of treatment and early adverse events as well as to identify prognostic factors for the achievement of overall survival (OS) and complete remission (CR) in AML patients older than 60 years that are eligible for intensive chemotherapy.

Methods: Data from 52 electronic charts of consecutive elderly patients with newly diagnosed AML, hospitalized in our ward between April 1st 2007 and January 1st 2013 for intensive induction treatment, were collected and analyzed. 38 patients received the 3+7 regimen and the remaining 14 patients received alternative intensive regimens such as the FLAG regimen. The primary endpoint of this study was OS and the secondary end points were the rate of complete remission (CR & CRi) and disease free survival (DFS). Factors associated with OS were examined.

Results: Table 1 shows patient characteristics. 38 patients (73%) achieved CR after 1-2 courses of induction chemotherapy and overall 14 patients (27%) were considered as treatment failure. Out of the 38 patients who achieved CR, 6 patients were ineligible for further treatment, 17 patients (45%) received consolidation with chemotherapy only and 15 patients (42%) proceeded to reduced intensity conditioning allogeneic hematopoietic cell transplantation (RIC allo- HCT). The OS at 2 and 3 years for all patients was 29% and 23%, respectively. Longer OS was associated with CR achievement (19.3 *vs.* 2.32 months), allo- HCT in CR1/CR2 (19.6 *vs.* 6.13) and ECOG 0-1 (14.4 *vs.* 5.9 months) according to univariate analysis (all p<0.05). Age, disease risk according ELN and NCCN and type of induction therapy were not significantly associated with survival.

Table 1. Patient characteristics.

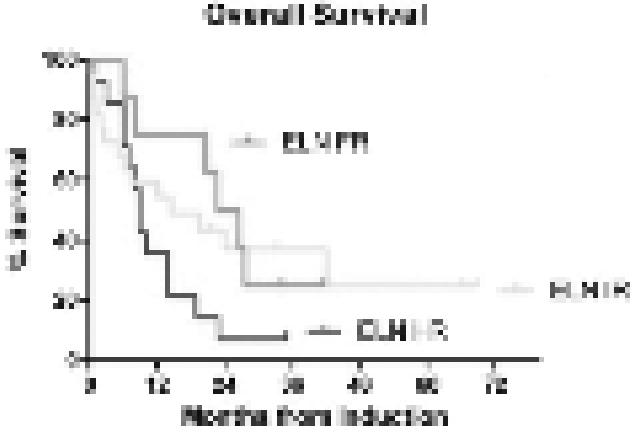

Figure 1.

Summary and Conclusions: Our results indicate that while the prognosis of elderly AML patients is poor, selected eligible patients might benefit from intensive treatment aiming for the achievement of CR. ECOG status before treatment rather than conventional prognostic markers should be used to triage elderly patients for intensive chemotherapy. While the role of post remission therapy in the elderly is still a matter of debate, RIC allo-HCT for patients who achieve CR should be considered.

PB1483

A PROPOSAL FOR MINIMAL DIAGNOSTIC PROCEDURES BASED DIAGNOSTIC ALGORITHM FOR ACUTE MYELOID LEUKEMIA (AML): SINGLE CENTER EXPERIENCE

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Background: Modern concept of the individual therapeutic approach for acute myeloid leukemia (AML) cases is based on exact classification and prognosis of the AML subtype at diagnosis. A multimodal diagnostic approach which combines different diagnostic techniques is needed to meet these requirements. The diagnostic process is becoming more demanding with respect to experience, time and costs due to the expansion of methods and algorithms, which guide the diagnostic procedure from basic to more specific methods and which finally lead to results that are essential for modern diagnostics and therapeutic concepts. There are numerous overlaps between different diagnostic methods.

Aims: In order to establish and standardize a diagnostic algorithm based on a

minimal diagnostic procedures and to improve the diagnosis and management of acute leukemia in Republic of Macedonia, we conduct a prospective study at the University Clinic of Hematology-Skopje.
Methods: A total of 67 (>15 years) adult patients with acute leukemia who were consecutively admitted at the Clinic were enrolled in the study. The aim of our study was to establish the correct lineage assignment of the blast cells and to evaluate the incidence of the favorable genetic markers PML/RARa, AML1/ETO, CBFb/MYH11 among the AML cases. Furthermore, the obtained results were correlated with patients' age, comorbidities, and performance status, and consecutively effective treatment strategy for each single acute leukemia patient was selected. The diagnosis of acute leukemia was made by standard morphological examinations and cytochemical analyses of bone marrow smears according to the criteria established by the French-American-British (FAB) Study Group and confirmed by immunophenotyping of bone marrow aspirates and/or peripheral blood samples following the criteria of the European Group for the Immunological Classification of Leukemia's (EGIL) and the British Committee for Standards in Hematology (BCSH).
Results: Morphological and cytochemistry analyses assigned myeloid lineage in 83.6%of the patients, but in 16,4%of cases additional immunological analyses were need for establishment of the lineage assignment. Furthermore, using a reverse transcriptase-polymerase chain reaction (RT- PCR) assay 12 pateints were classified in the prognostic favorable AML group; 5 patients were positive for PML/RARa, 5 patients for AML1/ETO and 2 patients for CBFb/MYH11 fusion trasncripts. The applied multimodal diagnostic approach enabled improved and more precise diagnosis and clinical stratification in 34.3% acute leukemia patients from our study. Moreover, when we correlate those results with the results obtained from the analyses of the ECOG performance status and the incidence of the comborbidities in our study group, an additional 12.5% of the patients were stratified to a different risk adapted therapy.
Summary and Conclusions: Our initial results are consistent with literature data and indicate that our applied multimodal diagnostic approach based on minimal diagnostic procedures enables definition of specific genetic entities of AML and allows further individual clinical stratification in treatment protocols of the patients.

PB1484

AZACYTIDINE THERAPY IN UNTREATED AML PATIENTS: RETROSPECTIVE MULTICENTRE REGIONAL EXPERIENCE

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Background: In patients with high risk myelodysplastic syndromes DNA methyltransferase inhibitors induce up to 40% responses and azacytidine (AZA) has shown to prolong survival when compared with conventional supportive therapy. AZA is increasingly used in acute myeloid leukaemia patients (AML) with low marrow blast count or considered unfit for intensive chemotherapy.
Aims: Herein we report a retrospective analysis of AML patients treated with AZA therapy in an Italian region (Liguria).
Methods: From June 2010 to December 2013, 34 AML patients received AZA as front line treatment. Median age was 75 years (range 46-89); males were 60%, females 40%. Thirteen patients had de novo (38%) and 26 (62%) secondary AML. Median marrow blastic infiltration was 30% (range 29-90%). Karyotype was available in 16 patients [normal in 12, del (Y) in one, complex in 3]. Relevant comorbidities were present in the majority of patients (70%), mainly diabetes mellitus, heart disease, hypertension, liver or lung diseases. AZA was delivered at a dosage of 75 mg/sqm s.c. with a 5+2 days schedule. Patients were given a median of 4 courses of treatment (range 1-27). Twenty-five patients (75%) received concomitant recombinant alpha erythropoietin therapy (40 000 U once weekly).
Results: Major infections were recorded in 23 patients (67%). Six patients (18%) died early, mainly for infection associated to disease progression. Twenty-eight patients received at least 3 courses of therapy and were evaluated for response. Complete (CR) and partial remission (PR) were achieved in 5 (15%) and 14 (41%) patients, respectively, for an overall response rate of 56% (19/34). Nine patients did not respond (26%). Hematological response was achieved after a median of 3 courses of AZA (range 1-6). A statistically significant reduction of transfusional support was observed in responding patients ($p<0.05$). De novo disease was the only factor that significantly impacted on response rate (all denovo AML patients were responsive to treatment). Disease progression was observed in 12 out of 19 responding patients. At the moment of analysis 19 patients have died (55%), 15 are alive (45%) with a median follow up of 20 months. Overall 12 months and 20 months projected survival was 48.8%, and 39.4%, respectively. Survival was not different in de novo and secondary AML patients. Survival, was significantly worse in older patients, in patients with high marrow blast count (20 months-projected survival was 57.1% and 18.3% in patients with <30% and > 30% marrow blasts, respectively, $p<0.05$) and in patients showing progression under AZA therapy (20 months-projected survival was 36.4% and 85.7% in patients with and without progression under treatment, respectively; $p<0.05$). Two out of five patients aged 65 or less underwent allogeneic BMT (one in PR and one in CR, after 11 and 6 courses of therapy).
Summary and Conclusions: Our data show the efficacy and feasibility of AZA therapy in the common clinical practice, for newly diagnosed AML patients. High marrow blast infiltration at diagnosis, older age and progression under AZA therapy are poor prognostic factors affecting survival in this patient population. Outcome and quality of life of AML patients treated with AZA seem to compare favourably with that of patients receiving conventional intensive chemotherapy or supportive therapy only.

PB1485

CYTOGENETIC PROFILE AND FLT3 MUTATION OF 1OO ADULT DE NOVO ACUTE MYELOID LEUKEMIA PATIENTS FROM SAUDI ARABIA: CORRELATION TO BIOLOGIC FACTORS AND OUTCOME

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Background: Diagnostic cytogenetic is regarded as one of the most valuable prognostic indicators in acute leukemia. Clonal chromosome abnormalities can be detected in approximately 55-78% of adult acute myeloid leukemia (AML) and in 78-85% of childhood AML. AML patients are categorized into good, intermediate and poor-risk groups according to cytogenetic changes.
Aims: No sufficient data exist regarding cytogenetic profile in Saudi AML patients. Most of the data reported were from western countries. Our aim was to define the frequencies and subtypes of chromosomal abnormalities among adult AML patients in the Saudi population and to compare the cytogenetic profile with those reported from other populations and did a correlation to other biologic factors.
Methods: From 2004 to 2013, we reviewed all cases with established diagnosis of Adult AML (100 cases). Informed consent was obtained.
Cytogenetic Analysis: Chromosome banding analysis and fluorescence *in situ* hybridization (FISH) were used to detect genetic aberrations. *Analysis of FLT3 mutations:* Bone marrow or blood samples were screened for *FLT3* mutations (internal tandem duplications, ITDs and point mutations, D835) using polymerase chain reaction methods (PCR).
Results: Table 1: Summarizes patient's characteristics and Cytogenetic and molecular findings. Our patients are categorized into good (13), intermediate (58), and poor-risk (29) groups according to cytogenetic changes.
Out of 100 cases, 20 cases were positive for FLT3 mutations with overall frequency of 20% (15/20, ITD and 5/20, D835), the frequency of cytogenetic abnormality in FLT3 positive group 8/20 (40%) while in FLT3 negative group was 34/60 (57%). Successful cytogenetic analysis 80/100 by FISH (80%) and 58/100 by Conventional cytogenetics (58%) showed chromosomal anomalies in 42 out of 80 cases with overall incidence 42/80 (53%). The most frequent cytogenetic abnormality was t(8;21) detected in 7/80 (9%) of successful cases, followed by trisomy 8 in 8/80 (10%) , t(15;17) in 5/80, (6%) , Inv 16 / t (16;16) in 4/80 (5%), and -5, +5, and 11q23/MLL each in 3/80 (4%) . The most frequent numerical abnormality was of chromosome 8, occurring in 9/80 (11%), 8 gain (+8) and one loss (-8) with or without additional abnormalities. This frequency was similar to that seen in most western countries (6–13.9%) but higher than most of Asian countries (3.8-7.1%). The mean age of patients with translocation abnormalities was significantly younger than that of patients with normal, deletion or trisomy anomaly. This study revealed the occurrence of false negative results by conventional cytogenetics analysis and highlighted the complementary nature of FISH in detecting cryptic genetic abnormalities in AML patients [one case in our study, t(15;17)]**.** Our data are in accordance with those published showed that *FLT3*-ITD mutation have a strong bad prognostic factor in AML patients and associated disease progression with high rate of relapse, shorter overall survival; (P=0.036) and event-free survival (EFS) was also worse (P=0.040**)** compared to standard risk group.
Summary and Conclusions: The frequencies of *FLT3* and cytogenetic abnormalities were slightly lower than those reported in the literatures but not reach significant, while its prognostic relevance was similar to those reported population from Asia and western countries. These data confirm that *FLT3* mutation occur in a significant percentage of Saudi AML and had bad prognostic relevance. More cases should be collected to clarify these findings.

PB1486

THE ROLE OF FLOW- CYTOMETRIC IMMUNOPHENOTYPING IN THE EARLY PROGNOSTIC STRATIFICATION OF ACUTE MYELOID LEUKEMIA PATIENTS: SINGLE CENTER EXPERIENCE

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Background: Flow cytometric immunophenotyping (FCI) has a well established role as a diagnostic modality in acute myeloid leukemia, particularly as a tool for assigning lineage and facilitating further pathologic classifications. So far, there is no clear consensus regarding the prognostic implications of FCI in AML in contest of predicting treatment response, induction death or overall survival.
Aims: In this study we evaluate possible role of the leukemia associated immunophenotype in the early prognostic classification of AML regarding the treatment response, especially the rate of early induction remission.
Methods: In our study we evaluate one hundred and fourteen (114) consecutive adult (>14 years) AML patients. Acute promyelocytic leukemia (APL) cases were excluded because they received different treatment. The diagnosis of AML was made by standard morphological examination and cytochemical analyses of peripheral blood/bone marrow samples to the criteria established by FAB Cooperative Study Group and confirmed by immunophenotyping of bone marrow aspirates and/or peripheral blood samples following the criteria of the European group for the immunological Classification of acute leukemia (EGIL).
Results: All patients received anthracycline plus cytarabine-based induction chemotherapy After 42 days from the initiation of the treatment 55.8% of the patients (male: female=29;20; median age 49, 8) achieved complete remission (CR), 17, 7% of the patients experienced early induction dead, and 30, 8% of the patients had residual disease. The expression of CD34 and HLA-DR or a combination of those markers was different between the remission and early induction dead group, with the statistical significance of $p<0,05$ for the CD34 expression. None of the other analyzed panmyloid markers or their combinations, nor the aberrant expression of the lymphoid markers was found to correlate with the induction treatment outcome. Moreover, our analyses showed that CD34 expression was associated with leukocyte count $>100 \times 10^{(9)}$ /l. A further analysis of the succumbed patients showed that risk factors for early induction death are also comorbidity index score ≥3 and age > 65 years.
Summary and Conclusions: Our results showed that results from FCI, especially expression of CD34 and HLA-DR alone, or their coexpression in correlation with age >65 years, and comorbidity score ≥3 can be used as early predictive marker for induction therapy outcome and may facilitate early individual therapeutical stratification of AML patients.

PB1487

ACUTE MYELOID LEUKEMIA IN PREGNANCY: A SINGLE CENTRE EXPERIENCE

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Background: The incidence of acute myeloid leukemia (AML) is estimated to be 1 in 50-70.000 pregnancies. Due to aggressiveness of disease, treatment cannot be delayed indefinitely and balance between chemotherapy (CTx) consequences on both fetus and mother, as well as the effects of postponing treatment must be carefully evaluated. In the 1st trimester the risks of CTx during organogenesis must be considered and discussed carefully. In the 2nd or 3rd trimester CTx with cytarabine and daunorubicin can be administered monitoring fetal cardiac function and growth.
Aims: To describe our experience consisting of 5 cases of AML diagnosed during pregnancy.
Results: -A 31 year old woman, 14 weeks pregnant, presented with severe leucopenia: bone marrow (BM) was consistent with AML. After 5 days of multidisciplinary consulting, the patient (Pt) asked for surgical abortion. Then she underwent induction CTx and consolidation with HSCT. Two years after the women is alive and in CR. A 36 year old woman, 32 weeks pregnant, was admitted with severe anemia, thrombocytopenia and LDH increase. An urgent cesarean section was performed and an healthy female was born. BM revealed AML and the patient received conventional induction and consolidation CTx. Eight months after, because of recurrence, she underwent autologous HSCT. Five years later she is alive and in CR. A 32 year old women, 26 weeks pregnant, presented with severe pancytopenia. US showed normal fetus growth. BM was consistent with RAEB-1. We decided for close monitoring, supportive Tx and betamethasone. Elective cesarean section was performed at 32+2 without complications. Soon after delivery, disease progressed to AML. The Pt received induction and consolidation CTx with HSCT. She is alive and well. A 34 years old woman, 31 weeks pregnant, presented with abdominal pain, asthenia and fever, thrombocytopenia, elevated LDH and D-Dimer with fibrinogen 167 mg/dl. At US examination fetus was still alive. However, an urgent cesarean section lead to extraction of a dead fetus of 1865g. Placenta resulted histologically involved by AML with hematoma and infarcts of surrounding parenchima. BM examination confirmed AML without maturation. She received induction and consolidation therapy and is still in CR. A 39 years old woman, 24 weeks pregnant, was referred to our hospital for management of AML. Blood values showed severe anemia, thrombocytopenia and leukocytosis. Betamethasone was administered and she underwent induction CTx with daunorubicin and cytarabine in continuous infusion (3+7 schedule). At 30 weeks of gestational age a caesarean section extracted a living male of 1495 grams. The Pt died after 4 months for sepsis during consolidation therapy.
Summary and Conclusions: From 2006 to 2012, at our Institution, 5 pregnant women were diagnosed with myeloid malignancies. One Pt had an intrauterine death at diagnosis; one chose surgical abortion before starting CTx, while 3 continued a regular pregnancy and delivered a healthy baby; one of them underwent CTx during pregnancy and died some months after delivery for septic shock in CR. In summary 4 women are currently living in CR and 3 healthy children were born. Our cases indicate that treating AML in pregnancy is demanding, but feasible. The clinical approach must be multidisciplinary: the specialists team must consider the nature of the disease (MDS *vs* AML), must discuss the appropriate timing for any intervention (both delivery and chemotherapy), with a continuous and careful evaluation of both maternal and fetal risk, during all the period of cure.

PB1488

ADDITIONAL CHROMOSOMAL ABNORMALITIES IN ACUTE PROMYELOCYTIC LEUKEMIA: A SINGLE-INSTITUTION EXPERIENCE

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Background: Additional cytogenetic abnormalities (ACAs) are reported in 23-39% of acute promyelocytic leukaemia (APL) cases. Even if most studies report no impact on clinical outcomes in patients treated with ATRA and chemotherapy, the prognostic significance of ACAs is still not clearly defined. In particular, recently, Wiernik et al demonstrated, in a cohort of 140 APLs, a reduced ATRA sensitivity in patients with karyotypic evolution, compared to those characterized by t(15;17) alone.

Aims: To describe the incidence and the prognostic value of the presence of ACAs in patients affected by APL referred to our Institution since 2002
Methods: From 2002 until 2013, a total of 27 patients were diagnosed as affected by APL at our Hematologic Unit. There were 13 males and 14 females, with a median age of 43 years (range 13-76 years). Six patients were classifiable as high risk (*i.e.* WBC > $10x10^9$/L), 9 were intermediate risk (PLT<$40x10^9$/L) and the remaining 12 were low risk (PLT > $40x10^9$/l). All the patients were treated with chemotherapy plus ATRA (in the majority of cases as per AIDA 2000 schedule). Only one patient received arsenic trioxide (ATO) plus ATRA as induction therapy followed by maintenance with the same drugs. One patient had clinical characteristics, bone marrow morphology and immunophenotype (HLA-DR+) not consistent with APL, and received ATRA only after cytogenetic results acquisition.
Results: Among the 27 consecutive APL cases (24 de novo, 3 therapy-related) patients, 18 (67%) had t(15;17) as the only chromosomal alteration, 1 (3%) had no metaphases analysable and 8 (30%) had an ACA at the cytogenetic analysis (Table 1).

Table 1.

No statistical differences were observed in the two groups in terms of age, sex or WBC count. Median follow-up was 34 months. We had a total of 5 relapse at a median time from diagnosis of 14 months. Only 1 relapse was observed in patients with ACA: this case carried t(5;21)(q31q22) in combination to t(15;17) in 3 metaphases and only t(5;21) in 17; the aquired , non constitutional nature of the translocation t(5;21) has been confirmed by a cytogenetically normal result of phytohemagglutinin-stimulated blood analysis. The patient obtained molecular remission and a normal karyotype after the first consolidation cycle; however he had a bone marrow molecular relapse with asymptomatic CNS involvement after 3 months maintenance therapy. Three patients of our cohort died: we had two early deaths, both for intracranial hemorrhage, while one patient died for progressive disease after a third relapse treated with familiar HSCT. All these 3 patients had t(15;17) as the only chromosomal alteration. Overall survival seem not to be influenced by the presence of ACA.
Summary and Conclusions: In conclusion, we report an incidence of 30% ACAs in APL, consistently with published studies. We did not observe any chromosome 8 trisomy, described as the most common ACA, and reported an additional balanced translocation, t(5;21), which, to our knowledge, has never been described in APL. This patient had an early cytogenetic relapse with CNS involvement. The analysis of additional cases may help to assess a possible prognostic role for this translocation. In our series, the presence of ACAs did not seem to impact on APL clinical characteristics and outcome.

PB1489

THE EVALUATION OF NO AND LDH SERA LEVEL REGARDING THE FAB SUBTYPES OF AML

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Background: Nitric oxide (NO) is a multifunctional molecule produced in a variety of cells. There are many other molecules which it can interact, that it is quickly consumed close where it is synthesized. At lower levels NO is associated with vasodilatation and neurotransmission. At higher levels, NO has tumoricidal and bactericidal effects .
Aims: Several lines of evidence indicate that NO has cytotoxic effects on human cell lines from patients with leukemia or lymphoma. We investigated the level of NO and LDH at diagnosis of acute myeloid leukemia and correlated its with FAB subtypes.
Methods: This study included 22 man and 18 woman of AML patients, aged 25 to 68 years (median 50 years). Bone marrow aspirate was analyzed with classic cytochemical staining and immunophenotyping of blast cells by flow cytometry (Becton Dickinson) with monoclonal antibodies. Cytogenetic examination of bone marrow cells revealed a normal karyotype in 20 pts. In 1 pts t(15;17), trisomy 8 in 1, del(9) in 1, hyperploidy in 1, del(16) in 2, inv(16) in 3 and Ph chromosome in 3 patients were found. *NO* analysis was performed with Griess reaction. The level of NO was determined with ELISA spectrophotometer (Behring, ELISA reader, Germany) on wave length 570 nm. LDH activity in sera of AML patients was determined using Ectachem clinical chemistry slides with a multipoint rate slides test (Jonson & Johnson Clinical Diagnositic, USA) on the Ectachem 259 Analyser
Results: Results show that patients with AML have significantly different values of sera NO depending on FAB subtype of AML . The patients with M0 subtype of AML have higher values of NO (27.17 mmol/L) than other subtype of AML which also correlates with other poor clinical and cytological characteristics of undifferentiated type of AML. In comparison to M0, patients with M4 subtype show values of N0 of 5.45 mmol/L, which is statisticaly significantly lower in comparison to M0 (Mann Whitney U test, p=0.009). Sera NO in AML patients with M2 subtype showed values of 14.12 mmol/L which is significantly different in comparison with M4 (Mann Whitney U test, p=0.02), but with no difference in comparison with M1 (Mann Whitney U test, p> 0.05). Lowest values of NO in M4 subtypes correlated with favorable cytogenetic findings (inversion and deletion of chromosome 16) and with good prognosis. Significant positive correlation between NO and LDH was found in this study. AML patients with sera LDH below 300 U/ml show at the same time lowest sera NO and AML patients with elevated LDH (over 1000 U/ml) have elevated sera NO. Correlation between percentage of blast cells in bone marrow and NO was also found. Pearson correlation (p<0.05) shows that increase of blast cells in bone marrow is accompanied with high sera NO level.
Summary and Conclusions: These data indicated that NO is one of indicators of metabolic disturbance in malignant cells and especially of undifferentiated cells types. We conclude that this parameter could be used simultaneously with other prognostic factors for analyses in AML patients.

Chronic lymphocytic leukemia and related disorders - Biology

PB1490

BENDAMUSTINE USED ALONE OR IN COMBINATION WITH RITUXIMAB MODIFIES THE EXPRESSION OF APOPTOSIS-INVOLVED GENES DEPENDING ON IGVH MUTATIONAL STATUS OF CLL CELLS

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Background: Bendamustine (BENDA) used either alone or in combination with rituximab (RIT) is effective in the treatment of chronic lymphocytic leukemia (CLL). However, the influence of these drugs on the genes expression profile is still not fully understood and the expression of apoptosis-involved genes depending on IGVH mutational status has not been studied yet.
Aims: The aim of the study was to evaluate the influence of BENDA used alone or jointly with RIT on the apoptotic genes expression of CLL cells *in vitro* and to answer the question if the expression of these genes depends on the IGVH mutational status.
Methods: Peripheral blood samples were collected from 11 newly diagnosed CLL patients (5 F, 9 M) aged 49-81 (mean 66) yrs. All specimens were collected after written consent of patients. The IGVH gene was mutated (IGVH+) in 7, and unmutated (IGVH-) in 4 patients. Peripheral blood CD19+ cells at the concentration of $1.0x10^6$ cells/ml were incubated in RPMI1640 containing 10% fetal calf serum and 10% autologous serum with addition of BENDA (40mg/ml), RIT (10 mg/ml) or BENDA (40 mg/ml)+RIT (10mg/ml), for 48 hours. Control cultures were incubated without the drugs. After standard RNA isolation and cDNA preparation the TaqMan Low Density Array for PCR analysis of 96 gene transcripts (93 examined and 3 housekeeping genes) was used. The results showing the mean expression level at least 100 higher than the remainder were excluded from the microarray analysis. The fold change (RQ) value was defined as the ratio of the gene expression level after incubation of cells with a drug to the expression level in a control culture. RQ <0.5 or >2.0 was considered as significant.
Results: In the IGVH+ group BENDA led to a significant increase of 19 genes expression. Eleven of them encoded proapoptotic proteins, including 7 (*BBC3, BAX, BOK, CARD-9, APAF-1, CASP9, LRDD*) involved in the mitochondrial pathway and 4 (*FAS, CASP10, TNFRSF10, TNFRSF10B)* in the extrinsic pathway. In the IGVH- group BENDA increased the expression of 24 genes: 17 of them *encoding* proapoptotic proteins, including 8 involved in the mitochondrial (*BAK, BAX, BBC3, BNIP3L, BOK, CARD15, DIABLO*, *PYCARD*), and 4 in the extrinsic apoptotic pathway (*CASP14, CASP6, FAS, PYCARD*). The expression of 4 genes (*BAX, BBC3, BOK, FAS)* increased in both IGVH+ and IGVH- groups. In the IGVH+ group RIT significantly increased the expression of 16 genes, 9 of them encoding the proapoptotic proteins. In the IGVH- group RIT modified the expression of 56 genes increasing it in *49* (32 proapoptotic and 13 antiapoptotic), and decreasing it in 7 (6 proapoptotic and 1 antiapoptotic).The expression of 13 genes (*BCL2A1, BCL2L10, BCL2L14, BOK, CASP4, CASP5, CASP7, CRADD, DAPK1, ICEBERG, RIPK1, TNFRSF10B, TNFRSF21*) changed independently of the IGVH mutational status. BENDA+RIT led to an increase of the expression of 34 genes and a decrease of one gene in the IGVH+, and to an increase of 10 genes and a decrease of 3 genes in the IGVH- group. Both groups showed an increase of the expression of 10 genes and a decrease of one gene, 9 of which were proapoptotic (*BAX, BBC3, BOK* involved in the internal pathway; *CASP14, FAS, TNFRSF10B* in the extrinsic pathway and *NFKB1, BCL2L14, CASP5* probably in both the pathways) and 2 antiapoptotic.
Summary and Conclusions: We suggest that BENDA used alone or with RIT induces preferentially the expression of proapoptiotic genes, especially those involved in the intrinsic pathway. This effect seems to depend on the IGVH gene mutational status.
The work was supported by Mundipharma Research LtD grant.

PB1491

ARRAYCGH ANALYSIS OF CHROMOSOME 13Q DELETIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The cytogenetic abnormality most frequently detected by fluorescence *in situ* hybridization (FISH) is deletion of 13q14 present in ~50% of patients with chronic lymphocytic leukemia (CLL). As a single aberration, deletion of 13q14 is associated with favorable prognosis. The prognostic value of other changes such as biallelic deletion of 13q14, monosomy 13 or translocation of chromosome 13 remains controversial. Efforts to clearly determine the extent of the deletion, significant genes located in the deleted region and clinical impact of the changes are still a matter of research.
Aims: To perform arrayCGH analysis of a group of patients with deletion of 13q14, to determine the extent of the deletion and to compare findings in patients with a single aberration, those with deletion of 13q14 and one additional change and those with complex aberrations. Furthermore, to determine the extent of the deleted region and to compare the extent of the deletions with certain clinical parameters.
Methods: A CLL FISH probe panel was used to investigate a group of 671 patients. Of those, 51 patients with detected deletion of 13q were examined with arrayCGH (peripheral blood in 46 pts, bone marrow in 4 pts and a lymph node in 1 pt).
Results: In the group of 671 patients, deletion of 13q14 was determined in 301 (45%) patients. Among those, arrayCGH was used to examine 14 patients with a single aberration of 13q14, 11 patients with two aberrations and 26 patients with complex changes. The subgroup comprised 38 males and 13 females with a median age of 62 years (range, 27–86 years). Seventeen patients had Binet's stage A, 10 patients had stage B and 17 patients had stage C; in 7 patients, the stage was not specified. Unmutated and mutated *IgVH* genes were found in 31 and 11 patients, respectively; in 9 patients, the mutational status was not studied. A total of nine patients died. Ten patients had biallelic deletion of 13q14; this was associated with monoallelic deletion of 13q14 in 8 cases. ArrayCGH determined the smallest deleted region of 850 kb at 13q14.2-13q14.3, containing the *MIR15a, MIR16-1, DLEU1, DLEU2* and *DLEU7* genes. In one patient, deletion of 13q was only determined by arrayCGH at 13q21.1-q21.2 sized 1.1 Mb. Two patients had monosomy 13. Comparison of the detected deletion with certain studied parameters such as *IgVH* mutational status, Binet's stage, type of deletion of 13q and additional chromosomal changes has not confirmed a direct association with the extent of the deletion as yet. Only patients with complex changes tended to have more extensive deletions.
Summary and Conclusions: The arrayCGH analysis of a group of 51 CLL patients with deletion of 13q determined by FISH confirmed a large heterogeneity of the extent of the deletion and tendency of association of large deletion with complex changes. Given the small number of patients in the study, the clinical significance of the investigated deletions of 13q cannot be clearly determined.
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PB1492

***IN VITRO* EFFECTS OF LENALIDOMIDE ON CELL SURFACE ANTIGENS EXPRESSION IN PRIMARY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) B-CELLS: A QUANTITATIVE FLOW CYTOMETRIC STUDY**

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Background: B-cells belonging to CLL clones are characterized by low density of CD20 antigen on their surface. Despite this, anti-CD20 monoclonal antibody Rituximab has been proved to be effective in this lymphoid disorder. Moreover, Lenalidomide (Revlimid, CC-5013, Celgene) has been shown to potentiate the therapeutic efficacy of Rituximab in the treatment of CLL (Badoux et al, 2013). Surprisingly, however, it has been reported that, *in vitro*, Lenalidomide induces a down-regulation of CD20 expression on CLL B-cells surface (Lapalombella et al, 2008). Other authors found no antigenic density changes following *in vitro* Lenalidomide treatment (Hernandez-Ilizaturri et al, 2005). In both these studies the expression of CD20 antigen was evaluated by a flow cytometric analysis using the mean fluorescence intensity (MFI).
Aims: We aimed to evaluate the antigenic density of CD20, and that of other surface antigens likely involved in the immunomodulating mechanism(s) of action of Lenalidomide, by means of a different, quantitative flow cytometric approach (QuantiBRITE™, Becton Dickinson), a more accurate technology to estimate the number of antigens expressed per cell as antibody binding capacity (ABC).
Methods: Lenalidomide was provided by Celgene (Summit, NJ) and dissolved in DMSO at a stock concentration of 1 M. Peripheral blood mononuclear cells (PBMC) were collected, after written informed consent, from 8 untreated CLL patients (6 males; 2 females; mean age 74 yrs; range 58-89 yrs). PBMC were cultured at $0,5x10^6$ cell/ml in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, L-glutamine (2 mM) and penicillin–streptomycin (100

U/ml) and treated with three different doses of Lenalidomide (0.1, 0.5 and 2 μM). For each patient, the same number of cells was cultured without Lenalidomide (controls). PE-conjugated monoclonal antibodies anti-CD20, CD44, CD52, CD80, CD86, and CD95 were employed to determine the antigen expression by flow cytometry (FACSCalibur, Becton Dickinson) at different times (24, 48, and 72 hours).

Results: The ABC value of CD20 antigen on B-cells surface, normalized to the relative control and expressed as fold-change, was found to be significantly increased (mean: from 1.13 at 24 hours to 1.2 at 72 hours) after treatment with 2 μM Lenalidomide (Figure). Changes found after 0.1 and 0.5 μM doses were less relevant. Regarding other antigens, a time-related decrease of CD52 expression levels was reported at all doses of treatment. Interestingly, CD44 and CD95 antigens levels showed a characteristic time-related trend. A decreased CD44 expression observed at 24 hours of treatment was in fact followed by an increase at 48 hours and by a new decrease to the basal levels at 72 hours. The CD95 expression trend, on the contrary, was precisely the opposite of the CD44 one. Finally, Lenalidomide augmented the expression levels of CD80 antigen starting from 48 hours of treatment, while the same effect on CD86 expression was observed at 24 hours.

Summary and Conclusions: Differently from previously reported data and using a more precise approach, a positive modulating effect of Lenalidomide on CD20 expression in CLL B-cells was found. The increase of antigen expression occurred at doses that are comparable to those that are effective *in vivo*. Although Lenalidomide is thought to be pleiotropic in modality of action on the CLL microenvironment, an increase in CD20 expression might contribute to explain the synergistic effect of rituximab in CLL when used *in vivo* in combination with Lenalidomide. We are also currently analyzing in detail the possible role of variable changes in expression of other co-stimulatory molecules we found: more mature data in this setting will be presented at the Meeting.

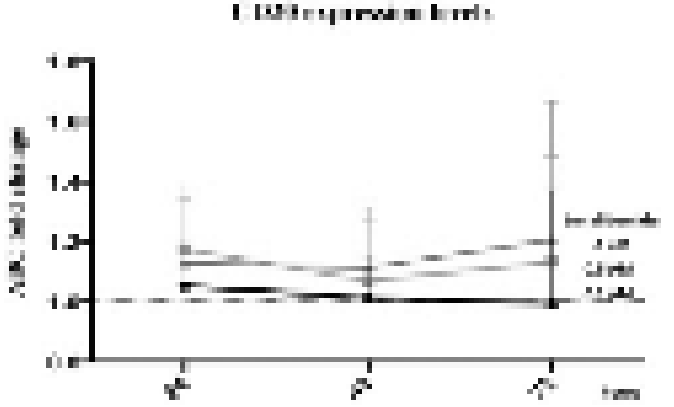

Figure 1.

PB1493

Abstract withdrawn

PB1494

THE SIGNIFICANCE OF KIR GENE POLYMORPHISMS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Natural killer (NK) cell receptors are polymorphic; and this might cause variations in tumour response among different subjects. The escape of leukemic cells from NK immunity might be possible because of the presence of certain Killer Immunoglobulin-Like Receptor (KIR) genes. KIR receptors might play activating or inhibitory roles depending upon the allelic polymorphism.

Aims: We studied KIR gene polymorphisms in chronic lymphocytic leukemia (CLL). We determined whether KIR polymorphisms had any associations with clinical features like stage, prognosis and survival in CLL patients.

Methods: We included 68 CLL patients (28 females, 40 males, mean age: 64 years) and 64 controls (29 females, 35 males, mean age: 66.6 years). Clinical features, laboratory data, treatment modalities and outcome of CLL patients were recorded from medical records. Ethical committee approval and consent from all subjects were obtained. Peripheral blood was drawn from CLL patients and controls; genomic DNA was extracted. PCR was used to determine KIR gene polymorphisms.

Results: Fifty-two CLL patients (76.5%) had early Rai stage (0, 1, 2) disease, 16 (23.5%) were in advanced stages (III, IV). The inhibitory KIR2DL3 genotype was significantly more frequent in CLL patients (66 patients, 97.1%) than in controls (55 subjects, 86.9%) (OR: 4.78, 95%CI: 1.07-21.3, p=0.02). Other evaluated genotypes (inhibitory KIR2DL1, KIR2DL2, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3 and activating KIR2DS1, KIR2DS2 KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1) were similar in two groups (p values >0.05). Early-stage CLL patients had significantly more frequent KIR2DL5 genotype than the advanced-stage group (57.7% *vs.* 6.3%, p=0.02). The former group had significantly more frequent KIR2DS1 genotype than the latter group (42.3% *vs.*12.5%, p=0.03). KIR2DS3 genotype was significantly more frequent in early-stage CLL patients (40.4% *vs.* 6.3%, p=0.01). Early-stage CLL patients had more frequent KIR3DS1 than advanced-stage patients (50% *vs.* 18.8%,p=0.03). The mean number of activating genes were significantly higher in early-stage CLL patients than in advanced-stage patients (3.1±1.6 *vs.*1.88±1.15,p=0.04).

Summary and Conclusions: The inhibitory KIR2DL3 gene might have suppressed activating signals of NK cells, probably preventing lysis of CLL cells, and contributing to disease pathogenesis. In addition, KIR2DL5, KIR2DS1, KIR2DS3, KIR3DS1 genes which were more frequent in early-stage CLL patients might have played preventive roles in disease progression and acted against more severe disease activity.

PB1495

THE NECESSITY FOR THE PARALLEL APPLICATION OF KARYOTYPE AND FISH ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Conventional and Molecular Cytogenetics is an important prognostic and diagnostic parameter in Chronic Lymphocytic Leukemia (CLL) investigation. Recently, the improvement of cultivation techniques, by using new mitogens, combined with fluorescence *in situ* hybridization (FISH) analysis provide the opportunity of the whole spectrum of CLL aberrations to be revealed that was not possible before due to the low mitotic activity of CLL cells *in vitro*.

Aims: We present a systematic cytogenetic study of 237 B-CLL cases using karyotypic and FISH analysis in order to define the cytogenetic aberrations in CLL and their frequencies in greek population as well as to identify the best method for the investigation of the disease.

Methods: Karyotypic analysis was performed on unstimulated and stimulated with the combination of oligonucleotide DSP30 plus IL-2 bone marrow cells derived from 237 patients, aged 32-88 years. The karyotypic analysis was considered successful when a minimum of 20 metaphases were analyzed. FISH studies were carried out in 126 patients using the commercial CLL set probes: LSI p53/LSI ATM και LSI D13S319/LSI 13q34/CEP12 Multi-Color Probe Sets.

Results: The sex ratio (males/females) was 1.63. The median age of the patients was 64.88 years. Karyotypic analysis was successful in 218 patients (92%). A normal karyotype was found in 82 patients (37.61%) and an abnormal in 136 patients (62.39%). Among the abnormal karyotypes, 43 (31.62%) were complex and 64 (47.06%) carried only one aberration. More than 60 different recurrent chromosomal aberrations were detected in our cohort. The most frequent chromosome aberrations in abnormal karyotypes were: +12 (34.56%), del(13q) (16.91%), -Y (13.24%), translocations of chromosome 14q (10.29%), del(11q) (9.56%), del(6q) (8.82%), -17 (7.35%), translocations of chromosomes 13 and 6 (5.88% each) and add(14q) (5.15%). Other not so frequent but recurrent chromosomal aberrations were also found in 50 patients (36.76%). The most common abnormalities found in karyotypes as sole aberrations were +12 (32.81%), -Y (9.38%), del(13q) (7.81%), del(6q) (6.25%), translocations of 14q (6.25%), and del(11q) (4.69%). FISH analysis was successful in all 126 patients while aberrations were detected in 87 (69.05%) patients. Among the abnormal cases, del(13)(q14.3) was found in 57 (65.52%) patients, +12 in 23 (26.44%), del(17)(p13.1)/p53 in 13 (14.94%), del(11)(q22.3)/ATM in 11 (12.64%) and del(13)(q34.3) in 2 (2.30%) patients. Moreover, a homozygous deletion of 13q14.3 was found in 13 (14.94%) patients.

Summary and Conclusions: Karyotypic analysis in CLL revealed a variety of recurrent chromosomal aberrations, most of which have not been identified as CLL specific abnormalities and consequently have still an unknown prognosis. The frequency of abnormalities found by karyotype and FISH was similar but differences in the number and frequency of specific chromosomal aberrations were detected. Trisomy 12 was the most common abnormality in karyotypes while the submicroscopic del(13)(q14.3) was the most common aberration detected by FISH. These differences can be attributed to the ability of FISH to detect submicroscopic aberrations not always detected in karyotypes and the ability of karyotype to provide an overview of all microscopically visible chromosome

abnormalities through the genome. Therefore, the above indicate the necessity for the parallel application of karyotype and FISH analysis in CLL investigation.

PB1496

INCIDENCE OF SECOND CANCER IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA: 13 YEARS EXPERIENCE FROM SINGLE INSTITUTE

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Background: Chronic lymphocytic leukemia (CLL) is most common malignancy in the west and relatively uncommon in India
Aims: Patients with CLL have a higher incidence of second malignancies (two times) than the general population. Our study aims to present the incidence and predicting factor for second cancer in patients of CLL at our institution.
Methods: We retrospective evaluated 320 patients of CLL registered at All India Institute of Medical Sciences (AIIMS) , New Delhi over a period of 13 years (2000-2012) . A second malignancy was defined as another malignancy at the time of diagnosis or during follow-up
Results: There were 230 males and 90 females. The median age was 59 years (28-90 years). The common presenting features were lymphadenopathy 63%, fatigue 25%, fever 20%, hepatomegaly 40%, splenomegaly 55%. Median total leucocyte count at presentation was $47x10^9$/L. Sixtey percent of patients were early (Rai stage 0 - 10%, stage I - 16%, stage II - 34%) and 40% of cases were in advanced (stage III -20% and stage IV - 20%) stage. Fifty percent of patients received treatment at presentation (one hundred twelve patients (70%) received chlorambucil and 40 patients (25%) received Fludarabine and eight patients received bendamustine based chemotherapy). With a median follow-up of 40 months ,total of 24 cancers were identified (18 hematological and 6 solid tumors) . Seventeen cases were transformation to diffuse B cell lymphoma (richter's syndrome)and rest were myelodysplastic syndrome -1, basal cell carcinoma -1, carcinoma of larynx -1, breast cancer -1, adenocarcinoma of lung -1 and locally advanced urinary bladder cancer -1 case each. According to correlation analysis, young age (<55 years, p=0.015) a high expression of CD38 positivity (>30%, p=0.001) and advanced Rai stage (III & IV, p<0.001) were found to be independent predictors of transformation to richter's syndrome (RS). Median time to progression to RS and overall survival was 26 months and 8 months respectively.
Summary and Conclusions: The incidence of second caner is 7.5 % in our population , richter's transformation is the most common and accounts for 5% of cases . Young age, advanced stage and high expression of CD 38 predict poor outcome

PB1497

CYTOKINE PROFILE IN PATIENTS WITH CHRONIC LYMPHOPROLIFERATIVE DISEASES

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Background: Modified cytokine production is especially important in the pathogenesis of hemoblastoses. However, only few studies are available concerning spontaneous and mitogen-stimulated production of pro-, anti-inflammatory cytokines, and colony-stimulating factors in patients with lymphoproliferative diseases.
Aims: Aim of the present study was to evaluate the level of spontaneous and mitogen-stimulated production of pro- and anti-inflammatory cytokines in lymphoproliferative diseases at initial presentation, progression and remission (complete or partial), as a result of chemotherapy.
Methods: Seventy-six patients with lymphoid malignancies: 42 patients (27 men, 15 women, mean age 58.3±9.6 years) with chronic lymphocytic leukemia and small lymphocytic lymphoma; 23 patients (10 men, 13 women, mean age 59.7±8.3 years) with multiple myeloma; 11 patients with diffuse large b-cell lymphoma (6 males, 5 females, mean age 52.0±11.1 years) were included in this study. In order to study spontaneous and mitogen-stimulated cytokine production by blood cells using reagent kit "Cytokine-Stimul-Best", immediately after the punction of the cubital vein 1 ml of blood was added to a vial containing 4 ml of maintenance medium (DMEM), heparin (2.5 U/ml), gentamicin (100 mg/ml) and L-glutamine (0.6 mg/ml). This vial has been used for the study of spontaneous cytokine production. For mitogenic stimulation, 1 ml of resulting diluted blood was transferred to a vial with a mixture of polyclonal activators (lipopolysaccharide, concanavalin A, phytohemagglutinin P and phytohemagglutinin M). Both flasks were incubated during 24 hrs at 37°C, then blood cells were pelleted at 10,000 g for 3 min, the supernatant after separation of the precipitate was frozen and stored at -40 °C until testing of cytokines. The concentration of cytokines in the samples were evaluated using enzyme immunoassay reagent kits manufactured by JSC «Vector-Best" (Novosibirsk). Informed consent to the study was obtained.
Results: Significant increase in the spontaneous production of colony-stimulating factors was shown in the initial presentation and progression phases of lymphoproliferative diseases. Increasing production of IL-18 and VEGF was found to be common for investigated diseases. Decreased secretion of IL-2 in addition to increased levels of cytokines was evidenced in the initial presentation and progression of lymphoproliferative diseases. We found that the remission of lymphoproliferative diseases is accompanied with reducing the level of proinflammatory cytokines (spontaneous production of IL-8, IL-6, IL-2, IL-17, INF-γ, mitogen-stimulated production of TNF-α, IL-6, IL-2) and anti-inflammatory cytokines (spontaneous production of IL-4, IL-10, mitogen-stimulated production of IL-10, IL-1RA). The only exception was an increase in the spontaneous production of GM-CSF that proved to be 4.1-fold (p=0.02) in chronic lymphocytic leukemia and small lymphocytic lymphoma.
Summary and Conclusions: The study has shown that the initial presentation or progression of lymphoproliferative diseases are accompanied with increased level of the majority of pro-inflammatory cytokines and colony-stimulating factors. The remission phase of lymphoproliferative diseases is characterized with reduced level of pro- and antiinflammatory cytokines and increased level of colony-stimulating factors.

PB1498

DOWNREGULATED MIR-181A/B EXPRESSION PREDICTS POOR PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Emerging evidence suggests that altered microRNAs (miRNAs) regulation is involved in the pathogenesis of cancers-mainly by regulating the translation of oncogenes and tumor suppressors. Aberrant expression of miRNA has been recently associated with chronic lymphocytic leukemia (CLL) outcome.
Aims: In this study, we aimed to investigate the role of miR-181a/b in Chinese patients of CLL, we assessed the expression of miR-181a/b and their correlation with other prognostic factors, including Binet stages, immunoglobulin heavy chain variable gene (IGHV) mutation status, TP53 mutation status, and CD38 expression level in CLL patients by using real-time polymerase chain reaction methods.
Methods: We assessed the expression of miR-181a/b and their correlation with other prognostic factors, including Binet stages, immunoglobulin heavy chain variable gene (IGHV) mutation status, TP53 mutation status, and CD38 expression level in CLL patients by using real-time polymerase chain reaction methods.
Results: Patients with unmutated IGHV genes had significantly lower expression of miR-181a/b than patients with IGHV mutations. The lower expression level of miR-181a/b was also significantly associated with higher level of CD38 and ZAP-70, and more aggressive Binet stage. Furthermore, the lower expression of miR-181a/b in CLL shows a strong association with shorter overall survival (OS) (p=0.016) as well as with reduced treatment free survival (TFS) (p=0.003). We also observed that miR-181a/b targets BCL-2, Mcl-1, and XIAP protein and that the decrease of its expression inversely correlated with negatively regulated expression of a BCL-2/MCL-1/XIAP 3' untranslated region-based reporter construct.
Summary and Conclusions: Our data suggest that miR-181a/b expression may play an important role in the progression and prognosis of CLL.

PB1499

YOUNG CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE OF 150 CASES

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Background: Chronic lymphocytic leukemia is rare in India and its incidence is 1% of all leukemias
Aims: In this study, the clinical characteristics, survival, and prognostic factors of 150 cases of young (less than 55 years) chronic lymphocytic leukemia (CLL) patients were analysed at IRCH, AIIMS, a large tertiary care centre of Northern India
Methods: Patients records collected from computer database using ICD code (C-91.1) between period of 2000-2013 were retrospectively evaluated
Results: Over a period of 14 years, 360 CLL patients (150 [41.6%]55 years of age) were evaluated. There were similar distribution of sex, lymphadenopathy, organomegaly, and absolute lymphocyte count in both groups At diagnosis, younger patients were less incidentally detected (p =0.01),

more B- symptoms (p=0.01) and advanced Rai stage (p <0.001), than older patients. Response rate and toxicity profile were same in both groups with chlorambucil and fludarabine based chemotherapy. Overall response rate (ORR) seen with chlorambucil and fludarabine was 69% and 88% with complete remission (CR) rate was 3% and 44% respectively. Richter's transformation was significantly higher in younger patients (3.41% v 0.5%; p =0.001). Median follow up period was 39 months. Younger and older patients showed a similar overall median survival but were characterized by a different distribution of causes of deaths. CLL unrelated deaths predominated in the older age group, as one third of patients have different co-morbities like diabetes, hypertension, coronary artery disease e.t.c, whereas the disease related death were prevalent in the younger age group. Multivariate analysis showed that for young CLL patients, advanced clinical stage (Rai III and IV) was associated with poor overall survival [HR 2.22 (95% CI 1.10-4.11) p =0.001].
Summary and Conclusions: Forty one percent our CLL population was young. Young CLL patients presented with more B symptoms and advanced stage (Rai III and IV) than elderly CLL patients.

PB1500

THE PTPN22 R620W POLYMORPHISM IS NOT ASSOCIATED WITH CLINICAL AND MOLECULAR CORRELATES OF CRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN PATIENTS ORIGINATING FROM REPUBLIC OF MACEDONIA

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Background: PTPN22 is a protein tyrosine phosphatase that modulates antigen-receptor signaling in T and B cells. A PTPN22 R620W polymorphic allele (C1858T, rs2476601) was associated with a major risk for the development of multiple autoimmune diseases in patients originating from northwestern Europe, whereas this association was less clear in patients originating from southern Europe. A higher frequency of the 620W risk allele has recently been reported in CLL patients originating from northwest Europe. These data adds to the recent findings that PTPN22 is markedly over expressed in the majority of CLL patients and that gene products of PTPN22 is involved in the pathogenesis of the disease by affecting apoptosis/survival of B-lymphocytes.
Aims: In order to extend further those observations we conduct a retrospective study on the frequency of PTPN22 R620W variant in 117 CLL patients and 108 age/sex matched normal controls without a history of malignant disease originating from the Republic of Macedonia (Southeastern Europe), which is a region with low prevalence of the autoimmunity risk variant. Moreover, we analyzed the possible association of the variant with various clinical/molecular correlates of the CLL patients.
Methods: Our study group consisted of 117 adult (>15 years) CLL patients that were diagnosed and followed at the University Clinic of Hematology-Skopje, Republic of Macedonia. Clinical and molecular data from the CLL patients were collected according the internal protocol that was approved by the University Clinic of Hematology Institutional review Board.
Results: Similar allele frequency of the minor T allele (0.06 and 0.063) and genotype distribution (0.88, 0.115 and 0.005 of CC, CT and TT genotypes) was found in CLL patients and normal controls, respectively. Both study groups were in Hardy-Weinberg equilibrium. Also, the PTPN22 R620W variant was not associated with any of the studied parameters in patients such as initial blood counts, Combs test, CD38 expression, IGHV mutation status, incidence of autoimmune disorders and immunoglobulin levels. Our data are comparable with the data reported for CLL patients originated from southeastern European region.
Summary and Conclusions: The PTPN22 R620W variant is not a risk factor for the development or clinical course of CLL in patients originating from the southeastern European region. The differences of our data with the reported findings for CLL patients originating from northwest Europe mirrors the differences observed in patients with various autoimmune diseases, suggesting a possible gene-environmental factor that affects the influence of R620 variant on the lymphocytic homeostasis. Our results are in line with the hypothesis that ethnic variations in the germline composition of the IGHV locus in correlation with environmental factors from particular geographic areas influence development of CLL.

PB1501

NUCLEOPHOSMIN (NPM) AND TELOMERASE (TE) IMMUNOPOSTIVITY IN CLL LYMPHOID CELLS

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Background: Nucleophosmin (NPM) is phosphoprotein that is ubiquitously expressed in tissues, resides in nucleolus and shuttles continuously between nucleus and cytoplasm. Genetic locus of human NPM1 is 5q35.1. The wild-type protein is mainly located in the nucleolus and on the nuclear membrane and in cytoplasm of mitotic cells. Major role of NPM is to mediate nuclear export of ribosome components to the cytoplasm and to control centrosome duplication and it also interacts with oncosupressors p53 and alternate–reading-frame protein (ARF) thus controlling cell proliferation and apoptosis. NPM promotes cell growth by enhancing ribosome biogenesis and transport to the cytoplasm, as its expression increase in response to mitogenic stimuli and above normal amounts are detected in highly proliferating and malignant cell. Telomerase (TE) is an RNA-dependent DNA polymerase that catalyzes the addition of telomeric repeat sequences to chromosome ends, and its genetic locus is 5p15.33. In most human somatic cells, TE activity is undetectable, and telomeres shorten with successive cell divisions. However, telomerase activity is detectable in immortal cells and in many human tumors. It was found that TE is sequestrated within the nucleolus during most of the cell cycle of normal cells and released into the nucleus only just before cell division. TE is not sequestrated in tumor cells and continually interacts with the chromosomes.
Aims: The aim of the study was to analyze NPM and TE immunocytochemical positivity in lymphoid cells and lymphocytes of patients with chronic lymphoid leukemia (CLL).
Methods: Immunocytochemical procedure was performed on cytological bone marrow (BM) specimens of five CLL patients and on effusion sediments in patient with benign reactive effusion lymphocytosis. Cytological specimens collected for immunostaining were obtained in patients at diagnosis, only as a part of standard diagnostic procedure and patients signed informed consent. Cytological specimens were stained with anti-NPM mouse monoclonal antibody (clone FC82291) raised against full length NPM, useful for identification of both wild-type and mutated NPM, and with anti-human goat polyclonal antibody (clone C-20) to TERT (telomerase reverse transcriptase). Immunostaining was further done with LSAB immuno peroxidase technique. NPM and TE nucleus immunopositivity was analyzed by microscope, determined were percentages of immunopositive cells, as well as score values od immunoreaction. Score values of NPM and TE immunopositivity were obtined by rating intensity of 100 cell immunopositivity using a scale of 0 to 4+ and then the number of cells counted in each cell rating is multiplied by the cell rating value and these values were summarized.
Results: Nuclei of almoust all CLL lymphoid cells were strongly NPM immunopostive (percentages median 100%, range 92-100%; score median 257, range 222-375). Telomerase was weakly immunopositive in most of nuclei of CLL lymphoid cells (percenatges median 74%, range 54-93%; score median 110; range 58-150). Opposite, in most of nuclei of reactive effusion lymphoid cells NPM was weakly positive (61%, score 91), and TE was completely negative. NPM and TE immunopostivity pattern in CLL lymphoid cells was different: TE was positive in form of fine small dots, and NPM was mostly positive in form of numerous coarse granules, coarse network or as diffuse coarse immunoreaction. Opposite, in most of benign reactive effusion lymphocytes NPM was weakly immunopositive in form of one fine dot.
Summary and Conclusions: In our five CLL patients most lymphoid cells were NPM and TE immunopositive, but in most of CLL lymphoid cells intensity of TE immunopositivity was weak in comparison to strong NPM immunopositivity. Moreover, benign reactive effusion lymphocytes were negative for telomerase and NPM was weakly immunopostive mostly in form of one fine dot. These results point that telomerase activity is very low and undetectable in benign lymphocytes and that NPM immunopositivity pattern is different in CLL lymphiod cells in comparison to benign reactive effusion lymphocytes. However, the number of analyzed patients is small and further analysis in larger groups of patients with CLL and patients with benign reactive effusion lymphocytes is needed to clearly determine NPM and TE cell immunopositivity differences between CLL lymphoid cells and benign lymphocytes.

PB1502

B CELL LYMPHOPROLIFERATIVE DISORDERS WITH CIRCULATING TETRAPLOID CELLS : FROM CYTOLOGY TO CYTOGENETIC

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Background: Cytogenetic abnormalities are frequent and recurrent in B cell chronic lymphoproliferative disorders (BCLPD). Thus, in chronic lymphocytic leukemia (CLL), the most frequent recurring chromosomal aberrations include

trisomy 12 and deletions of 13q, 11q and 17p, the translocation t(11;14) is closely associated to mantle cell lymphoma (MCL) and t(14;18) is observed very frequently in follicular lymphoma. Some of these cytogenetic abnormalities can be predicted by the morphological aspects of the lymphoid cells, particularly in follicular lymphoma, MCL and CLL with trisomy 12. In MCL, tetraploid clones are more frequently observed in blastoid variant (36%) and pleomorphic variant (80%) (Ott, 1997). In CLL, Quijano et al (2008) showed, by using flow cytometry, DNA hyperdiploidy in 10 out of 180 CLL cases. However no cytogenetic analyses were performed to confirm the flow data. At this time, only few studies reported a correlation between hyperploidy and specific morphological aspects. Pui et al (1990) described a striking morphologic finding with the presence of clumped chromatin and grooved nuclei in acute lymphoblastic leukemia.

Aims: The aim of the study was to relate specific cytologic aspect to tetraploid cells and/or other cytogenetic abnormalities in 9 cases of BCLPD (6 CLL, 2 splenic marginal zone lymphomas (MZL) and 1 unclassified chronic lymphoproliferative syndrome) with peripheral blood circulating tetraploid cells.

Methods: Blood cell counts were performed using XE 2100 or XE 5000 (Sysmex, Japan) and Advia 2120 (Siemens, USA). Total white blood cells (WBC) and basophils are counted in a specific channel in which all WBC except basophils are lysed. Then, resistant cells are counted as basophils. Parallely, corresponding blood smears were performed and stained using the classical method of May Grunwald Giemsa. Microscopic analyses for leucocyte differential count and cytological analysis were performed by 2 different cytologists. Cytogenetic analyses were performed using conventional caryotype and fluorescence *in situ* hybridization (FISH).

Results: Results showed a good correlation between automated hematology analyzers, optical microscopy and cytogenetic studies for detecting tetraploid cells. Results are summarized in the following Table 1.

Summary and Conclusions: In conclusion, this report showed that tetraploidy is associated with a specific morphological aspect that could be suspected by using the hematologic analyzers and well recognized using optical microscopy. In addition, tetraploidy was closely associated with +12, del6q and t(14;19)

Table 1.

Chronic lymphocytic leukemia and related disorders - Clinical

PB1503

BENDAMUSTINE IN COMBINATION WITH RITUXIMAB AS FIRST LINE THERAPY FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A RETROSPECTIVE REAL PRACTICE ITALIAN MULTICENTRE STUDY

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Background: Recent trials have shown that the combination schedule of rituximab, fludarabine and cyclophosphamide as first-line therapy for chronic lymphocytic leukemia (CLL) patients improved progression free survival (PFS) and overall survival (OS). However, this therapy was associated with increased toxicity: 76% of the patients experienced at least one grade 3 or 4 event. Recently, encouraging clinical results have been obtained using bendamustine in combination with rituximab (R-B) in relapsed and/or refractory, as well as in untreated CLL patients in terms of safety and efficacy.

Aims: We performed a multicentre retrospective study to assess safety and efficacy of R-B in a large group of untreated CLL patients.

Methods: One hundred and eighteen untreated CLL patients satisfying the inclusion criteria were recruited from 11 Italian Institutions and included in the present analysis. The median age was 71 years (range 43–85), with 52% of patients older than 70 years; 51% of cases were male. All patients had active disease as defined by the NCI-WG, and 28% of patients were at Binet stage C at study entry. FISH data, available in 78/118 cases, identified a del(17p) in 6.4% of the patients and del(11q) in 10.3%. Fifty-four patients (46.0%) had a creatinine clearance ≤70 mL/min and 41.5% of the cases showed an elevated lactate dehydrogenase (LDH) value. Fifty-five% of cases showed a WHO performance status (PS) of I-II and 62% a CIRS comorbidity index >0.

Results: Among the 118 patients, 80 cases (68%) received B at the dosage of 90 mg/m^2 on day 1 and 2 every 28 days, the remaining 38 cases received B at the dosage of 70 or 80 mg/m^2. R was administered at the dosage of 375 mg/m^2 on day 1 of all cycles in 59% of cases, while 41% received 375 mg/m^2 on day 1 of first cycle and 500 mg/m^2 on day 1 of the other cycles. A total of 582 cycles were administered with a median number of 4 cycles per patient. Thirteen patients (11%) required early discontinuation of therapy before the sixth cycle for: serious infections (n=6), persistent hematological toxicity (n=3), grade 4 dermatological toxicity (n=2) and withdrawal of consent (n=2). Grade 3 or 4 adverse events for neutropenia, thrombocytopenia, and anemia were documented in 16.1%, 27.1%, and 16.1% of patients, respectively. Grade 3 or 4 severe infections occurred in 6.8% of patients. Out of 93 cases evaluable for response, 64 patients (69%) achieved a complete response (CR), 25 (27%) a partial response (PR) and 4 (4%) a stable disease (SD). Of the clinical and biologic parameters only, age <70 years (P<0.0001), WBC <50x10^9/L (P=0.038) and creatinine clearance >70 ml/min (P<0.0001) were statistically associated with achievement of CR. High-risk FISH (del11q and del17p), as well as PS and CIRS CI >0 did not impact on achievement of CR. After a median follow-up of 8 months, 2-year PFS was 87% and 2-year OS 80% (10 deaths occurred). Five of 10 deaths were CLL-related (infections in 4 cases due to infections and disease progression in 1).

Summary and Conclusions: The clinical practice of the Italian centers taking part in the study confirms that chemoimmunotherapy with R-B was an effective and well-tolerated treatment for untreated CLL patients, producing a remarkably high CR rate and mild toxicity.

PB1504

BENDAMUSTINE-BASED FIRST LINE THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN ROUTINE CLINICAL PRACTICE: INTERIM ANALYSIS OF THE GERMAN NON-INTERVENTIONAL STUDY BE-CELL1ST

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Background: Bendamustine is an established treatment option in chronic lymphocytic leukemia (CLL) and frequently used in Germany. Current guidelines recommend a Bendamustine-based therapy in patients (pts) at Binet stage C or at Binet stages A (not covered in product label) and B with symptomatic disease and notably physically non-fit pts. In addition to results from clinical trials Be-CeLL1st reveals treatment modalities and outcome of Bendamustine-based therapy of CLL in daily practice in Germany.
Aims: This trial is conducted to gain further insight into therapy modalities, efficacy and safety of Bendamustine treatment in non-selected patients treated in clinical routine in Germany.
Methods: A total of 400 B-CLL patients are to be enrolled over a period of 43 months at about 70 study centres. Interim results of more than 200 pts are presented. The documentation covers patient demographic and tumor characteristics, treatment modalities, side effects and investigator assessed response evaluation (best response). Main entry criteria are indication for 1st-line therapy with Bendamustine, no pre-treatment with Interferone or Rituximab, unsuitability for Fludarabine-based therapy and absence of contraindications according to the market authorisation for Bendamustine.

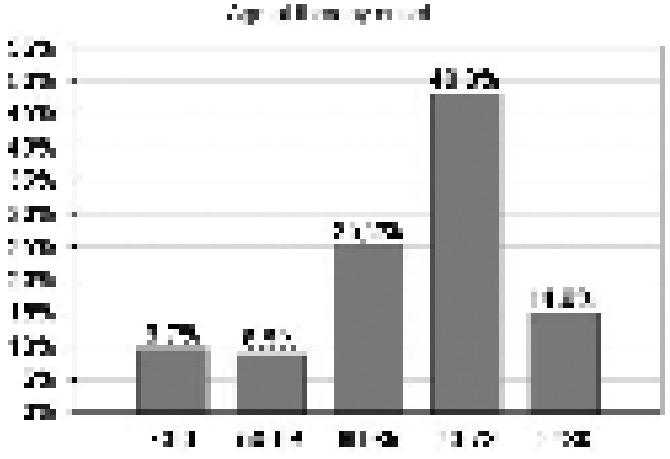

Figure 1.

Results: 264 pts were registered from 10/2011 until 12/2013. 202 pts were enrolled at least 6 months before data cut-off and were analyzed with regard to treatment modalities and efficacy. For safety, data of 253 pts with at least one documented treatment cycle were evaluated. Patients had a median age of 73 years (32-90 years) and performance status ECOG 1. 63% were 70 years or older. 65% of pts were male. 26% of pts presented B-symptoms. Tumor stages were 21% Binet stage A, 44% Binet B and 31% Binet C. CIRS-Score was ≤ 6 in 87% and > 6 in 13% of pts. The majority of pts (90%) was treated with the combination of Bendamustine and Rituximab, complemented by steroids in 3% of these pts. 7% of pts received Bendamustine monotherapy and 3% other combinations. Median time to treatment from primary diagnosis was 25 months. Most pts received Bendamustine on day 1 and 2 of the cycle (83%). In 10% of pts, Bendamustine was administered once per cycle only. The median treatment duration was 6 cycles (Bendamustine monotherapy: 3 cycles). The median dose of Bendamustine was 174 mg/m^2 per cycle. Median dose intensity was 38 mg/m^2 per week. At least one dose modification occurred in 36% of pts. Therapy was delayed at least once in 45% of pts. The most common reasons for therapy modification were side effects and comorbidities. In 16 pts (6%) side effects led to therapy discontinuation. Overall response rate (ORR) for evaluable pts (n=158) was 89% (complete response 38,6%, partial response 50,6%, stable disease 7,6%, progressive disease 3,2%). In 124 of 253 pts (49%) a total of 313 side effects were documented (all grades). Most frequent side effect was neutro-/leukopenia (25%). A total of 61 grade 3/4 adverse events (19%) occurred in 36 pts (14%). Serious adverse reactions occurred in 24 pts (10%). One fatal outcome was reported (sepsis).
Summary and Conclusions: The interim data of Be-CeLL1st shows that Bendamustine (+/- Rituximab) is an effective and safe treatment option for patients with B-CLL in daily clinical routine. These data seems to be in line with efficacy and safety data from recent clinical trials and demonstrates the broad applicability of Bendamustine (+/- Rituximab) in 1st-line CLL also in a wider, non-selected patient population.

PB1505

AN UPDATE ANALYSIS OF RITUXIMAB BASED CHEMOTHERAPY IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS: AN INDIAN SCENARIO

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Background: Rituximab (Anti- CD 20) has been reported to be an effective drug for the treatment of chronic lymphocytic leukaemia (CLL) with combination of fludarabine and bendamustine. Its safety and efficacy in Indian patients has not been studied.
Aims: We carried out analysis of 111 patients of CLL who received two different regimen FCR (fludarabine , cyclophosphamide, and rituximab) and BR (bendamustine and rituximab) for treatment of CLL in upfront and relapsed setting at I.R.C.H, AIIMS, New Delhi, India.
Methods: The records of all patients (N=360) with diagnosis of CLL between January 2000 and December 2013 were analysed. Rituximab was used in only 19% of cases (N=70, upfront) in combination ,due to financial constraints. Response evaluation was done as per the National Cancer Institute-Working Group guidelines, in those patients who received at least 3 cycles of chemotherapy. Toxicity was graded as per the common terminology criteria for adverse events, version 3.0. Median event-free survival was obtained using Kaplan-Meier survival analysis. Events were described as relapse, progression and death due to any cause.
Results: One hundred eleven patients (70-upfront/de novo, 41 relapsed) were included in the study and 421 cycles were administered (median: 3 cycles per patient). Fifty five patients received FCR and 55 received BR . Thirty five had treatment delay in FCR and 6 patients in BR subgroup due to prolonged myelosuppression. The complete (CR), partial (PR) and overall response rate (ORR) of FCR and BR was 43%, 43%, 86% and 40%, 50%, 90% respectively those treated upfront . ORR was 50% in those with relapsed disease in both subgroup. Grade 3/4 myelosuppression occurred in 65% with FCR and 20% with BR. One third patients had developed overt tuberculosis during treatment period or subsequent follow-up in patients who received FCR . Four patients died of pneumocystis jiroveci carinii pneumonia (PCP) in FCR group. Median event-free survival for patients treated upfront with FCR was 30 months and BR was not achieved.
Summary and Conclusions: In our patient population, response to FCR and BR is same and similar to that in the published literature but toxicity profile is different. Prolonged myelosuppression (two third of patients leads to treatment delay) and tuberculosis (one third of patients) in FCR regimen. BR is better option to treat CLL patients in our group of population where the prevalence of subclinical tuberculosis is high.

PB1506

LONG-TERM FOLLOW-UP OF A COHORT OF PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH LOW-DOSE ALEMTUZUMAB

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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a variable clinical course. Despite the use of highly effective chemo-immunotherapy, CLL remains incurable. Alemtuzumab has been found to be effective in CLL as both front-line and salvage therapy, also in patients (pts) with worse prognosis carrying 17p deletion and/or TP53 mutations.
Aims: We report long-term results of efficacy and tolerability of low-dose subcutaneous alemtuzumab therapy in pts with relapsed and/or refractory CLL treated at our institution.
Methods: Pts with active relapsed and/or refractory CLL treated with low-dose alemtuzumab were evaluated. Alemtuzumab was administered subcutaneously at the dose of 30 mg weekly for 18 weeks with a maximum cumulative dose of 540 mg.
Results: Sixty-two consecutive pts, treated between January 2003 and June 2013 at our Centre, were included in the analysis. The median age was 68

years (40-83), 26 (42%) pts were older than 70 years. FISH analysis and IGHV mutational status were performed in 52 and 26 pts, respectively. Twelve (23%) pts showed del(11q), while 14 (27%) pts had del(17p); 18 (69%) pts had unmutated IGHV genes. Twenty-six (42%) pts were exposed to fludarabine, single agent or in combination, and 57% of pts were pretreated with at least 2 lines of therapy. Thirty-five (57%) pts concluded the scheduled 18 weeks of treatment; median administered dose was 510 mg. Overall response rate was 62% (95% CI, 49%>73%), with 26% of complete responses. After a median follow-up of 40 months (2-128), median overall survival (OS) was 40.6 months (95% CI, 34.1-52.1) and median progression free survival (PFS) was 16 months (95% CI, 9.8 -22.9). None of prognostic markers or pre-therapeutic parameters was predictive for inferior response. In multivariate analysis, OS was significantly associated only with ZAP-70 expression (p0.004) and PFS with bulky disease (p0.03), CD38 expression (p0.03) and response (p<0.0001). Twenty-two pts of our cohort were fludarabine refractory. These pts had a median OS of 29.5 months (95% CI, 23.8-35.1), 10 obtained at least a PR and 4 are still alive with a survival longer than 36 months. In our analysis, we identified a subset of 34 pts with an OS longer than 36 months from the start of therapy. Their median age was 68 years (40-79). Sixteen (47%) pts were treated with at least 2 lines of therapy and 7 (25%) pts carried del(17p). In multivariate analysis, long-term survival was associated with ZAP-70 expression (p 0.03) and obtained response (p 0.02). Fifteen percent of long-term survivors was pretreated with fludarabine *versus* 39% of no long-term survivors (p 0.03); 6% had lymph-node ≥ 5 cm *versus* 25% (p 0.03); 46% were ZAP-70 positive *versus* 83% (p 0.007); 29% were CD38 positive *versus* 61% (p 0.02); 35% had Binet stage C *versus* 50% (p 0.02). Hematologic toxicities were the most common adverse events. Forty-nine (79%) pts showed peripheral blood cytopenia, but this was grade 3 or greater in 15 (24%) pts. The main non-hematologic adverse effects were CMV reactivation (31%) and non-CMV infections (19%), which together were the main reasons for treatment interruption and/or premature termination. Grades 3 or 4 non-CMV infection occurred in only 4 (7%) pts, while no one had grades 3 or 4 CMV events.

Summary and Conclusions: Low dose subcutaneous alemtuzumab showed a good tolerability and long-term efficacy. We emphasize that alemtuzumab still remains a valid therapeutic option in subsets of relapsed/refractory CLL pts.

PB1507

EXTENDING THE MATUTES' SCORE FOR DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA: THE ROLE OF NEW MARKERS

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Background: the diagnosis of chronic lypmhocytic leukemia (CLL) is based on data of peripheral blood (PB) counts and immunophenotyping. Due to frequent occurrence of cases with atypical morphology and abnormal phenotypes, the Matutes' score has been established for the differential diagnosis between CLL and other leukemic mature B-cell neoplasms, but even this score takes into account the variability of phenotypic features. So, the utility of new markers have been tested in the last years.
Aims: we examined the relation between the amount of atypical lymphocytes in PB and the occurrence of abnormal phenotypes in CLL. We also tested how the addition of more markers to the Matutes' score could improve the accuracy of the differential diagnosis between CLL and other leukemic mature B-cell neoplasms.
Methods: we examined 75 consecutive cases of PB lymphocytosis entering our Laboratory between june 2012 and November 2013. PB lymphocyte morphology was analyzed. For immunophenotyping, a four color panel of monoclonal antibodies was used. Diagnosis was confirmed by histology of bone marrow or lymph node whenever necessary.
Results: the final diagnosis of CLL was made in 62 cases. Besides, there were 4 cases of mantle cell lymphoma (MCL), 3 cases of lymphoplasmacytic lymphoma (LPL) and 6 cases of splenic marginal zone lymphoma (MZL). For CLL cases, median age: 68 years (45-88); median PB lymphocyte count: $16.9x10^9$/L (5.8-141.6); atypical lymphocytes: median 12% (3-37), prolymphocytes: median: 2% (0-10). Matutes' score was 5 in 14 cases; it was 4 in 31 and 3 in 17 cases. Concerning other markers, expression of CD200 had a median MFI of 129, 163, 7 and 32 respectively for CLL, LPL, MCL and MZL. CD19 had a median MFI 162, 321, 113 and 259 respectively for CLL, LPL, MCL and MZL, and CD20 had a median MFI of 229, 1263, 812 and 1718. In CLL, there was an inverse correlation between the IMF of CD200 and the percentage of atypical lymphocytes (r = -0.34; p=0.003) and prolymphocytes (r = -0.327 p=0.01). In a discriminant analysis using the IMF of CD19 in CLL), CD20 and CD200, the predictive value for CLL x non-CLL was 97%.
Summary and Conclusions: the intensity of expression of CD19, CD20 and CD200 can increase the diagnostic accuracy in atypical cases of CLL.

PB1508

Abstract withdrawn

PB1509

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PB1510

VENOUS THROMBEMBOLISM: A FREQUENT COMPLICATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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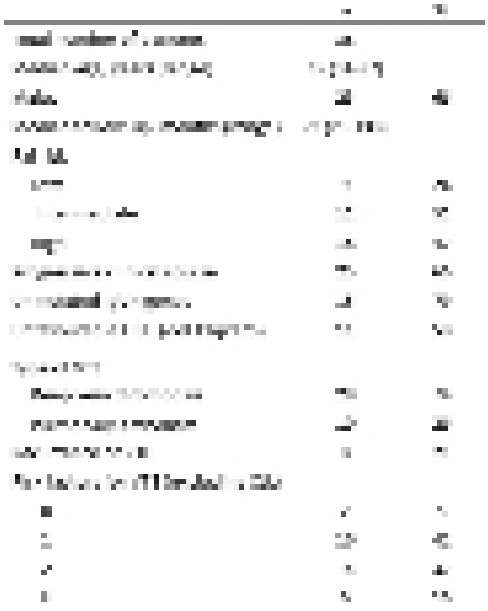
Background: Venous thromboembolism (VTE) is a major cause of morbidity and mortality in patients with malignant tumors. Increased risk of VTE is well described in a variety of hematologic malignancies , especially in myeloproliferative disorders, plasma cell malignancies, and lymphomas. However, data regarding risk of VTE in chronic lymphocytic leukemia (CLL) is very scarce. A recent study (Whittle *et al.*, Leuk Res 2010) reported high incidence of TEN in CLL patients compared with the general population.
Aims: To evaluate the occurrence and risk factors of VTE in patients with CLL.
Methods: We retrospectively analyzed clinical and laboratory data of consecutive patients (pts) with CLL followed up at 4th Department of Internal Medicine – Hematology, University Hospital, Hradec Kralove, diagnosed between 1999 and 2013. In all, 346 patients were analyzed (222 males; median age at diagnosis, 64 years; low/intermediate/ high Rai modified risk in 41/47/12%). Only patients with radiologically confirmed deep vein thrombosis (ultrasonography) or pulmonary embolism (computer tomography) developed after diagnosis of CLL were considered as having VTE.
Results: After a median follow-up of 72 months (range, 26-138), at least one episode of VTE occurred in 38 patients (11%). Basic characteristics are listed in Table 1. VTE developed after with a median of 34 months since CLL diagnosis. There was a high proportion of unfavourable prognostic factors (advanced Rai stages, unmutated IgVH genes, unfavourable cytogenetics). There was no association of VTE with hyperleukocytosis or other parameters of blood count. An apparent precipitating factor for VTE was present in 4 patients (acute infection). The presence of 0/1/2/3 risk factors for VTE was identified in 2/16/14/6 patients. Seventeen pts were previously untreated for CLL; 14 cases of VTE occurred during treatment for CLL and 7 cases after CLL treatment. The most common risk factors for VTE besides age (n=24) were corticosteroid therapy (n=13), other malignancies (n=9) and obesity (n=7). Recurrence of VTE was diagnosed in 8 pts (21%). Overall survival of patients who developed VTE was not different from those without VTE.

Table 1.



Summary and Conclusions: Based on our results, VTE is a relatively frequent complication in patients with CLL. Although most patients had other known risk

factors for VTE including treatment of CLL, 29% had no risk factors or only age≥ 65 years. These findings support the role of CLL in the development of VTE. Updated data will be presented.

PB1511

THE ROLE OF SERUM FREE LIGHT CHAINS IN PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Currently, serum free light chains (sFLC) are considered as an additional factor that can more accurately predict the course of chronic lymphocytic leukemia (CLL) and response to therapy.
Aims: Evaluate the prognostic value of sFLC in patients (pts) with CLL and identify an association with unfavorable prognostic factors.
Methods: The study involved 153 pts with newly diagnosed CLL at the age of 33-75 years (a median age – 59 years). Among the pts were 90 men and 63 women. In 64 (42%) pts stage A was established, in 63 (41%) pts – stage B and 26 (17%) pts – stage C by Binet. Concentration of sFLC (kappa and lambda) was determined by immunoturbidimetric method with the help of reagent kits «Freelite». At the time of diagnosis the expression level of Zap-70 by flow cytometry, was investigated, the cut-off level of the positive expression of ZAP-70 was 20%. The content of serum thymidine kinase (TK) was determined by a radioenzyme method.
Results: In 80 (52%) pts normal sFLC ratio was detected. In 73 (48%) pts abnormal sFLC ratio was revealed due to the predominance of one of the chains, moreover, there were 15 (10%) pts there with an increased production of the set of lambda chain, and in 58 cases (38%) the kappa chain was found. Median follow-up was 32 months. In pts with abnormal sFLC ratio median time from diagnosis to first treatment was significantly shorter than in pts with normal sFLC ratio (3 months *vs.* 26 months, respectively, p<0.001). In this case a correlation of abnormal sFLC ratio with the known unfavorable markers of the course CLL was revealed: positive expression of ZAP-70 (r=0.323; p=0.019) and the level of serum TK ≥ 20 U/L (r=0.386; p=0.027). Furthermore, it is found that the response to immunochemotherapy of FCR (fludarabine, cyclophosphamide, rituximab) depended on the sFLC ratio. Thus, in pts with normal sFLC ratio complete remission (CR) was achieved in 78 (98%) pts, partial remission (CR) – in 2 (2%) pts. Whereas in pts with an abnormal sFLC ratio CR was achieved in 22 (30%) pts, PR – in 41 (56%) pts and 10 (14%) pts were non-responders (p<0.001).
Summary and Conclusions: Production of light chain of kappa or lambda in CLL pts associates with positive expression ZAP-70, high serum TK, rapidly progressive course of the disease and low response to treatment. Determination of sFLC ratio can be used as an additional criterion for prognosis of CLL.

PB1512

FIRST-LINE TREATMENT CHOICE FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS: WHAT DO CLINICIANS CARE ABOUT? THE CLL FITNESS STUDY

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Background: In the last decade, fludarabine+cyclophosphamide+rituximab (FCR) has become the standard first-line treatment for young fit CLL patients, showing great unprecedented efficacy, but also relevant treatment-related toxicities. Though a number of approaches, considering age, comorbidities and organ impairment, have been proposed in clinical trials to define the most appropriate group of patients who can benefit from FCR, none has been specifically validated and little is known on how CLL patients should be managed at best in the daily clinical practice.
Aims: We designed an investigator-driven multicenter retrospective observational non-interventional study in order to collect data on the criteria applied in the clinical practice when selecting first-line treatment for CLL patients and on the outcome in terms of tolerance and toxicities to the initial therapy. The primary objective of the study is to define the most relevant parameters influencing physician's choice and their correlation with treatment tolerance. The most reliable factors will be included in a "Fitness score" that could become the basis for a prospective study aiming at validating its use in day to day clinical practice.
Methods: The study is based on a retrospective data collection from the medical records of consenting patients treated by participating centers. Patients enrolled into the study had previously untreated CLL requiring first-line treatment between January 1st, 2009 and December 31, 2010. The accrual just started and will be completed by Spring 2014, reaching a study target population of 700 subjects.
Results: Thirty-seven Italian sites with long-standing experience in CLL patient management were invited to participate in the study. The main requested clinical information includes anthropologic parameters, global health status, disease information, biological parameters, treatment and follow-up data.
To date, 104 patient records were reported in the dataset. Median age at time of first treatment was 66 years (39-90), 35 were females and 69 males. Reasons for starting treatment were reported as follows: increased lymph nodes (50%), lymphocyte doubling time <6 months (40%), massive splenomegaly (25%), bone marrow failure (27%), autoimmune phenomena not responsive to steroid treatment (3%). The most frequent first line treatment options included: FCR (43%), alkylating agent (22%), alkylating agent+purine analog (15%), alkylating agent+anti-CD20 antibody (13%), other combinations (7%). Thirty-five percent of patients were enrolled in clinical trials. Treatment choice was based on age (relevant parameter in 99% of the cases), comorbidities (98%), functional (82%), performance (80%), and mental (79%) status, need for caregiver (68%) and polypharmacy (65%). Biological factors (*e.g.* IGHV, FISH and CD38) influenced treatment decision in 39%of cases. Thirty-nine percent of patients experienced grade 3-4 adverse events (AEs), with only 1 patient experiencing a fatal AE. The most frequent grade 3-4 AEs were represented by neutropenia (22%), thrombocytopenia (3%), anemia (2%), infusion-related reactions (2%).
Summary and Conclusions: Our study is shedding light on the criteria considered most relevant in the clinical practice and will identify those that more significantly associate with a reduced tolerance to intensive treatments like FCR. This knowledge will allow to develop a comprehensive index of the patient's fitness status towards chemoimmunotherapy, helping the physicians to better stratify patients in order to maximize benefits and avoid potentially harmful overtreatment.

PB1513

Abstract withdrawn

PB1514

CENTRAL SCREENING ENABLES IDENTIFICATION OF HIGH RISK CLL PATIETNS FOR LENALIDOMIDE MAINTENANCE THERAPY WITHIN THE CLLM1 TRIAL OF THE GERMAN CLL STUDY GROUP

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Background: Long lasting remissions can be achieved with first-line chemoimmunotherapy (FCR or similar regimens). Research now focuses on the attainment of more durable remissions. In general, a response duration of less than 24 months is regarded as unsatisfactory for patients who received chemoimmunotherapy. Identifying patients with a high likelihood for early relapse after intensive therapy is particularly important since they might be candidates for maintenance and consolidation strategies.
Aims: Based on an analysis of data of the CLL8 trial of the German CLL Study Group a subset of CLL patients was observed with a high risk for early relapse after first-line therapy (median progression free survival of 22 months) and also

a considerably shorter overall survival. These patients can be defined by the presence of minimal residual disease (MRD) levels of ≥10^{-2} or a combination of MRD levels of ≥10^{-4} to <10^{-2} with either an unmutated *IGHV* status, del(17p) or TP53 mutations. CLLM1 is a phase 3, randomized, placebo-controlled study investigating the efficacy and safety of lenalidomide as maintenance therapy for high risk patients with chronic lymphocytic leukemia (CLL) following first-line therapy.
Methods: For identification of high risk patients central screening procedures prior to randomization are performed. Inclusion and exclusion criteria including patients' comorbidities based on the Cumulative Illness Rating Scale, the need of treatment, ECOG performance status and disease stage are centrally reviewed. In addition blood samples are to be sent to the central laboratories to confirm the diagnosis of CLL and to assess cytogenetic aberrations prior to start of first-line therapy. After first-line therapy MRD levels are centrally analyzed. A repeated central review covers the final risk assessment of all risk factors and eligibility, in particular response to first-line therapy and the ability to receive the maintenance treatment. Each patient provided written informed consent before enrolment.
Results: Between July 2012 and February 2014 442 patients were registered. 31 (7.0%) patients were excluded before start of first-line treatment due to other B-cell lymphoma (14/3.2%), withdrawal of consent (4/0.9%), comorbidities (2/0.05%), Hep B infections (2/0.05%) and other reasons (9/2.0%). 72 patients failed to proceed after first-line therapy due to patients' decision (34/47.2%), physicians decision (4/5.6%), non response to first-line (4/5.6%), death (3/4.2%) or other reasons (32/44.4%). A site survey using a questionnaire completed by the treating physicians revealed that the main reasons for patients' withdrawal have been tiredness of treatment, concerns regarding the study drug and frequent visits for maintenance therapy. 171 pts were screened after responding to therapy with FCR (86/ 50.3%), BR (82/ 48.0%) or other (3/1.7%). 130 (76.0%) of them were identified as low risk patients. 36 pts (21.1%) were randomized to receive study drug so far.
Summary and Conclusions: The screening process supports the identification of eligible high risk patients and allows a risk adapted approach to maintenance therapy.

PB1515

EFFICACY OF SPLENECTOMY IN CHRONIC LYMPHOCYTIC LEUKEMIA ASSOCIATED WITH IMMUNE CYTOPENIAS

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Background: Autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP) complicate the course of chronic lymphocytic leukemia (CLL) in 15-25% of patients. In case of ineffective steroid-based and chemotherapy and in the presence of overt splenomegaly, splenectomy is performed in such patients.
Aims: To analyze retrospectively the efficacy of splenectomy in patients with CLL associated with AIHA and/or ITP.
Methods: Splenectomy has been undertaken in 40 patients with CLL. In 25 (62.5%) of them, immune cytopenias were diagnosed: AIHA warm type in 10 (25%), ITP in 11 (27.5%), and AIHA + ITP in 4 patients (10%). The patients were under observation during 4-324 months after splenectomy.
Results: In 8 patients with CLL complicated by AIHA, splenectomy proved to be effective. A large tumor mass has been removed, and hemolysis has been ceased. An immediate postoperative death was stated in two patients due to an acute adrenal insufficiency and acute liver impairment, respectively. The median post-splenectomy survival reached 51.2 months. In all 11 patients with CLL and ITP, a normalization of platelet number after splenectomy was noted. One patient died due to disease progression at 2 months following surgery (having a normal platelet count). The median survival in those patients was 113.1 months. In 4 patients, CLL was associated with both AIHA and ITP. Two patients died due to disease progression within a year following splenectomy; one patient had been alive for 21 month; another one has been living 168 more months after surgery. The median survival in that group of patients was 49.1 months.
Summary and Conclusions: Splenectomy is an effective treatment option in CLL associated with overt splenomegaly and immune cytopenias. Presence of AIHA and/or ITP is found to be an additional risk factor of surgical intervention.

PB1516

SECONDARY MALIGNANCIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: OUR EXPERIENCE.

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Background: There is a general consensus in the literature that the risk of developing a second cancer (SC) is increased in those patients with chronic lymphocytic leukemia (CLL), regardless of treatment. Several mechanism have been discussed for the association between CLL and SC, including disease- and treatment- immunosuppression and shared risk factors.
Aims: We evaluated a small group of patients with diagnosis of CLL and a secondary primary solid neoplasia; we recognized some elements that could be important to make a CLL profile (RAI STAGE, treatment-related immunosuppression) in order to increasing surveillance for an early detection and/or prevention of SC.
Methods: We evaluated at the Division of Hematology of Federico II University in Naples, between 1980 and 2013, n= 476 patients with CLL; 2.7% (F=2 and M= 11) of these, with a median age (yrs) of 64.5 at the diagnosis of CLL, developed a SC. We observed a total of n= 14 SCs (5 non -melanoma skin cancers; 2 gastrointestinal cancers; 3 lung cancers; 1 prostate cancer and 3 bladder cancers): the real incidence was difficult to assess because many patients were lost in follow-up; in 4/13 patients chemotherapy free, we observed 1 lung adenocarcinoma stage IV, 2 urothelial carcinoma G1 and G3, 1 gastric cancer, with a median interval (yrs) between first and second diagnosis of 6.5; 1 patient died 2 months later the diagnosis of lung cancer, at the age of 70 years old. 9/13 patients, on the other hand, received different chemotherapy treatment as first line: fludarabine-based (n=7) and chlorambucil (n=2). Median interval between first and second diagnosis was 5.5 and between the first line treatment and the diagnosis of a SC was 5 years; in this group we observed: 6 skin-cancers (3 basocellular carcinoma, 1 squamous carcinoma, 1 Kaposi's sarcoma), 2 non small cell lung cancer stage IIIA, 1 colorectal cancer stage IIA, 1 urothelial cancer, 1 prostate cancer; one patient presented with the coexistence of 2 different cancer (prostate and skin).
Results: There was an excess in patients with a RAI STAGE IV at diagnosis (43.8% IV, 7.1% III, 14.2% II, 21.4% 0). Smokers in the chemotherapy free group were 15%, and 31% into the other one; 43% of all patients were positive for Hepatitis virus C.
Summary and Conclusions: In our patients with CLL we observed first of all an excess of skin malignancies in the subgroup of treated patients; median interval between first and second diagnosis (5.5 *vs* 6.5 years) was inferior in the treated group, regardless of the histotype of the SC. We observed an increased number of SCs in the subgroup of patients with a RAI STAGE IV, all treated with at least one chemotherapy cycle, and we concluded that there was a very important bias related to a prevalence of advanced stage requiring inevitably a treatment: further research in this area will be necessary to understand the real influence of the chemotherapy. In conclusion, it's important for physicians to be alert to the occurrence of SCs especially whether new symptoms or physical findings arise. This surveillance and a correct cancer screening seems to be important first of all for patients with a RAI STAGE IV at diagnosis.

PB1517

STRATIFICATION MODEL OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The pathogenesis of chronic lymphocytic leukemia (CLL) is complex and not completely understood. Currently, interaction aspects of leukemic cells, reflecting their cells' proliferation, survival and resistance to the effects of pro-apoptotic signals play a significant role in it. It is known that the characteristics of the heterogeneous clinical course of CLL depend on a series of prognostic factors, reflecting proliferative activity, apoptosis, and mutation status of tumor cells. The question about the stratification of CLL patients (pts) into risk groups at the time of diagnosis, with a value in predicting the course of the disease and in the selection of program of therapy remains unresolved.
Aims: Develop a model of stratification of CLL pts based on biological markers of tumor cells.
Methods: The study involved 230 pts with a newly diagnosed CLL at the age of 35-79 years (a median age – 59 years). In 81 (35%) pts stage A was established, in 120 (52%) pts – stage B and in 29 (13%) pts – stage C by Binet. Status by ECOG was from 0 to 3 points. At the time of diagnosis the expression level of ZAP-70, Bcl-2 and Ki-67 in tumor cells clone was investigated by flow cytometry. The cut-off level of the positive expression of Zap-70 was 20%. The content of serum thymidine kinase (TK) was determined by a radioenzyme method. Enzyme content which was twice as large as the maximum rate of its normal level (20 U/l) was used as a threshold value of TK.
Results: Depending on the presence of biological markers of tumor cells all CLL pts were divided into 3 risk groups. The low-risk group consisted of pts with negative expression of ZAP-70, Bcl-2 < 30% and Ki-67 < 10% and the level of serum TK < 20 U/l. The intermediate-risk group included pts with positive expression of Zap-70 and the presence of one or two of the following factors:

Bcl-2 ≥ 30% but < 50%, Ki-67 ≥ 10% but < 15%, level of serum TK ≥ 20 U/l. The high-risk group included pts with positive expression of Zap-70, Bcl-2 ≥ 50%, Ki-67 ≥ 15% and the level of serum TK ≥ 20 U/l, *i.e.* with the presence of all four factors. The median of overall survival in the low-risk group was 113 months, in the intermediate-risk group – 73 months and in the high-risk group – 48 months (p=0.027 and p=0.019, respectively). Median time from diagnosis to first treatment was significantly shorter in the high-risk group than in the intermediate and low risk ones (1.4 months *vs.* 11 months, p=0.012; 1.4 months *vs.* 28 months, p=0.004, respectively).

Summary and Conclusions: The results showed that the model stratification of CLL pts, including Zap-70, Ki-67, Bcl-2 and serum TK, predicts the indolent or progressive course of the disease, overall survival that have significance for choosing a therapy program.

PB1518

RISK STRATIFICATION OF CHRONIC LYMPHOCYTIC LEUKEMIA ACCORDING TO THE NUMBER OF FACTORS OF POOR PROGNOSIS

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Background: Chronic lymphocytic leukemia (CLL) is one of the most common adult leukemia, presenting a remarkably variable clinical course. The Rai and Binet staging systems have been used to risk-stratify patients; however, for each stage there is still heterogeneity in the clinical evolution. In addition to factors used in clinical staging, several other patient characteristics and laboratory tests have been correlated with overall survival.

Aims: Risk stratification of CLL according to the number of factors of poor prognosis.

Methods: We retrospectively analyzed 178 patients with CLL in a single center between 2000 and 2013. We have considered as factors of poor prognosis: male sex, advanced stage (III-C or IV-C Rai-Binet), absolute lymphocytic count greater than 50 000/µL, elevated serum levels of β-2 microglobulin, elevated serum lactate dehydrogenase (LDH), presence of chromosome abnormalities such as 17p deletion or 11q deletion and expression of ZAP-70 or CD38 by leukemia cells. Patients were divided into 3 groups according to the number of poor prognostic factors: group A (0-1), group B (2-3), group C (>3).

Results: The median age at diagnosis was 69 (range, 30-91) and 64% (n=114) were men. The median follow-up time was 3.6 years (range, 28 days to 12 years). The overall survival (OS) at 3 years was 98.9% in the group A (n=115), 83.4% in the group B (n=54) and 63.5% in the group C (n=9) and these differences were statistically significant (group A *versus* B, p=0.049; A *versus* C, p<0.001; B *versus* C, p=0.026). According to multivariate regression analysis group C presented a risk of death 32.5 times superior than group A (p <0.001, HR 32.5, 95% CI 6.4 - 166.5). The median time from diagnosis to treatment was 2 months for group C, 20 months for group B and 91 months for group C (group A *versus* B, p<0.001; B *versus* C, p=0.03; A *versus* C, p<0.001).

Summary and Conclusions: Accounting for poor prognosis factors allowed us to stratify patients into 3 groups (A, B, C). Mainly, patients with more than 3 poor prognostic factors (group C) had clearly worse OS than patients with 0-3 poor prognosis factors and that determined early treatment.

PB1519

Abstract withdrawn

PB1520

PENTOSTATIN, CYCLOPHOSPHAMIDE AND RITUXIMAB (PCR) IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Three purine nucleoside analogs have shown single-agent efficacy in lymphoid neoplasms: fludarabine, 2-chlorodeoxyadenosine and pentostatin. Their use, however, can be limited by bone marrow suppression and increased risk of infectious complications.

So far, the gold standard in the treatment of fit patients with B-CLL is the combination of rituximab with fludarabine and cyclophosphamide (FCR).

Pentostatin is toxic to lymphocytes as it causes the accumulation of deoxyadenosine-triphosphate and compared to the other purine analogs, it can be less myelosuppressive. We present the results of a retrospective analysis carried out on patients with relapsed/refractory CLL treated with pentostatin, cyclophosphamide, and rituximab (PCR).

Aims: The purpose of this study was to evaluate the toxicity and the efficacy of PCR chemoimmunotherapy in terms of response and survival in unfit and/or relapsed/refractory patients with B-CLL.

Methods: Between February 2008 and December 2010, 29 patients (19 males, 10 females) were treated at 3 italian hematology centers. Their median age was 70 years (range 46-84). Three patients were in 1st line therapy, 12 in 2nd, 8 in 3th and 6 in 4th line. Median number of previous lines was 1. Performance status (PS) was 0 in 4 pts, 1 in 5 pts, 2 in 5 pts, missing in 13 pts. Stage was A/2 in 3 pts, B/2 in 4 pts, B/4 in 1 pt, C/2 in 2 pts, C/3 in 2 pts, C/4 in 5 pts. Cumulative Illness Rating Scale (CIRS) was grade 0 in 15 pts, 1 in 8 pts, 6 in 1 pt. Median peripheral lymphocytosis was 30.8 x 10^9/l (2.6-153.8). Treatment schedule consisted of 4-6 cycles of pentostatin 4 mg/m^2 on day 1, cyclophosphamide 600 mg/m^2 day 1, rituximab 375 mg/m^2 day 1 at the 1st cycle, 500 mg/m^2 for cycles 2-6. The cycle interval was 28 days. Age, gender, stage, performance status, extranodal sites, serum lactate dehydrogenase, B symptoms, bulk, erythrocyte sedimentation rate, serum albumin level, hemoglobin level, white blood cells and platelets count were considered for clinical assessment and prognosis. Responses were classified according to the criteria of the NCI working group. Response and survival durations were calculated according to the method of Kaplan and Meier.

Results: After 3 cycles of PCR, 26 patients were evaluable for response: 9 (31%) were in complete remission (CR), 11 (38%) partial remission (PR), 1 (3%) minimal response (MR), 1 (3%) non-response (NR), with 4 (14%) early death (ED). Overall response rate (ORR) was 69%. The final response was CR in 11 (38%) pts, PR in 7 (24%) pts, stable disease (SD) 1 (3%) with ORR of 62%. More than 50% of patients completed 6 cycles of treatment. Discontinuation of therapy was due to early death in 4 cases , 1 to severe obstructive broncopneumopathy , 1 to an unknown cause. At median time of observation (17 months, range 0.5 to 44), 46% of the patients were alive in CR, overall survival (OS) was 72% and progression free survival (PFS) 67%.

Summary and Conclusions: The PCR regimen showed a good level of response in relapsed or refractory patients, without important side effects, excessive marrow toxicity and/or severe infections. It has shown efficacy in patients pretreated with fludarabine as well. We did not observe the development of myelodysplastic syndromes or acute myeloid leukemias in any of our patients. Despite the encouraging therapeutic advances, CLL remains so far an incurable disorder with many repeated relapses. Therefore, the evidence that, among all other drugs, pentostatin can be successfully and safely used in combination with cyclophosphamide and rituximab in every phase of the disease, even in older or unfit and/or refractory patients, can help to broaden our therapeutic arsenal and further improve our healing potential.

PB1521

SMUDGE CELL ON BLOOD SMEAR IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: A PROSPECTIVE SINGLE INSTITUTIONAL STUDY FROM INDIA.

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Background: Smudge cells are ruptured lymphocytes seen on routine blood smears of chronic lymphocytic leukemia (CLL) patients

Aims: We evaluated significance of smudge cells percentage on a blood smear in CLL patients.

Methods: We calculated smudge cell percentages (ratio of smudged to intact cells plus smudged lymphocyte) on blood smears of 165 consecutive untreated CLL patients registered at I.R.C.H, AIIMS, New Delhi over a period of 5 years (2006-2010).

Results: There were 125 males and 40 females. The median age was 59 years (30-88). Median absolute lymphocyte count was 40 X10^9/L. Clinical Rai stage distribution was: stage 0 - 5%, stage I - 25%, stage II - 40%, stage III -10% and stage IV - 20%. The median smudge cells percentage was 28% (4% to 76%). There was no correlation of proportion of smudge cells with age, sex, lymphocyte count, lymphocyte doubling time, beta 2 microglobulin , organomegaly, ZAP 70 + or CD 38 + CLL patients, but there was significant correlation with stage of disease. Median smudge cell percentage in stage 0 & I -36% (12-76), stage II-30% (12-61) and stage III&IV-20% (4-51) [p <0.001]. Eighty five patients of early stage (0, I &II) patients required treatment during follow up [65% required treatment with smudge cell<30%, against 35% patients requiring treatment with smudge cells > 30% , p=0.01]. The percentage of smudge cells as a continuous variable correlated with OS [HR 0.96, p< 0.001]. The 5-year survival rate was 51% for patients with 30% or less smudge cells compared with 76% for patients with more than 30% of smudge cells. Median OS was 4.8 years with median follow up period of 3.6 years. Smudge cells percentage (<30% *vs.* >30%) had significant association with OS [HR 0.97, 95%CI (0.62-1.21), p=0.001)].

Summary and Conclusions: Simple and inexpensive detection of smudge cells on blood smears on routine diagnostic test useful in predicting progression free and OS in CLL patients and may be beneficial in countries with limited recourses.

PB1522

SOLUBLE TACI SERUM CONCENTRATIONS AT DIAGNOSIS IS A POWERFULL PROGNOSTIC MARKER IN CLL

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Background: TACI (transmembrane activator and CAML interactor) is one of the 3 BLyS (BAFF) receptors and is expressed by B and T cells. It can also bind APRIL. Both BLyS and APRIL were shown to regulate B-CLL cells proliferation and survival. Serum BLyS levels were found considerably decreased in CLL patients and their low concentrations related to a shorter time to first treatment (TFT) but not to overall survival (OS). It was suggested that low BLyS levels in aggressive CLL were reflecting its binding to its receptors on B-cell surface, sequestrating it from circulation. TACI can be shed from cells' surface and circulate in its soluble form. Very few studies so far investigated soluble TACI (sTACI) in CLL.
Aims: To investigate the impact of sTACI levels at diagnosis in CLL and to compare them with soluble BLyS levels.
Methods: Sixty CLL patients, followed and treated in our hospital were studied; 58%, 25%, and 17% were in Binet stage A, B and C respectively. Patients' median TFT was 34 months (range 1-157), while median OS was 79 months (range 1.8-174). Frozen sera aliquots drawn at diagnosis were tested for sTACI. Measurements were performed by ELISA (R&D, Quantiquine duo set), according to the manufacturer's instruction. Corresponding soluble BLyS levels ranged from undetectable to 680 pg/ml (median 65) and were statistically significantly lower in patients with a shorter TFT. Statistical analysis was performed by standard methods.
Results: Median serum sTACI levels were 2,52 ng/ml (range undetectable - 17,39) in patients and 0,865 ng/ml in HI (range undetectable - 4,14). sTACI concentrations strongly correlated inversely with soluble BLyS (p=0,000021), while with regard to CLL parameters of disease activity, they correlated with b2-microglobulin (p=0,005), inversely with anemia (p=0,03), thrombocytopenia (p=0,04), Binet stage (p=0,02) and with free light chains ratio (p=0,0003). In addition, serum sTACI values above median were related to a shorter TFT compared to values below median (p=0.007) and, importantly, also to OS (p= 0,048).
Summary and Conclusions: sTACI serum concentrations at diagnosis is a powerfull prognostic marker in CLL, related to parameters of disease activity and stage and more importantly to TFT and OS.

PB1523

SYSTEMATIC REVIEW OF THE RECENT EVIDENCE OF EFFICACY AND SAFETY OF CHLORAMBUCIL IN PREVIOUSLY UNTREATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chlorambucil was historically considered as treatment of choice for previously untreated patients with chronic lymphocytic leukemia (CLL). Emergence of agents as purine analogue (as fludarabine) limited using of chlorambucil to elderly and/or co-morbid patients who may not tolerate aggressive approach due to high risk of unacceptable toxicity.
Aims: Provides recent evidence on efficacy and safety of chlorambucil used alone or in association with other treatment in CLL patients.
Methods: This review attempted to collate all recent empirical evidence that assess efficacy and safety of chlorambucil in CLL. In a systematic research prospective, controlled, randomized, studies on human investigating the benefits and harms of chlorambucil to treat CLL and including at least one group with chlorambucil will be searched (1rst January 2000 to 30 January 2014) in PubMed, Medline databases. Abstracts (less than 2 years) from the 4 main congresses in hematology were also searched (ASCO, ASH, ESH, IWLLC).
Results: Eight studies were identified. Most of studies included co-morbid or unfit patients. In 2 studies all stages of the disease were included (Raï stage I to IV). Each study comprised a group with chlorambucil using alone. Dose (40 mg/m^2 D1, 10 to 12 mg/ m^2 D1 through D7, 0,4 mg/kg to 0,8 mg/kg D1 and D15 each 28 days) and duration (6 to 12 cycles of 28 days) of chlorambucil regimen was very heterogeneous between studies. Two studies included 3 groups of treatment. The comparisons were done either *versus* an association of chlorambucil with fludarabine (1 trial), with ofatumumab (1 trial), with rituximab (1 trial), with obinotuzumab (1 trial) or *versus* a monotherapy: cladribine (1 trial), fludarabine (3 trials), alemtuzumab (1 trial), bendamustin (1 trial). Primary end points were overall survival (OS) (2 trials), progression free survival (PFS) (3 trials), PFS and OS (1 trial), overall response rate (ORR) and PFS (1 trial) and no defined in 1 trial. Median time of follow up ranged from 23 months to 62 months. In chlorambucil groups, ORR ranged from to 31% to 72% and complete response rate (CRR) from to 0% to 10,8% (without steroid) to 12% (with prednisone). Median time for PFS ranged from 8.8 months to 20 months and for OS from 46 months to 78 months. Chlorambucil was used as comparator in 2 more recent RCT assessing efficacy and safety of monoclonal anti-CD20 drugs in association with chlorambucil. In a recent randomized clinical trial (RCT) conducted in advanced age or with co-morbidities untreated CLL patients considered inappropriate for fludarabine-based therapy the median PFS (primary end point) in chlorambucil group was 13.1 months and 22.4 months in ofatumumbab + chlorambucil group (p<0.001). In an another RCT conducted in untreated active CLL with CIRS > 6, the median PFS (primary end point) was 11.1 months in chlorambucil group, 26.7 months in obinotuzumab + chlorambucil group (p<0.001 *vs* chlorambucil) and 16.3 months in rituximab + chlorambucil group (p<0.001 *vs* chlorambucil).
Summary and Conclusions: Recent trials showed that chlorambucil using alone has a low toxicity and acceptable ORR with low level of CR. In unfit patient inappropriate for fludarabine-based therapy, chlorambucil remains the backbone of treatment particularly with the new monoclonal anti-CD20 drugs.

PB1524

PROGNOSTIC SIGNIFICANCE OF IMMUNOPHENOTYPING AND QUANTIFICATION OF CLONAL LYMPHOID CELLS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: With the advent of highly effective treatment options, minimal residual disease (MRD) measurements gained interest as a prognostic marker in chronic lymphocytic leukemia (CLL). With the advent of routine bench-top cytometers capable of simultaneously analyzing ≥ 4 colors and with improved standardization, multicolor flow cytometry (MFC) has become the method of choice for MRD assessments.
Aims: To determine the pretherapeutic immunophenotype and the quantification of clonal lymphoid cells (CLC) and their dynamics after immune-based combination therapies in patients with CLL.
Methods: Thirty-one adult pts (median age 64 years, range 31 - 80; male 20, female 11) with newly diagnosed CLL were included. The diagnosis of CLL was made according to standard diagnostic workup (complete blood count with differential, MFC analysis of blood and bone marrow (BM), lymph node and BM immunohistochemistry (IHC), computered tomography). CLC were prospectively quantified in 31 BM samples based on relative quantity of typical CLL cells (CD5+CD19+CD20+CD23+) and quantity of myelocariocytes. Immunophenotype (IFT) of CLL cells assessed with combinations: CD3/CD19, CD19/CD5, CD19/CD11c, CD19/CD20, CD19/CD22, CD19/CD23, CD19/CD25, CD19/CD38, CD19/CD43, CD19/CD81, CD19/HLA-DR, and CD19/CD5/CD23. We have used NCI revised guidelines (Hallek M, *et al.*, 2008) for treatment initiation and assessment of response after completion of primary therapy with rituximab (RTX)-based regimens (FCR, RB, and RChl). Patients with partial response (PR) and complete response (CR) treated subsequently with RTX maintenance. The quantity of CLC were measured in 6 months (9/17 pts), and in 12 months (5/17) from the start of RTX maintenance.
Results: CD5+CD19+CD23+ cells were detected in 93.5% cases. In cases of CD5-negative CLL (6.5%) assessed with MFC, there were CD5+ slightly positive (IGH) lymph node samples. High expression level of pan-B-cell markers (CD20, CD22) detected with use of MFC (93.5%, and 54.8%, respectively). Elevated expression of CD43 was detected in 90.3%, HLA-DR – 25.8%, CD81 – 22.6%, and CD25 – 6.5% of cases. High expression level of CD38 – 29.0%, CD11c – 25.8%. CLC quantity at diagnosis without indications for treatment was 50.48±10.94x10^9/L (n=9), and 92.27±19.71x10^9/L, when primary therapy was indicated (n=22). The measurement of CLC at the end of induction therapy (n=17) revealed statistically significant differences (*p<.05*): CR (n=10) – .004±.002 x10^9/L, and PR (n=7) - .718±.369 x10^9/L. CLC quantity in 6 months of RTX maintenance - .085±.047x10^9/L, in one year - .002±.002 x10^9/L (*p<.05*). MRD was not detected (<10^{-4} tumor cells) in five (50%) pts at time of CR documentation. IFT of tumor cells at relapse/progression (n=4) did not changed (CLC quantity - 13.9±8.58x10^9/L).
Summary and Conclusions: Detection of IFT and quantification of CLC at diagnosis may help in decision-making process in newly diagnosed CLL patients. RTX maintenance leads to the more complete eradication of tumor clone. It seems that the treatment planning aimed toward achieving long-lasting CRs should include at least one test to assess MRD because the lack of leukemia persistence using these sensitive tests seems to have a strong, positive prognostic impact.

PB1525

EFFICACY OF THE COMBINATION OF BENDAMUSTINE AND RITUXIMAB IN PATIENTS WITH RELAPSED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Currently, one of the treatment's options of relapsed and refractory chronic lymphocytic leukemia (CLL) is the combination of bendamustine and rituximab (BR).
Aims: Evaluate the efficacy and safety of the combination of BR in patients (pts) with relapsed and refractory CLL with regard to the presence of unfavorable prognostic factors.
Methods: Thirty six pts with CLL at the age of 35-72 years (a median age – 56 years) were included in the study. All pts had ECOG status less than 3. Seventeen pts (47%) had B stage and 19 (53%) – stage C by Binet. In 32 (89%) pts a positive expression of ZAP-70 was revealed during the diagnosis and in 24 (67%) pts – positive expression of CD38. In 28 (78%) pts the level of serum thymidine kinase (TK) was ≥ 20 U/l. According to FISH-analysis, 8 (22%) pts had del17p and 15 (42%) pts had del11q. In 14 (39%) pts refractory to prior therapy was registered, 22 (61%) pts had relapsed CLL. The prior therapy included regimens containing fludarabine (FC, FCR and FCMR) and alemtuzumab monotherapy. Median lines of the previous therapy were 2 (from 1 to 4). Pts were treated with BR therapy: bendamustine was administered in a dose of 90 mg/m^2 i.v. on the first and second days, rituximab – in a dose of 375 mg/m^2 i.v. on the first day of course. Therapy BR was carried out every 28 days up to 6 courses. Response to the treatment was assessed according to the criteria IWCLL.
Results: With the combination of BR the overall response rate was 64% (23 pts). Complete remission was achieved in 6 (17%) pts, partial remission – in 17 (47%) pts. Thirteen pts (36%) were non-responders. Response to the treatment was achieved in 6 (43%) pts with refractory CLL and in 18 (82%) pts with relapsed CLL (p=0.040). In pts with refractory CLL more unfavorable prognostic factors were revealed such as del17p, del11q, serum TK ≥ 20 U/L and positive expression of ZAP-70, in comparison with the pts having a relapse (86% *vs.* 45%, p=0.039). With a median follow-up of 27 months median overall survival has not been reached. The median progression free survival (PFS) was 19 months. In the process of a multivariate analysis including age, gender, ECOG status, serum TK, the expression of ZAP-70 and CD38, del11q and del17p, independent prognostic factors for PFS identified serum TK (p=0.031), ZAP-70 (p=0.034) and del17p (p=0.013). Neutropenia, thrombocytopenia, anemia of the 3-4 grade were detected in 8 (22%), 7 (19%) and 5 (14%) pts, respectively. The infection of the 3-4 grade (2 cases of pneumonia, 1 case of sepsis) occurred in 3 (8%) pts.
Summary and Conclusions: Thus, the combination of BR is a safe and effective therapy for pts with relapsed and refractory CLL. The most expressed efficacy was observed in pts with relapses who lacked unfavorable prognostic factors, in comparison with pts with refractory CLL and the presence of unfavorable prognostic factors.

PB1526

PROGRESSION-FREE SURVIVAL AS A SURROGATE ENDPOINT FOR OVERALL SURVIVAL IN RELAPSED-REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: Advancements in treatments for end-stage diseases, specifically in oncology, have made collecting overall survival (OS) as the primary endpoint in clinical trials more challenging. As a result, other endpoints to demonstrate the effectiveness of drugs are becoming increasingly important.
Aims: The purpose of this research is to investigate the potential of progression-free survival (PFS) as a surrogate endpoint predicting overall survival in relapsed and/or refractory (R/R) chronic lymphocytic leukaemia (CLL).
Methods: A systematic literature review was undertaken using MEDLINE and EMBASE, to identify clinical trials investigating the effectiveness of treatments in adult R/R CLL patients. Trials included in the analysis had to report median OS and median PFS. To minimise the risk of attrition bias, studies with substantial loss to follow up (*i.e.* >30%) were excluded. The level of surrogacy was quantified using Pearson and Spearman rank correlation statistics, and weighted least squares (WLS) regression using sample size weights. Correlation statistics were also evaluated within different patient subgroups.
Results: A total of 34 trial arms were analysed from 28 clinical studies. Both correlation analyses demonstrated a strong association between median PFS and median OS in R/R CLL (Spearman=0.891; Pearson=0.877, 95% CI: 0.711 to 0.951). WLS regression also showed positive results (R^2=0.805) with a one month extension of median PFS contributing to 1.42 (95% CI: 1.076 to 1.768) month extension in median OS (p<0.01). The endpoints showed the strongest correlation in trials where the mean age of patients was over 65 (0.922; 95% CI: 0.214 to 0.995), and in trials where patients were treated with a chemo-immunotherapy combination (0.922 95% CI: 0.258 to 0.953).
Summary and Conclusions: These results give convincing evidence about the validity of PFS as a surrogate for OS at the individual level. In fact, our findings demonstrate the potential of the median PFS to predict the median OS in this population of patients. This is necessary in CLL where patients are having increasingly long OS and PFS, justifying the need to establish a suitable surrogate. Further research is necessary to quantify the strength of surrogacy based on treatment effect data (*e.g.* hazard ratio for OS *vs.* hazard ratio PFS) using aggregate or individual patient level data. This research provides the first step in establishing the use of PFS as an acceptable primary clinical endpoint for R/R CLL patients in decision making in the future.

PB1527

RITUXIMAB PLUS BENDAMUSTINE IN ELDERLY PATIENTS AFFECTED BY RELAPSED OR REFRACTORY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a highly variable clinical course. Among the biological features underlying this heterogeneity, genetic lesions and the mutational status of the immunoglobulin heavy chain variable genes (IGHV) are of importance. Therapeutic options in CLL have been considerably expanded during recent years. The combination of fludarabine, cyclophosphamide and rituximab (FCR) has become gold standard in the first-line treatment of physically fit patients. Bendamustine demonstrated clinical activity in pre-treated hematological malignancies due to its unique mechanism of action distinct from standard alkylating agents.
Aims: We have assessed the efficacy and safety of bendamustine plus rituximab in elderly patients with pre-treated chronic lymphocytic leukaemia.
Methods: In the last 48 months we treated 24 elderly patients (12 F and 12 M, median age: 77 years, r.: 72-88 years) with relapsed/refractory CLL who had been heavily pretreated (more than 4 lines of treatment). Patients received Bendamustine 90 mg/m^2 on days 1 and 2 and Rituximab 375 mg/m^2 on day 3 of a 4-week cycle. A dose escalation of Rituximab to 500 mg/m^2 was performed from second course. 20/24 patients received six cycles, while the remaining two received four cycles.
Results: Overall response rates were 75% (18/24 patients), with clinical complete response rates of 50%. At present (+36 months after the end of treatment), 20/24 patients are alive and show no disease progression. One patient died due to respiratory infection six months after the end of therapy. Thrombocytopenia and gastrointestinal toxicities were more frequent adverse events. Grade ≥ 3 adverse events were infrequent and most commonly included thrombocytopenia (20%), anemia (10%), and infection (10%).
Summary and Conclusions: Our results suggest that bendamustine plus rituximab is effective and well tolerated in the treatment of relapse/refractory CLL in elderly patients. Although further data are required to fully establish the efficacy of this combination therapy in the management of this subset of patients, it appears to be a useful addition to the armamentarium of currently available therapies for this haematological malignancy.

PB1528

ROLE OF PERIPHERAL BENZODIAZEPINE RECEPTOR IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background: The peripheral benzodiazepine receptor (PBR) is a critical component of the mitochondrial permeability transition pore (MPTP), a multiprotein complex placed at the contact site between inner and outer mitochondrial membranes, which is intimately involved in the initiation and regulation of apoptosis. PBR is physically associated with the voltage-dependent anion channel (VDAC) and adenosine nucleotide translocase (ANT) that form the

backbone of MPTP. Consistent with its localization in the MPTP, PBR has been involved in the regulation of apoptosis, but also in the regulation of cell proliferation and other related processes. Transfection-enforced overexpression of PBR attenuates apoptosis induced by oxygen radicals or ultraviolet light. Together, these observations led several authors to consider the PBR as a promising drug target, especially in the field of cancer.
Aims: Chronic lymphocytic leukemia (CLL) cells have increased levels of radical oxygen species (ROS). In this prospective study, we have investigated PBR expression in order to use the latter as therapeutic target and prognostic marker.
Methods: We have investigated PBR expression in leukocyte subsets from healthy donors and CLL patients before and after chemo-immunotherapy. Moreover, we evaluated in lymphocytes of healthy donors and CLL patients the levels of toxic aldehydes (MDA) by thiobarbituric acid reactive species (TBARS) assay as expression of oxidative stress.
Results: In 30 CLL patients the CLL cells showed an increased number of PBR receptors normalized for mitochondria expression if compared to normal lymphocytes. Moreover, CLL cells show low levels of TBARS, NO and caspase-3 activity compared to the lymphocytes of healthy donors. After 6 months from the beginning of treatment PBR/mitochondria ratio resembled that one of healthy controls in 24/30 CLL patients. In addition, we evidenced an increase of toxic aldehydes (TBARS assay) and nitric oxide levels, both markers of oxidative stress, and a potentiation of caspase-3 activity in all responder patients. Interestingly, the 6 patients who resulted resistant to treatment displayed also higher PBR levels and lower caspase-3 activity and TBARS levels.
Summary and Conclusions: These data suggest that PBR expression could be a molecular prognostic factor predictive of response in CLL patients. Moreover, PBR could also represent a useful therapeutic target in order to increase treatment activity in CLL patients.

PB1529

BENDAMUSTINE AND RITUXIMAB AS FIRST OR SECOND LINE THERAPY IN ELDERLY PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: Current standard therapy for fit young patients with chronic lymphocytic leukemia (CLL) is rituximab, fludarabine, cyclophosphamide (RFC). This therapy is highly effective in standard risk patients with CLL, however in the elderly it can be troublesome since it can give unacceptable myelotoxicity and increase risk of infection. Because median age at diagnosis for CLL patients is now 72, the majority of patients cannot receive RFC, the best therapy available.
Aims: We investigated the safety and efficacy of Bendamustine plus Rituximab (RB) in elderly patients with CLL as second or first line of therapy at three centers in Tuscany, Italy
Methods: Twenty-six patients were treated, 9 at diagnosis and 17 at first relapse. Patients were M/F 14/9, median age was 72 (range 67-80). Six cycles of R-B were planned. Rituximab was given 375 mg/m2 day 3 in the first cycle and then 500 mg/m^2 day 1 of each subsequent cycle. Bendamustine was given 70 mg/m^2 day 1-2 cycle 1 and then day 2-3 in cycles 2-6. Antibiotics prophylaxis was given days 1-10 of each cycle. Biological prognosticators were assessed at diagnosis and at disease relapse. In particular IGHV gene use and mutational status, ZAP-70 protein and CD38 expression by flow cytometry, deletions of 11q22.2, 13q14.1, 17p13.1 loci and chromosome 12 trisomy were determined by fluorescent *in situ* hybridization (FISH) as previously reported.
Results: Results.up is 18 months. Overall response was observed in 22/26 (84%) of the patients, being 6 CR (23%, 4 at diagnosis, 38%), 16 PR (61%). No differences were seen in response and PFS for patients with del 11q , trisomy 12 and del 13q abnormalities; del17p responded less as well as patients with unmutated IGHV. Median PFS for patients treated at diagnosis has not been reached. PFS for patients treated at first relapse is 17 months. Hematologic toxicity was evident but manageable: grade 3-4 in 10/26 (38%) patients, grade 1-2 in 7/26 (27%). Extra hematological toxicity was mild : grade 1 gastrointestinal in 19/26 patients, grade 1 cutaneous in 5/26 patients. Four patients developed pneumonia.
Summary and Conclusions: RB in extremely effective therapy in elderly patients with CLL and particularly at diagnosis. Hematological toxicity is the most important toxicity but can be easily managed with growth factors and antibiotics prophilaxis. These results are important because obtained in a selected aged population of patients.

PB1530

BONE MARROW FINDINGS IN CLL-LIKE MBL AND CLL RAI STAGE 0: CORRELATION WITH MAIN DISEASE FEATURES AND OUTCOME
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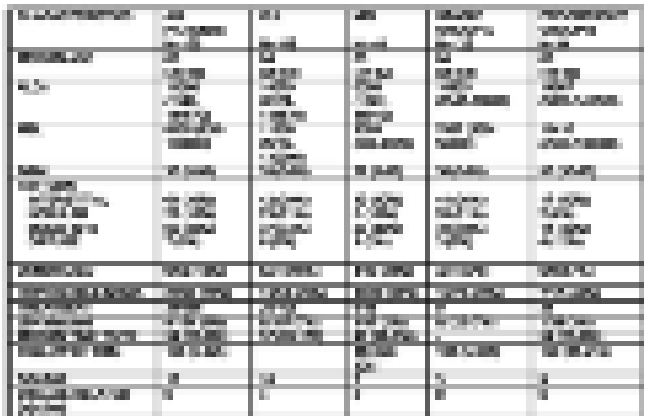
Background: CLL-type MBL and CLL Rai 0 share many clinical and biological similarities. Data on bone marrow histopathology findings in these entities are limited.
Aims: To evaluate the histopathological and immunohistochemical findings of bone marrow biopsies (BMB) in a series of patients with MBL and CLL Rai 0 and to correlate these findings with other disease features.
Methods: 156 pts were included in this study. At diagnosis all pts underwent whole body CT scan and BMB. Evaluation of the extent and the pattern of BM infiltration, the cytology and the immunohistology were performed. Clinical and laboratory data were analyzed.
Results: 48 pts had MBL and 108 CLL Rai 0. The main clinical and laboratory characteristics are shown in table 1. There was significant difference in the extent of BM infiltration between the two groups (p=.003). Furthermore, in MBL cases a mixed (nodular and interstitial) pattern predominated (48%), while in CLL cases the interstitial pattern (39%). The diffuse pattern was uncommon and was correlated with worse outcome. All cases but one exhibited neoplastic infiltration consisting almost exclusively by small lymphocytes. Only one case exhibited plasmacytoid differentiation. All cases presented weak expression of surface immunoglobulin, were CD20, CD79a, CD5 and CD23 positive, were negative for cyclin D1 while most of them were negative for cytoplasmic immunoglobulin. Most cases had preserved hematopoietic reserves, while in 13 cases BMB revealed hyperplasia of erythroid (7 cases) and megakaryocytic (6 cases) series. At a median follow up time of 106 mos (5-325), 122 remained stable (group A) while 34 progressed to CLL requiring therapy (group B). The median lymphocytic infiltration of group A and B was 30% (5-95%) and 50% (30-80%), respectively (p<.001). The nodular pattern was uncommon in group B (6% *vs* 21%). Hypoglobulinemia was evidenced in 22%and 41% of pts in group A and B respectively (p=.18). Unmutated IgVH genes were found in 7% and 67% of group A and B respectively (p<.001).
Summary and Conclusions: BMB evaluation provides important information regarding the tumor load of the disease which usually cannot be identified by the absolute B-cell counts only. BMB evaluation also correlates significantly with other disease features.

Table 1. Clinical characteristcs and BM findings in MBL and CLL Rai O patients.



PB1531

AUTOIMMUNE HEMOLYTIC ANEMIA IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES
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Background: Autoimmune haemolytic anaemias (AIHA) are diseases characterized by increased degradation of erythrocytes due to the presence of plasma proteins (globulin) that have character of antibodies' reacting with antigens of erythrocyte membranes of patients. According to the temperature at which the auto-antibodies bind to antigens in erythrocytes, AIHA are divided

into: 1) AIHA caused by warm antibodies. 2) AIHA caused by cold antibodies. 3) Mixed (mixing) type. Depending on the presence or absence of the underlying disease, AIHA are divided into primary and secondary.
Aims: The aim of this study is to monitor the occurrence of secondary AIHA in patients with lymphoproliferative diseases.
Methods: During the period of five years, we followed up 91 patients of both sexes, aged between 18 and 84 years, with the diagnosis of lymphoproliferative disease. The group consisted of 15 patients with acute lymphoblastic leukemia (ALL), 24 patients with chronic lymphocytic leukemia (CLL), 20 patients with Hodgkin's lymphoma, and 32 patients with non-Hodgkin's lymphoma. We used methods of clinical, laboratory and ultrasound examination.
Results: During the monitoring, 18% of patients were reported with autoimmune haemolytic anaemia occurrence. Anaemia is characterized by reticulocytosis, increased levels of lactate dehydrogenase, indirect hyperbilirubinemia, reduced levels of haptoglobin and positive direct Coombs antiglobulin test (DAT). In 80% of patients AIHA was caused by warm antibodies and in 20% of patients with cold antibodies.
We have discovered that the AIHA can occur earlier, together with the occurrence of lymphoproliferative disease, in the course of or after the end of chemotherapy, or as the first sign of disease relapse. The most common occurrence of autoimmune hemolytic anemia was recorded in the group of patients with CLL. Slightly higher incidence was found in the group of patients with Hodgkin's lymphoma, as compared to a group of patients with non-Hodgkin's lymphoma. In patients with CLL, AIHA was usually an early sign of disease relapse. The secondary AIHA is a well known paraneoplastic phenomenon in the lymphoproliferative disorders, closely associated with the development and progression of the disease.
Summary and Conclusions: AIHA may precede the appearance of lymphoproliferative disease, which has great diagnostic significance. AIHA also may be the first, early sign of the disease relapse. Fast definition of the AIHA cause is of great importance for the diagnosis, monitoring and further treatment of lymphoproliferative disease.

Chronic myeloid leukemia - Biology

PB1532

2-HYDROXYPROPYL-B-CYCLODEXTRIN ACTS AS A NOVEL ANTICANCER AGENT

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Background: Molecular-targeted drugs have superior anticancer effects compared to those of conventional chemotherapeutic agents, and have less adverse effects. However, resistance to chemotherapy, such as that resulting from point mutations or the existence of cancer stem cells, still hinders the treatment of cancer patients. Thus, alternative therapeutic approaches that enhance neoplastic cell death are required for successful cancer treatment. Cholesterol accumulation and/or dysregulated cholesterol metabolism is reported in various malignancies, including leukemia. Thus, modulation of cholesterol homeostasis might be a rational target for the development of anticancer agents. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CyD) is a cyclic oligosaccharide that is widely used as an enabling excipient in pharmaceutical formulations, but also as a cholesterol modifier. HP-β-CyD has recently been approved for the treatment of Niemann-Pick Type C disease, a lysosomal lipid storage disorder, and is used in clinical practice. However, the potential anticancer activities of HP-β-CyD have not yet been noted.
Aims: We hypothesized that HP-β-CyD itself might have anticancer effects, and investigated anticancer activities of HP-β-CyD using BCR-ABL-induced leukemia models.

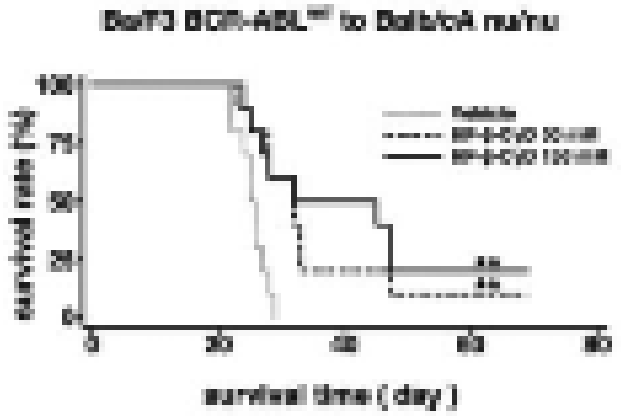

Figure 1. Administration of HP-β-CyD prolongs the survival in mouse model of BCR-ABL-induced leukemia. **P<0.01, compared with vehicle-treated mice.

Results: HP-β-CyD significantly inhibited the growth of mouse pro-B cell Ba/F3 cells expressing wild-type BCR-ABL (Ba/F3 BCR-ABL^{WT}), and BV173 cells, in a dose- and time-dependent manner. The IC_{50} values of 13 leukemic cell lines (Ph^+ leukemia; BV173, K562, KBM-5, KBM-5/STI, KCL22, MYL and MYL-R, AML; HL-60, pre-B cell leukemia; NALM-6, T-cell leukemia; Jurkat and MOLT-4, adult T-cell leukemia; MT1, and MT2) for HP-β-CyD after 72 hours exposure were in the range of 3.86-10.09 mM. Interestingly, HP-β-CyD-induced cell growth inhibition of imatinib-resistant chronic myeloid leukemia (CML) cell lines, such as KBM-5/STI and MYL-R was equivalent to that of respective parental cells. Subsequent *in vitro* analyses demonstrated that HP-β-CyD inhibits the proliferation of leukemic cells by induction of apoptosis and G_2/M cell-cycle arrest. Next, to clarify whether apoptosis induced by HP-β-CyD is related to cholesterol depletion, we evaluated the effect of CyDs on cholesterol efflux from leukemic cells, and revealed that HP-β-CyD treatment increased cholesterol release in a time and dose-dependent manner. In light of the data showing the inhibitory effect of HP-β-CyD on the proliferation of CML cell lines, we were prompted to evaluate HP-β-CyD for their potential to overcome tyrosine kinase inhibitor (TKI) resistance. The IC_{50} of HP-β-CyD against Ba/F3 BCR-ABL^{T315I} cells was comparable to that of the Ba/F3 BCR-ABL^{WT}, and similar results were obtained in cell proliferation assays that used trypan blue staining. These results suggest that HP-β-CyD can inhibit the

proliferation of TKI-resistant cell lines and may be of use against TKI resistance. Importantly, HP-β-CyD also inhibited proliferation of hypoxia-adapted CML cells that have characteristics of leukemic stem cells, and human primary leukemic cell colony formation. Finally, to investigate the *in vivo* efficacy of HP-β-CyD, we generated mouse models of BCR-ABL-induced leukemia, and administered HP-β-CyD. As a result, HP-β-CyD-treated mice survived longer than vehicle-treated mice, and the log-rank test for overall survival showed statistically significant differences between vehicle- and HP-β-CyD-treated recipients (Figure 1). HP-β-CyD treatment also prolonged the survival in human leukemia xenograft model. Systemic administration of HP-β-CyD to mice had no significant adverse effects.
Summary and Conclusions: These data suggest that HP-β-CyD is a promising anticancer agent. This study is the first report to describe the efficacy of HP-β-CyD in BCR-ABL-induced leukemia, including mutated TKI-resistant clones and hypoxia-adapted cells. Early-phase clinical trials are planned to verify the efficacy and safety of HP-β-CyD for treatment of leukemia.

PB1533

PEROXIREDOXIN EXPRESSION COULD BE INVOLVED IN THE INCREASE OF REACTIVE OXYGEN SPECIES AND DRUG RESISTANCE TO TYROSINE-KINASE INHIBITORS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Peroxiredoxins (Prdx) are a family of multifunctional antioxidant thioredoxin-dependent peroxidases that not only protect cells against oxidative stress but also modulate signaling cascades that apply hydrogen peroxide as a second messenger molecule and regulate cell proliferation. Thereby the Prdx are related to tumorigenesis while acting as cellular antioxidants, regulating cell apoptosis and proliferation, modulating signaling cascades and interacting with oncogene products and molecules related to tumorigenesis. The relation between Prdx and Chronic Myeloid Leukemia (CML) has not been deeply studied up to now; however, there are evidences that the increase of oxidative stress by Reactive Oxygen Species (ROS) could contribute to genomic instability, leading to accumulation of mutations and chromosomal aberrations that could lead to resistance to Tyrosine Kinase Inhibitors (TKI) used to treat the ailment.
Aims: This study aims to correlate gene and protein expression of Peroxiredoxins with oxidative stress, development of Chronic Myeloid Leukemia and the promotion of Tyrosine Kinase Inhibitors (TKI) resistance, allowing better understanding of the disease and helping to develop new targets to improve the treatment for patients with TKI resistance.
Methods: Peroxiredoxin gene and protein expression was evaluated in 17 CML patients in the chronic phase and treated with TKI, and in 8 healthy volunteers. All patients were classified according to criteria of the European Leukemia Net as responsive (6 patients), resistant (6 patients) or with sub-optimal response (5 patients) to TKI. The CML patients were treated with Imatinib 300-800 mg/day, Dasatinib 70-140 mg/day or Nilotinib 800 mg/day. Dasatinib and Nilotinib were used in patients with resistance to Imatinib. All samples were collected at the blood center at the Hematology and Hemoterapy Center at University of Campinas. RNA samples were submitted to the synthesis of cDNA using the kit RevertAid™ HMinus First Strand cDNA Synthesis Kit (Fermentas, Life Sciences). The gene expression was evaluated by quantitative real time PCR using β-Actina as endogenous control, and the results were analyzed using $2^{-\Delta\Delta CT}$; statistical analysis were made by using student's T test. This study was supported by the FAPESP agency.
Results: The results showed that there is a difference in the Peroxiredoxin gene expression between patients with CML and control individuals. The *PRDX 1* expression was significantly reduced in all groups with CML when compared to the healthy volunteers. The *PRDX2* expression was reduced in responsive patients, but did not exhibit significant difference in the other groups. *PRDX6* had a decrease in its expression in responsive and suboptimal response patients. To evaluate the proteins oxidative stress and levels, Western Blot analysis will be made.
Summary and Conclusions: Our results indicate that Peroxiredoxin gene expression is lower in patients with CML when compared to healthy volunteers. Results presented in literature indicated that the increase of ROS in CML leads to an increase of DNA damage, triggering genomic instability and resulting in accumulation of mutations and chromosomal aberrations (Nieborowska-Skorska, Blood, 2012). These alterations could be involved in the mechanism of acquisition of resistance to TKI inhibitors observed in CML patients. The decrease of Peroxiredoxins expression observed, especially in the responsive group, could contribute to this process, since the detoxification of these species are compromising and the effects caused by oxidative stress are even more drastic, leading to mutations that could be followed by TKi resistance.

PB1534

HIGH RESOLUTION MASS SPECTROMETRY OF IMATINIB BIOTRANSFORMATION PRODUCTS

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Background: Therapeutic drug monitoring is widely applied useful tool for treatment individualization in order to achieve optimal clinical response and avoid toxicity. Due to drug metabolizing enzymes several bioactive or toxic metabolites may be produced. Therefore determination of not only parent drug but also of metabolites may have clinical relevance and could be helpful for treatment adjustment.
Aims: To assess a detailed profile of imatinib (IMA) metabolites in plasma of patients with chronic myeloid leukemia (CML).
Methods: Before analysis plasma proteins were precipitated by methanol and supernatant was injected. Separation was performed on Phenomenex Kinetex C18 column (100 x 2.1 mm; 1.7 µm) using UltiMate 3000 RS (Thermo Scientific) liquid chromatography. For detection Orbitrap Elite mass spectrometer (Thermo Scientific) based on exact mass measurement was used. Scan range of *m/z* 350 – 1200 was chosen and the resolution was set at 60,000 FWHM. MS/MS data were acquired in data-dependent strategy based on fragmentation of 25 selected exact *m/z* values of potential metabolites. Collision energy for CID was set at 35 eV. Dynamic exclusion duration of 3 s was enabled; mass accuracy was 5 ppm. Data were evaluated using Excalibur 2.2 SP1, MetWorks 1.3 SP3 and Mass Frontier 7.0 software. Eight samples of 7 CML patients with optimal or suboptimal response to treatment were analyzed (age 41-77 years, 400 mg IMA/day; sampling time 20-27 h after IMA ingestion).
Results: Contrary to previously described 14 IMA metabolites in plasma (Rochat B. *et al.* Mass Spectrom. 2008;43:736) we have found 100 potential metabolites in concentration range of 4 orders of magnitude (0.001 – 6.630 %) related to IMA plasma levels (635 – 1360 ng/mL). All metabolites found in plasma were identified on the base of exact *m/z* values and confirmed by MS2 and MS3 fragmentation experiments. Importantly, differences in the profiles of oxidized and dioxidized metabolites in optimally and sub-optimally responding patients were observed.
Summary and Conclusions: The new generation of high resolution mass spectrometry offers sensitive tool for detail study of drug metabolisation in biofluids. IMA is metabolised to many metabolites which have the potential to be diagnostically useful. Further studies should be focused on elucidation of clinical significance of newly discovered metabolites.
Acknowledgements: The work was supported by grant LF UP 2013-004 and 2013-010, grant of IGA Ministry of Health, Czech Republic NT12218-4/2011. Infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from the Operational Program Research and Development for Innovations; Project CZ.1.05/2.1.00/01.0030.

PB1535

IMATINIB AND PLATELET DERIVED GROWTH FACTOR COOPERATE IN INHIBITION OF MESENCHYMAL STEM CELL PROLIFERATION AND SUPPRESSION OF PLATELET DERIVED GROWTH FACTOR RECEPTOR EXPRESSION

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Background: Imatinib is a tyrosine kinase inhibitor (TKI), which is effectively used in the treatment of gastrointestinal stromal tumors (GIST) and chronic myeloid leukemia (CML). Imatinib selectively inhibits Platelet Derived Growth Factor Receptor (PDGF-R), c-kit and BCR-ABL. PDGF/PDGFR Signaling has been shown to be important for tumor progression by modulating the tumor microenvironment through promoting angiogenesis and tumor invasiveness.
Aims: Here, we wanted to assess the effects of Imatinib on proliferation of Mesenchymal Stem Cells (MSCs), the effect on differentiation of MSCs and expression of the PDGF-R.
Methods: Mesenchymal Stem Cells were isolated from healthy donor bone marrow and cultured in complete medium (CM), consisting of 60% MCDB201/40% DMEM-LG basal media, supplemented with 10% heat inactivated fetal calf serum, 1% penicillin/streptomycin and 2 mM L-glutamin. Proliferation was measured for 10 days using the XCelligence Real Time Cell Analyser (Roche, Turkey) using seeding cell doses of 1000-8000 per well in presence of 2,5 – 20 µM Imatinib (Novartis, Turkey). FACS analysis was performed for CD140b (PDGFRb) using a FACSARIA (Becton Dickinson,

Turkey). Adipogenic and osteogenic differentiation was assessed after three weeks of induction in appropriate differentiation media. Cultures were stained with Oil Red O or Alizarin Red S for adipogenic and osteogenic differentiation, respectively. Oil Red O dye extraction and Calcium assays (DICA-500) were performed for quantitation of differentiation. All tests were performed using Passage 3 MSCs.

Results: Imatinib inhibited MSC proliferation at all doses tested, but inhibition was not further increased at doses exceeding 5 μM. Initial seeding cell doses were found to be optimal at 1000 to 2000 cells/well. Increasing cell doses in presence of 5 μM Imatinib, resulted in competition and cell doses of 8000 cells/well in presence of 5 μM Imatinib showed similar proliferation as 2000 MSCs without Imatinib. Unexpectedly, Imatinib aggrevated PDGF induced inhibition of proliferation of MSCs. FACS analysis demonstrated suppression of CD140b after 24 hours of incubation with 5 μM Imatinib from 94.4±6.2% before Imatinib to 77.3±10.5% after Imatinib ($p<0.02$). Addition of 5 or 10 ng/mL PDGF-BB resulted in a further decrease of CD140b expression to 43.9% and 24.9%, respectively. Similar results were obtained after incubations in presence of Imatinib and PDGF for 48 hours or under low serum conditions (2% FCS). Differentiation assays confirmed lack of effect on adipogenic differentiation, but a prominent stimulative effect on osteogenic mineralization.

Summary and Conclusions: Imatinib inhibits PDGF through interference with tyrosine kinases. However, PDGF and Imatinib may reinforce each others actions by simultaneous suppression of the PDGF receptor and interfering with its function. This may result in inhibition of proliferation of MSCs, but may also be the case in GISTs. PDGF has been implicated in regulation of invasiveness of cancers and TKI have been used in the treatment of several types of cancer. Our results suggest that, counterintuitively, combined treatment with Imatinib and PDGF may be even more effective in suppression of cancer growth/invasiveness and may have important implications for future clinical treatment protocols.

PB1536

ADDITIONAL CHROMOSOMAL ABNORMALITIES IN PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKEMIA PATIENTS APPEARING DURING THERAPY WITH TYROSINE KINASE INHIBITORS

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Background: Clonal cytogenetic aberrations of the Philadelphia chromosome (Ph) positive hematopoiesis most include a second copy of the Ph chromosome, i(17q), trisomy 8 and 19. They are non-random and have been associated with the natural evolution of chronic myeloid leukemia (CML) to advanced disease. Therefore, these aberrations are of clinical and biological importance.

Aims: This study evaluates the frequency of recurrent Ph-positive abnormalities and novel aberrations in Ph-positive metaphases.

Methods: Standard R-banded cytogenetic analysis of metaphases was performed to detect additional chromosomal abnormalities.

Results: Among 600 patients with CML,who were treated with tyrosine kinase inhibitors, 136 (22,66%) developed additional chromosomal aberrations. Of these chromosomal abnormalities, 13,23% include additional copy of the Ph chromosome, 13,23% with trisomy 8, 8,82% with nullisomy Y, 3,67% with isochromosome 17q and 2,94% with monosomy 7. We have also reported novel structural chromosomal aberrations in 52 patients (38,23%), 55,76% of them were isolated with Ph chromosome and 44,23% were associated with other aberrations. They include translocations t(2;12)(p21;p13), t(3;3)(q21;q26), t(3;12)(q21;p13), t(4;9)(q21;p21), t(6;9)(p23;q34), t(7;12)(q11;p13) , t(11;12)(p14;q12), t(12;17)(q14;p13) and rob(14;21)(q10;q10), deletions on chromosomes 3,6,7,9,11,13,14,15,16 and 20, additions on chromosomes 1, 20, 21 and duplication on chromosome 1.

Summary and Conclusions: The presence of additional chromosomal aberrations may reflect genetic instability and, therefore, intrinsic aggressiveness of the disease which will be less amenable to subsequent alternative treatments. Conventional cytogenetic analyses remain mandatory during follow-up of patients with chronic myeloid leukemia under tyrosine kinase inhibitor therapy.

PB1537

RESISTANCE TO TYROSINE KINASE INHIBITORS OF CML PATIENTS MONITORED BY RTQ PCR AND DIRECT SEQUENCING

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Background: Chronic myeloid leukemia (CML) is monitored by the detection of BCR-ABL1 fusion gene on the molecular level. The protein fusion product BCR-ABL is created by the reciprocal chromosomal translocation t(9;22) and play the role of functional tyrosine kinase, which is constitutively activated by autophosphorylation and drives unchecked cellular signal transduction resulting in excess cellular proliferation. The treatment of the disease is managed by tyrosine kinase inhibitors (TKI). However, primary and secondary resistance is occurred and causes not responding or loss of response to the drug which results in relapse of the disease. Resistance can be caused by many mechanisms (*e.g.* efflux of the drug and it inactivation in plasma, mutations on DNA level). Malignant clones resistant to TKI may be acquired or selected during the treatment by several mutations in kinase domain of BCR-ABL1.

Aims: To monitor CML patients treated by TKI (imatinib in first line) by quantification of BCR-ABL1 transcripts by reverse real time PCR (RTQ PCR) and evaluate optimal and suboptimal response according ELN guidelines. In case of increasing levels of BCR-ABL1-trascripts asses the mutation analysis of the kinase domain of the fusion gene (KD BCR-ABL1) by direct sequencing.

Methods: Whole peripheral blood samples (or bone marrow) from CML patients treated by TKI (n=329) were obtained from 6 hematological clinics in Slovakia with patient´s informed consensus. RNA was extracted after separation of WBC and stabilization in RNAlater solution and cDNA was prepared by using TaqMan reverse transcriptase system. Quantification was performed by real time PCR system using a specific BCR-ABL and ABL TaqMan probes. The quantification of fusion transcripts were evaluated according to ELN guidelines as BCR-ABL/ABL ratio in% IS (international scale). Mutation analysis of KD BCR-ABL1 was assessed on seminested PCR product of fusion gene by direct sequencing using BigDye terminator chemistry for capillary electrophoresis in Genetic analyzer.

Results: Our molecular laboratory of Clinical genetics has assessed quantification of BCR-ABL1-trancripts of TKI-treated patients by real time PCR particularly every 3 months for five years. The suboptimal response (without Major molecular response MMR, or it´s lost) to TKI in 18 month was observed in 26% (77 from 329). In case of inadequate initial response to treatment or any sign of loss of response evaluation of ABL kinase domain mutation of BCR-ABL1 was done. From the cohort of 77 resistant patients mutations were detected in 28 cases (36%) (Figure 1) . Point mutation T315I (highly resistant to TKI) was confirmed 5 times, which is 18% from all detected mutations in resistant clones. In one case the CML patient with T315I mutation has undergone allo-SCT, but in 1 year he relapsed and the resistant clone with T315I reappeared.

Summary and Conclusions: The management of Ph^+, $BCR\text{-}ABL1^+$ CML patients is based on recommendations of European LeukemiaNet (ELN). The response to TKI is the most important prognostic factor. Monitoring of CML patients by RTQ PCR and direct sequencing is an important tool for clinicians, mainly in the case of resistance to TKI and in their choice of alternative doses of the drug or switch on TKI of 2th and 3th generation or undergo allo-SCT.

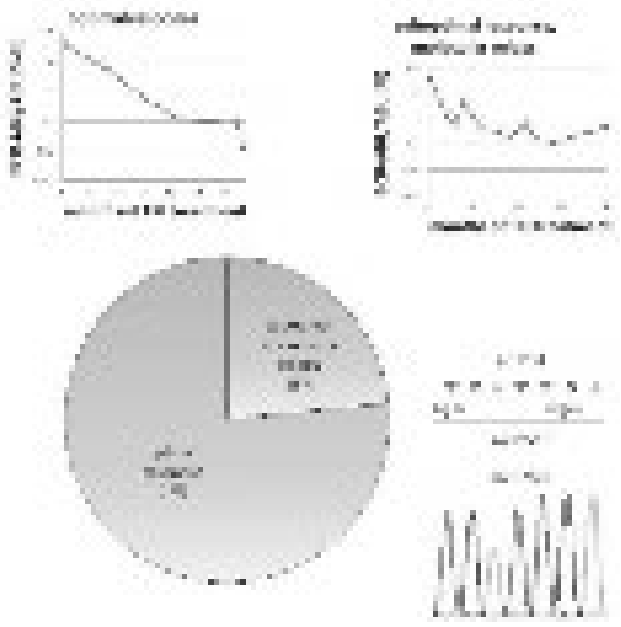

Figure 1.

PB1538

NILOTINIB INDUCES APOPTOSIS THROUGH JAK/STAT PATHWAY MEMBERS STAT5A & STAT5B IN K562 CML CELL MODEL

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Background: Signal transducers and activators of transcription (STAT) proteins function in the JAK/STAT signaling pathway and are activated by phosphorylation. As a result of this signaling event, they affect many cellular processes including cell growth, proliferation, differentiation, and survival. Increases in the expressions of STAT5A and STAT5B play a remarkable role in the development of leukemia in which leukemic cells gain uncontrolled proliferation and angiogenesis ability. Besides, these cells acquire ability to escape from apoptosis and host immune system. For chronic myeloid leukemia (CML) therapy, tyrosine kinase inhibitors are widely being used and one of them is nilotinib.
Aims: In the current study, we aimed to determine transcriptional and translational differences of STAT5A and STAT5B and also apoptosis rates following nilotinib treatment in CML model K562 cells.
Methods: Cell proliferation was assessed by WST assay in order to determine cytotoxicity of nilotinib upon leukemic cells. While mRNA expression levels of STAT5A and STAT5B were analyzed by real time qRT-PCR; protein expressions were detected via western-blot method following nilotinib treatment for duration of 24 – 96 hours also with untreated control group. Apoptosis was performed by "Caspase-3" and "Cell Death Detection" assays following the same treatments with same time intervals. Statistical analyses were done by GraphPad prism software with a significance of $p<0.05$
Results: While number of apoptotic cells were increased by 2.1 fold, 47.36% ($p=0.045$) as a result of measurement of caspase 3 activity; a 1.89 fold, 52.8% increase was detected in apoptosis rate following Cell Death assay ($p=0.0012$) in nilotinib treated group. As for mRNA expression results, while STAT5A expression was significantly decreased by 65.12% [(2.87 fold; $p= 0.0033$)] and by 90.9% [(10.99 fold; $p<0.0001$)] at 72^{th} – 96^{th} hours respectively; STAT5B was downregulated by 80.95% [(5.25 fold; $p= 0.0032$), and 93.36% (15.08 fold; $p<0.0001$)] for 72^{th} and 96^{th} hours in nilotinib treated group compared to control group. As for protein results, both STAT5A and STAT5B protein expression levels were inhibited in a time dependent manner but dramatic suppressions were detected especially at 96^{th} hour for each protein.
Summary and Conclusions: : In conclusion, target genes STATs expressions were suppressed at mRNA and protein levels and also leukemic cell apoptosis was induced following nilotinib treatment of IC_{50} dose. The underlying success mechanism of nilotinib treatment in CML might be due to inhibiting STAT5A & STAT5B transcription and translation. Therefore, targeting JAK/STAT pathway components and evaluating new candidate target genes responsible for CML pathogenesis has an accelerating importance in therapeutic application area.

PB1539

CABOZANTINIB INDUCE ERYTHROID DIFFERENTIATION OF K562 ERYTHROLEUKEMIA CELLS THROUGH DOWN-REGULATING PI3K/AKT SIGNALING PATHWAY

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Background: Chronic myeloid leukemia (CML) is a pluripotent hematopoietic stem cell disease which arises from the *bcr-abl* oncogene. The Bcr-Abl oncoprotein, as a constitutively activated tyrosine kinase, could activate multiple signaling pathways for the malignant transformation. Therefore, specific inhibitors of tyrosine kinases are attractive therapeutic agents. Cabozantinib (XL184, *N*-(4-((6, 7-Dimethoxyquinolin-4-yl) oxy) phenyl)-*N*-(4-fluorophenyl) cyclopropane-1, 1-dicarboxamide), a multiple tyrosine kinase inhibitor, has recently been approved by US FDA for the treatment of medullary thyroid cancer (MTC). The recommended daily dose and the maximum-tolerated dose (MTD) of cabozantinib are 140mg and 175 mg, respectively. Pharmacokinetics study revealed that the steady-state plasma levels were 2.8μM when administrating MTC patients with 175mg daily. In our preliminary results revealed that treating K562 cells harboring *bcr-abl* oncogene with 1μM cabozantinib for 3 days demonstrated an erythroid differentiation phenotype. We wonder that differentiation therapy could be an alternative strategy for treating CML.
Aims: This study was aimed to clarify the therapeutic potency of cabozantinib in CML and speculate its molecular mechanism.
Methods: Human leukemia cell line K562 was used as an *in vitro* model for this study. MTS assay was used to determine the effect of cabozantinib on cell viability. Morphology examination, quantitative real-time PCR, benzidine staining, flow cytometry, and western blotting were used in this study.
Results: Treatment with plasma-reachable dose (1μM) of cabozantinib in K562 leukemia cells for 72 hrs induced erythroid differentiation but not megakaryocytic maturation. We observed a significant population of cells with cytological features of early erythroid differentiation and significantly increased benzidine-positive cells (up to 50%) accompanying with significant accumulation of γ-globin. Expression of transferrin receptor (TfR, CD71) and glycophorin A (GPA, CD235a), both well documented erythroid-specific gene, were induced 3 to 4-fold relative to untreated control cells. Cell cycle analysis revealed that cabozantinib could also result in G_0/G_1 cell cycle arrest without inducing cell death in K562 cells. Signaling pathway analysis revealed that cabozantinib could decrease phosphorylation of ERK, STAT5 and AKT at 6 hours in a dose-dependent manner. Further analysis revealed that cabozantinib treatment could decrease c-Myc with a concomitant induction of p27 and decreased p21, cyclin D and cyclin E in a time- and concentration- dependent manner. Taken together, we proposed that cabozantinib could induce K562 cell differentiation toward erythroid lineage through inhibiting PI3K/AKT pathway, of which Myc, p27, cyclin D and cyclin E were involved (Figure 1).
Summary and Conclusions: We demonstrated cabozantinib with plasma-reachable dose could induce erythroid differentiation. Of note, down-regulating PI3K/AKT signaling pathway may be involved in the molecular mechanisms of cabozantinib-induced erythroid differentiation. This study uncovered the potential of cabozantinib for being a novel differentiation agent for treating patients with CML.

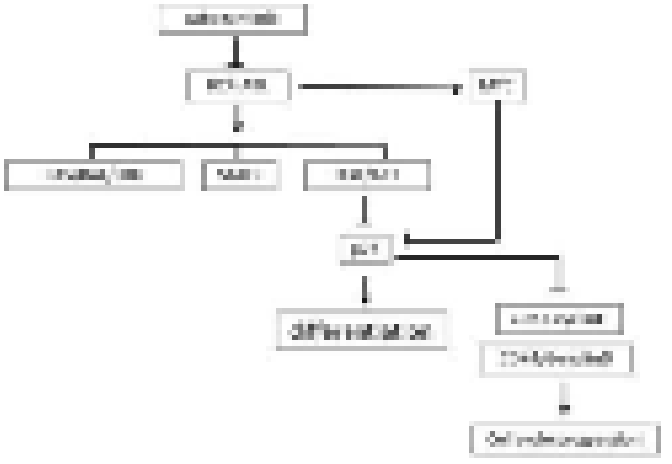

Figure 1.

PB1540

ABL KINASE DOMAIN MUTATIONS IN IMATINIB-TREATED EGYPTIAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Point mutations within ABL kinase domain (AKD) of the BCR ABL gene are the most common cause of resistance ti Imatinib methylate (IM) treated Chronic Myeloid Leukemia (CML) patients.
Aims: To assess the frequency, type and impact of AKD mutations on prognosis in a cohort of Egyptian CML patients.
Methods: Mutation screening was performed by allele specific oligonucleotide polymerase chain reaction (ASO-PCR) in 76 patients including all 46 non optimal responders; 28 resistant patients, 18 suboptimal responders in addition to 30 patients randomly selected with stable/ decreasing transcript level representing an optimal responder category.
Results: From 16 positive patients, P loop mutations were detected in 9 patients; Q252H in 3 patients (19%), Y253H in 2 patients (12%), Y253F in 2 patients (12%) and E255K in 2 patients (12%). T315I was detected 1/16 patient (6%). Regarding non-p loop mutations; V299L was detected in 1 patient (6%), M351T in 4 patients (25%), F359V in 2 patients (12%). One patient had both Y253H and E255K mutations. Ten/16 (62%) patients carrying mutations experienced disease progression *versus* 1/56 (2%) in non mutation group ($p=0.001$). Median Progression Survival (PFS) and Overall Survival (OS) of the mutation group was 13.5 months and 37.5 months, respectively *versus* 42.6 months in non mutation group ($p=0.001$). The estimated PFS and OS at 49 months in patients with mutations were 37.5% and 56.3% respectively *versus* 98.2% in non mutations carriers ($p<0.001$). Patients harboring P-loop mutations/T315I showed poorer PFS and OS; 14 months (7.5-38) and 10 months (3-40) *versus* 42 months (39-45) and 42 months (9-45) in non P-loop mutations carriers, respectively ($p=0.003$ and $p=0.017$).

Summary and Conclusions: P-loop mutations are significantly associated with advanced CML phases and poorer OS than non P-loop mutations. ASO PCR is a valuable tool for detection of mutations in countries where sequencing facilities are not available.

PB1541

INVESTIGATION OF EFFECTS OF FISETIN, VITEXIN AND HESPERETIN ON CHRONIC MYELOID LEUKEMIA CELLS

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Background: Chronic Myeloid Leukemia (CML) is characterized by Philadelphia chromosome encoding BCR-ABL1 oncoprotein. Despite therapeutic efficacy of tyrosine kinase inhibitors targeting tyrosine kinase activity of BCR-ABL1 in the clinic, the development of resistance and several side effects are stil challenging problems for most patients. Therefore, alternative therapeutic approaches are required and natural products have been investigated because of their potential in cancer prevention and treatment. Fisetin, viteksin and hesperetin are natural plant flavonoids and commonly found in several fruits and vegetables. Although their anticancer effects has been shown on several cancer types, there is no study related with their effects on K562 CML cells.
Aims: In this study, we aimed to identify the cytotoxic, apoptotic and cytostatic effects of fisetin, vitexin and hesperetin on CML cells.
Methods: K562 CML cells were treated with increasing concentrations (1-200 µM) of fisetin, vitexin and hesperetin and their cytotoxic effects on K562 cells were conducted via MTT cell proliferation assay. Changes in caspase-3 activity of the cells were examined by caspase-3 colorimetric assay kit (BioVision Research Products, USA). The loss of mitochondrial membrane potential (MMP) in response to them was examined by JC-1 Mitochondrial Membrane Potential Detection Kit (Cayman Chemicals, USA). The translocation of phosphatidylserine was determined by Annexin V/PI double staining method. The cell cycle profiles of K562 cells after treatment were analyzed by PI staining by flow cytometry.
Results: The MTT data showed that there were dose- and time- dependent decreases in proliferation of K562 cells. IC_{50} values of fisetin at 48 and 72 h were calculated from cell proliferation plots and were found to be 163- and 120 µM, respectively. On the other hand, IC_{50} values of hesperetin and vitexin were found to be 179- and 162 µM, and 153- and 147 µM at indicated time points, respectively. There were 5.4-, 9.5- ve 11.6 fold increases in percentage of apoptotic K562 cells in response to 50-, 100- and 200 µM fisetin, respectively, as compared to untreated controls. Same hesperetin concentrations resulted in 1.27-, 1.8- ve 4.3- fold changes while there were 1.02-, 1.5- ve 3.6- fold increases in reponse to same concentrations of vitexin. There were 1.03-, 1.25- ve 80-fold increases in loss of MMP in response to 50-, 100- and 200 µM fisetin, respectively. On the other hand, there were 1.7-, 2.4- ve 2.5 fold increases in loss of MMP after hesperetin treatment, and vitexin led to 2.2-, 2.6- ve 2.8 fold increases. There were 1.3-, 1.6- ve 1.8- fold increases in caspase-3 enzyme activity in response to 50, 100- and 200 µM fisetin, respectively, as compared to untreated controls. Hesperetin and vitexin treatment resulted in 2.8- ve 1.4- fold increases in caspase-3 enzyme activity, respectively only at higher concentrations. Hesperetin and vitexin treatment arrested cell cycle at G0/G1 phase.
Summary and Conclusions: All together these findings demonstrated cytotoxic, apoptotic and cytostatic effects of fisetin, hesperetin and vitexin on human K562 CML cells. Fisetin could be evaluated as the most effective flavanoid among others based on these preliminary data. These flavanoids could have therapeutic potentials if supported with *in vivo* studies.

PB1542

STUDY OF ADDITIONAL GENETIC ABNORMALITIES IN PHILADELPHIA-NEGATIVE CLONES IN CML PATIENTS USING FLUORESCENCE IN SITU HYBRIDIZATION

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Background: The Philadelphia chromosome (Ph), the cytogenetic hallmark of chronic myelogenous leukemia (CML), is known to play a central role in the pathogenesis of CML, but other cytogenetic abnormalities have been reported in Ph-negative (Ph-) cells of CML, mostly after treatment. Several mechanisms have also been suggested, such as the possible mutagenic effect of imatinib, the proliferative stress on a small pool of Ph- cells after the eradication of Ph-positive (Ph+) cells by imatinib, and the masking of the presence of concomitant Ph- clonal abnormalities by the presence of a majority of Ph+ cells at the presentation of CML.
Aims: In this study, we tried to detect abnormal cytogenetic abnormalities in Ph- clones in Ph+ CML patients at the time of initial diagnosis using fluorescence *in situ* hybridization (FISH), which is more sensitive than conventional cytogenetic analysis.
Methods: We examined 57 cryopreserved specimens from patients who were newly diagnosed as having BCR/ABL1-positive CML between January 2001 and December 2013. The diagnosis of CML had been established based on peripheral blood and bone marrow examination, supported by conventional chromosomal analysis and FISH to detect the BCR/ABL1 fusion gene.
FISH was performed according to the manufacturer's instructions, using the LSI AML1/ETO Dual Color Dual Fusion Translocation Probe, PML/RARA Dual Color Dual Fusion Translocation Probe, CBFB Dual Color Break Apart Rearrangement Probe, MLL Dual Color Break Apart Rearrangement Probe, and D7S486/CEP7 Dual Color Probe (Vysis/Abbott Molecular, Des Plaines, IL, USA). A minimum of 500 interphase/metaphase nuclei were scored.
Results: With regard to CML diagnosis, one case (1.8%) was in the blast phase, four cases (7.0%) were in the accelerated phase, and 52 cases (91.2%) were in the chronic phase. A total of 47 cases (82.5%) exhibited classical Philadelphia chromosomes, whereas three cases (5.3%) had variant Philadelphia chromosomes, and another four cases (7.0%) had additional chromosome abnormalities at the time of diagnosis. Only one case was classified as positive for abnormality in a Ph- clone in cytogenetic analysis and FISH studies. However, this case was diagnosed as having acute myelogenous leukemia with CML because the blasts were increased (62%) in the bone marrow, and monosomy 7 was positive in the blasts. In that case, BCR/ABL1 rearrangement was positive in 30.0% of the cells. With the exception of this case, the minimum percentage of cases with a positive BCR/ABL1 rearrangement pattern was 89.8%. One case with a three-way translocation as the sole abnormality in a chromosome study exhibited a minor clone with a classical Philadelphia chromosome pattern (1R1G2F) in FISH analysis.
Summary and Conclusions: The results of the present study indicate that the occurrence of masked BCR/ABL1-negative cells with other cytogenetic abnormalities, if present, is rare, particularly in cases with a high BCR/ABL1-positive rate. Larger studies involving more sophisticated methods are needed to fully elucidate this finding.

PB1543

ASSOCIATION OF MDR1 GENE POLYMORPHISM (G2677T) WITH IMATINIB RESPONSE IN EGYPTIAN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Background:
Despite the excellent efficacy results of IM treatment in CML patients, resistance to IM has emerged as a significant problem. Genetic variations in genes involved in drug transportation might influence the pharmacokinetic and metabolism of IM. The genotype of a patient is increasingly recognized in influencing the response to the treatment.
Aims: to investigate the genotype frequencies of SNPs G2677T in CML patients undergoing IM treatment and to determine whether different genotype pattern of these SNPs have any influence in mediating good response and resistance to IM.
Methods: A total of 96 CML and 30 control samples were analyzed for MDR1 gene (G2677T) polymorphism using PCR- RFLP technique.
Results: Genotype distribution revealed increase in GG, GT (34.4%, 46.9%) and significant decrease in TT (18.8%) genotype frequencies in CML patients compared to controls(p=0.257, 0.326, 0.017 respectively). No significant association of G2677T polymorphism was observed with respect to age and sex (p=0.259, 0.556). Patients in accelerated and blastic phases had higher GT genotype frequency compared to patients in early phases (p<0.001). The distribution of MDR1 2677 genotypes was significantly different among the resistance and sensitivity patients. The resistance incidence correlated with G allele. GG and GT genotypes were higher in CML patients showing imatinib resistance compared to sensitive CML patients (p=0.893, 0.002). Meanwhile TT genotype was shown significantly to be higher among imatinib sensitive group with p<0.001. GT genotype was found to be a significant predictor of IM resistance risk (OR= 4.55; p=0.002). GG genotype was not proved to be significant indicator of resistance risk (OR= 1.06; p=0.893). On the other hand, TT may be a protective factor against imatinib resistance in CML patients (OR= 0.119; P<0.001).
Summary and Conclusions: ABCB1 (MDR1) has been demonstrated to display high affinity for IM and confer IM resistance *in vitro* by extruding IM from hematopoietic cells. The G2677T polymorphism was significantly associated with increased or decreased plasma concentration of P-gp substrates. Carriers of the GT genotype are more at risk of blastic crisis than other individuals. 2677G allele carriers might be at risk for drug resistance. The G allele was reported to have enhanced P-gp expression and was observed to

be associated with increased efflux of P-gp substrates. Since imatinib is one of the P-gp substrate, the present results indicated that G allele was associated with poor response to imatinib in CML patients. Determination of G2677T MDR1 polymorphisms might be useful in response prediction to therapy with Imatinib in patients with CML.

PB1544

THE PROGNOSTIC VALUE OF THE DUAL CD34 AND CD7 EXPRESSION ON MARROW STEM CELLS AMONGE EGYPTIANT CML PATIENTS

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Background: For various reasons, chronic myeloid leukaemia (CML) is probably one of the most comprehensively studied human malignancies. CML was the first human cancer to be associated with a consistent chromosomal abnormality that responds to molecular target therapy, tyrosine kinase inhibitors (TKIs). The previous observational studies suggesting that the origin of CML clone may occur at 2 levels: The origin of CML from myeloid progenitors cell is supported by several proof such as the presence of a single glucose-6-phosphate dehydrogenase isoenzyme in red cells, neutrophils, eosinophils, basophils, monocytes, and platelets, but not in fibroblasts or other somatic cells in women with CML who are heterozygotes for isoenzymes A and B. Other studies suggesting the primitive pluripotent stem cell origin could be the origin of CML clone, which is supported by detection of the BCR-ABL fusion gene in approximately 25 percent of B lymphocytes in some but not all patients in chronic phase CML. Among CML patients who achieve BCR-ABL transcript-undetectable status; few remain transcript negative after molecular target therapy is stopped while others show re-appearance of transcripts; it may be explained by the variable effect of imatinib on either CML cell origin; Imatinib may eradicate the myeloid progenitors but not the primitive pluripotent stem cell.
Aims: The variant CML stem cell origins may be an explaining factor for the heterogenic course of CML and its effect on survival. The t(9;22) is supposed to occur at either the level of more primitive pluripotent stem cells that express CD7 on CD34+ve cells ; or at the level of more differentiated myeloid progenitors which does not express CD7 on CD 34 +ve cells. We are suggesting that; the more differentiated CML cell origin, the better response to TKIs , TKIs may be capable of total eradication of the more differentiated stem cells origin (CD34+ve, CD7 –ve); however the more primitive stem cells origin (CD34+ve, CD7+ve) are resistance to TKIs, which explain minimal residual disease with an inferior survival. We studied the relation between the expression of early myeloid progenitor marker,CD7, on CD34 +ve bone marrow CML cells and the prognostic Sokal score, to find out if the origin of stem cells is related to this prognostic score or not.
Methods: This study was conducted on a group of 31 Egyptian patients newly diagnosed to have CML in chronic phase who were presented to clinical hematology unit of Kasr Al Ainy teaching hospital. They did not receive TKIs or interferon prior to sampling. Dual culture Epics XL Flucytometry; using dual color FITC for CD34 and PE for CD7 on bone marrow aspirate samples was performed. The dual expression of CD34 and CD7 on bone marrow cells was compared to the Sokal prognostic score.
Results: Patients were catergorized into 3 groups according to Sokal risk score: low risk (19.4%), intermediate risk (38.7%) and high risk (35.5%). Out of thirty one patients; twenty nine patients (94%) have expressed CD34 +ve on bone marrow cells, with mean expression 14.1603%; twenty six patients (86%) have dual CD7 and CD34 expression with mean expression 13.29590%. Dual CD7 and CD34 expression did not correlate with Sokal score. Interestingly, we found CD34 expression is significantly related to Sokal score (p value 0.002).
Summary and Conclusions: The degree of CD34 expression in CML bone marrow cells may have a prognostic value for newly diagnosed CML patients. We are recommending studying CD34 expression on CML bone marrow cells for newly diagnosed patient and correlate it with Sokal risk score among different ethnicity and population, as it may be integrated in Sokal risk score, if it shows a similar significance as in our study.

PB1545

THE EFFECT OF INHIBITING THE SPLICE FACTOR KINASE CLK1 ON ALTERNATIVE SPLICING IN K562 CELLS

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Background: The alternative splicing of pre-mRNA is a ubiquitous and extremely versatile mechanism controlling the expression of human genes[1]. In humans, 92–94% of multi-exonic genes are alternatively spliced[2]. Alternative splicing provides cells with many protein isoforms with often radically different properties. In leukaemia the preferential expression of anti-apoptotic isoforms promotes the progression of disease[3]. Several receptor tyrosine kinases are involved in the pathogenesis of leukaemia, including RON. RON is alternatively spliced; skipping of exon 11 (DRON) results in a constitutively active isoform in malignancies. RON alternative splicing is altered in acute myeloid leukaemia[4]. The nuclear Cdc2-like protein kinase 1 (Clk1) plays an important role in the regulation of the activity of several splice factors[5], including the oncogenic splice factor SRSF1 known to promote DRON expression. The potential role of Clk1 in the regulation of alternative splicing of RON and of other critically important genes in leukaemia has not yet been investigated.
Aims: To determine whether or not Clk1 might contribute to the regulation of alternative splicing of key genes associated with leukaemia.
Methods: Clk1 was inhibited in K562 cells (CML) with the compound TG003 at 150 μM for 24 hours. Standard RT-PCR was performed to investigate the effect of Clk1 inhibition the expression of splice isoforms of the tyrosine kinase RON, and for comparison of the apoptotic gene caspase 9 and the transcription factor RUNX1 (AML1). The relative intensity of PCR amplicons was quantified using ImagJ software.
Results: The present study demonstrates that the inhibition of the splice factor kinase Clk1 has a significant effect on the alternative splicing of RON, reducing the amount of exon 11 skipping. Ultimately, inhibition of Clk1 would promote the expression of the RON splice isoform in K562 cells.
Summary and Conclusions: Targeting splice factor kinases may offer potential new treatment options. Inhibiting Clk1 cause significant shift in splicing patterns of genes associated to leukaemia.

Chronic myeloid leukemia - Clinical

PB1546

CLINICAL SIGNIFICANCE OF COMBINING BCR-ABL1 TRANSCRIPT LEVELS AT 3 AND 6 MONTHS IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH FRONTLINE SECOND-GENERATION TYROSINE KINASE INHIBITORS

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Background: The efficacy of frontline second-generation tyrosine kinase inhibitors(2G-TKI) in chronic phase chronic myeloid leukemia (CP CML) has been established. Frontline 2G-TKI threrapy demonstrated faster and deeper responses than imatinib(IM. Recent studies have demonstrated that measurements of BCR-ABL1 transcript levels at 3 and 6 months are able to identify high-risk patients and an achievement of early molecular response (EMR; BCR-ABL1 ≤ 10% at 3 months or BCR-ABL1 ≤ 1% at 6 months) predict overall survival (OS) and progression free survival (PFS).
Aims: The aim of this study was to evaluate clinical significance of combining BCR-ABL1 transcript levels at 3 and 6 months in CML patients treated with frontline 2G-TKI.
Methods: A total of 125 newly diagnosed CP CML patients were treated with radotinib (n=51), nilotinib (n=40), dasatinib (n=21), and bosutinib (n=13) as a frontline therapy. Among them, molecular analysis at 3 and 6 months was conducted in 114 and 106 patients respectively. We classified patients according to their transcript levels at 3 months (lower or higher than 10%) and 6 months (lower or higher than 1%).
Results: 81 men and 44 women were included and th median age was 41 years (range, 18-73). The percentages of patients with low, intermediate and high Sokal risk scores were 39%, 38% and 22%, respectively with unknown risk score in 1%. For 114 patients with molecular data at 3 months, the patients showed BCR-ABL1≤10% (n=106, 93.6%) or >10% (n=8, 6.4%). At 6 months, 8 patients did not yet followed-up. 106 patients with 6-month data showed BCR-ABL1≤1% (n=88, 83%) or >1% (n=18, 17%). A total of 81 patients achieved EMR both at 3 and 6 months; these patients had CI of MMR by 2 year (82.9%), 2 year EFS (75.2%), 2 year FFS (76.4%), and 2 year PFS (98.8%). 5 patients had no EMR on both occasions; these patients had a poor outcomes in terms of 2 year EFS (0%), 2 year FFS (0%), and 2 year PFS (53.3%). 13 patients had EMR at 3 months but no EMR at 6 months; their 2 year EFS, FFS, and PFS were 66.5%, 66.5%, and 92.3%, respectively. Only 2 patients had no EMR at 3 months but EMR at 6 months; until now, they continued frontline 2G-TKI during 9 and 21 months, respectively.
Summary and Conclusions: Our data showed that the patients with no EMR at both time points had poor outcomes. The patients who had EMR at 3 months but no EMR at 6 months showed no difference of EFS, FFS, and PFS, compared to those of patients who had EMR at both time points. It suggested that the combining assessment at 3 and 6 months may apply more prognostic information in CML patients treated with frontline 2G-TKI. However, further clinical investigations in a larger patient population with longer follow-up are needed.

PB1547

CAUSES OF ANEMIA AFTER LONG-TERM TREATMENT WITH IMATINIB IN CHRONIC MYELOID LEUKEMIA

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Background: In the IRIS Study, an incidence of 3% of anemia after 19 months of treatment with imatinib in newly diagnosed chronic myeloid leukemia (CML) (chronic-phase (CP) was reported. This finding was seldom examined by other centers. .
Aims: We evaluated the incidence of anemia after at least two years of imatinib treatment of CML patients in CP and tried to identify other contributing causes of anemia in this population
Methods: consecutive patients with CML, still in CP after completing 2 years of imatinib treatment entered the study. Patients with blast crisis at diagnosis, discontinuation of imatinib before 2 years of treatment or poor adhesion were excluded. Anemia after treatment was defined by the National Cancer Institute (NCI) Common Toxicity Criteria - Version 4.0 (grade 3 - hemoglobin = 6.5 - 8.0 g/dL and grade 4 - hemoglobin <6.5 g/dL). The normal values at diagnosis were considered >12.0 for women and >14.0 for men. Status of cytogenetic and molecular response was also recorded. Besides, renal toxicity and other causes of anemia were investigated.
Results: among 157 patients treated at our Center between 2003 and 2010, 97 fulfilled the inclusion criteria. Median age: 53 years (24-86), 54 male and 43 females. Comorbidities were found in 60 patients. Sokal score was low risk in 39%, intermediate in 36% and high risk in 25% of the patients. Twenty one patients had received interferon and one received bosutinib prior to imatinib. Eighty seven patients achieved complete cytogenetic response (CCR) and 41 had a major molecular response, but at the time of the analysis of anemia, 4 had lost cytogenetic remission and 8 had lost molecular response. After the second year of treatment, 80.8% of the cases presented grade 1, 17.9% grade 2 and 1.3% grade 3 anemia. Of these, no etiological causes for the anemia was found in 52 patients. The etiology of anemia was identified as iron deficiency (n=5), and one of them had simultaneously renal failure. Hypothyroidism was found in 2, B_{12} vitamin deficiency in 2), HIV infection in 1, pulmonary tuberculosis in 1 and renal failure in 1. Twenty-eight patients had normalization of hemoglobin without medical intervention but decrease in hemoglobin persisted in 40 cases. Six patients had resolution of the anemia after switching to a second-generation inhibitor and three showed clinical improvement after treatment of the specific cause. Renal toxicity was observed in 8.9% of patients, most of these patients due to mild TKI toxicity.
Summary and Conclusions: several degrees of anemia may occur in patients with CML on long-term imatinib therapy, most of them not severe. Regular hematological follow-up is required to identify causes not related to CML that can be corrected. In cases related to imatinib toxicity, dose modification or change of TKI is necessary. It should be emphasized the importance of investigating secondary causes for anemia, especially in patients with good adherence to treatment and satisfactory therapeutic response.

PB1548

EARLY COMPLETE CYTOGENETIC RESPONSE (CCGR-6M) CAN PREDICT LONG TERM OUTCOME IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED BY IMATINIB ACCORDING TO EUROPEAN LEUKEMIA NET RECOMMENDATIONS (ELN2006/2009)

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Background: Recent ELN 2013 guidelines changed milestones of response in CML patients treated with TKI, mainly imatinib, from previous ones published in ELN 2006 and 2009 recommendations. Also, recent reports emphasize prognostic value of early molecular response at 3 months (German CML IV trial). It is known that most of patients with CML in chronic phase achieving complete cytogenetic response (CCgR) up to certain time period have excellent long term results. But in some countries, like in Serbia, molecular monitoring was not feasible procedure in all patients, and regularly it was scheduled after achievement of CCgR, measuring depth of residual disease.
Aims: To analyze retrospectively prognostic impact of new optimal response milestone, CCgR at 6m (ELN 2013 recommendations).

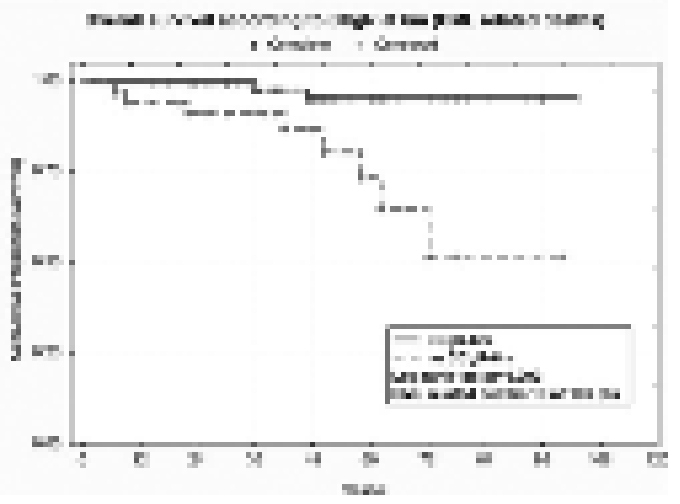

Figure 1.

Methods: Cohort analysis of 110 patients with CML in chronic phase treated with frontline imatinib (IM, Gleevec®) in period of 8 years (2005-2012) in single academic center. Patients were escalated to IM800 in case of suboptimal

response or failure at 6 and 12 months according to ELN 2006&2009 proposals and then subsequently with other TKI (nilotinib). All patients had at least 18 months of follow-up.
Results: Results. From 110 analyzed patients, new 6 month milestone, CCgR-6m was achieved in 72 of them, 64.9%, and others, 35.1% should now be considered as failure or warning (39/110). In that group, 19 patients were escalated to IM800mg due to suboptimal response or failure at 6m (8 pts) or 12m (11 pts) according to ELN 2006&2009 recommendations. Patients not responding to escalated dose of imatinib were switched to nilotinib (5 pts) together with other 5 pts with subsequent loss of response in 2012. In escalated patients, 4 of 8 pts escalated at 6m and 5 of 11 pts escalated at 12m achieved further optimal response (CCgR), comprising roughly 50%. Patients responding to escalated dose had lower Ph+ burden in time of escalation (Ph+ in 40% of mitoses), and many of them were in nowadays defined "warning" area. In our group, 14 pts died, 10 from CML related causes, and 4 from other reasons. Further analysis included only CML related deaths. Overall survival at 5 yrs, OS5y was 87%, but patients achieved CCgR-6m had much better survival, OS5y was 95.5% *vs.* 74.6% (log rank p=0.002). Only two patients achieved CCgR-6m, died due to sudden transformation in 3rd and 4th year from diagnosis. Overall survival at 8 yrs, OS8y was 95.5% for those achieved CCgR-6m but those without CCgR-6m had OS8y 52.3% (log rank p=0.002).
Summary and Conclusions: Even our data are based on small number of patients from single center, new optimal milestone, early complete cytogenetic response at 6 months could also be good and robust prognostic indicator, and therefore could also be used together with other early milestones like bcr-abl/abl<10% at 3 months, especially in circumstances where regular molecular monitoring is not feasible in all patients.

PB1549

METABOLIC SYNDROME AND TKIS TREATMENT IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterised by a pathognomonic chromosomal translocation t(9;22)(q34;q11.2). The introduction of Tyrosin-Kinase Inhibitors (TKIs), with the aim of targeting this translocation, has dramatically improved life expectancy of CML patients. First (imatinib) and second generation TKIs (dasatinib and nilotinib) act on various molecular targets and this functional difference could explain the various adverse reactions. Besides non haematological side effects, the three groups of TKIs have different effects on glucose and insulin metabolism (GIM), but no data are available on the prevalence of Metabolic Syndrome (MS). MS is a cluster of interrelated metabolic disorders associated with insulin resistance and increased risk of cardiovascular disease.
Aims: To evaluate the presence of MS defined by the National Institute of Health Cholesterol Education Program-ATP III in a cohort of CML patients treated with the three different classes of TKIs.
Methods: A total of 90 unselected CML patients treated with TKIs and referred to our Hospital were included. Blood samples and other clinical variables were collected for each patient. We evaluated anthropometric parameters to define the presence of MS according to ATP III (≥3 of following abnormalities): waist circumference ≥102 cm in men and ≥88 cm in women, serum tryglicerides level ≥150 mg/dl, HDL cholesterol level <40 mg/dl in men and <50 mg/dl in women, blood pressure ≥130 or ≥85 mmHg, or serum glucose level ≥100 mg/dl. We studied Beta-cell function assessed by HOMA-IR e HOMA-beta to estimate insulin-resistance and insulin-secretion, respectively.
Results: Among our patients, 26 (31%) were confirmed with MS. The nilotinib treated group presented the highest percentage of MS (p<0.01). Adjusting data for sex and age, total (p<0.01) and LDL (p<0.01) cholesterol levels were significantly higher in the nilotinib group when compared to the imatinib and dasatinib groups, while the homocysteine level was significantly higher in the dasatinib treated group (p<0.01) and higher, but not significantly, in the nilotinib treated group, when compared to the imatinib one. The mean levels of HOMA-beta were lower in patients with MS compared to the non-MS patients (p<0.01) in the three groups, while HOMA-IR, weight and BMI were higher (p<0.01).
Summary and Conclusions: In our series MS is highly prevalent and CML patients treated with nilotinib presented a higher cardiovascular risk profile compared to the others. On the basis of our results, when nilotinib is the selected drug, we suggest a multidisciplinary approach with the patient, with the aim to reduce cardiovascular risk and improve long term clinical outcomes.

PB1550

LABCEUTICS BCR-ABL1 EXTERNAL QUALITY ASSESSMENT STUDY-TWO ENDS OF THE (INTERNATIONAL) SCALE

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Background: Relative quantitative polymerase chain reaction (RQ-PCR) testing for *BCR-ABL1* is the recommended method for monitoring Chronic Myeloid Leukaemia (CML) patients receiving Tyrosine Kinase Inhibitor (TKI) therapy. Current guidelines anchor therapeutic decisions to *BCR-ABL1* levels reported on the International Scale (IS) at specific time points post initiation of therapy. Therefore there is great need to reduce inter-laboratory variation to ensure results are comparable between centres. Recent studies and guidelines have shown the importance of achieving *BCR-ABL1* levels below 10%^{IS} at 3 months as an early indicator of prognosis, whilst at the other end of the scale, many clinical trials are investigating cessation of TKI therapy in patients who demonstrate undetectable disease *e.g.* MR$^{4.5}$.
Aims: Labceutics conducted an EQA modelled study with 15 EU laboratories, investigating the conversion and correction to the international scale. As part of this study laboratory variation was assessed at both ~10%^{IS} and ~0.0032%^{IS}, addressing 2 important and current trends for diagnostic laboratories.
Methods: 15 participating laboratories (enrolled by Labceutics via an open call in EU5) each tested lyophilized cell line dilutions representing 5 levels of *BCR-ABL1* positivity. Five batches of test samples were prepared by the UK National External Quality Assessment Scheme (UKNEQAS) for Leucocyte Immunophenotyping (UK NEQAS LI) and distributed as randomized blinded triplicates (total 15 samples/laboratory) designed to represent clinically relevant *BCR-ABL1/ABL1* ratios between 5% and 0.0005%. Each laboratory extracted and tested the samples using their own laboratory procedures and in addition used the Asuragen ARQ calibrators to report results on the IS. Labceutics coordinated the study, via the CONNECT^{TM} communication portal and double-blinded data analysis was performed by the UKNEQASLI.
Results: Almost half of the laboratories reported at least one sample from batch 1 (consensus median 7.59%^{IS}) to be greater than 10%^{IS}, whilst only 20% of the laboratories reported all of the triplicates to be greater than 10%^{IS}. In total, 35% of samples analysed from this batch were reported as greater than 10%^{IS}, according to the current ELN guidelines this would be defined as a suboptimal response and a change in therapy would be recommended.
5 laboratories detected *BCR-ABL1* at least one of the triplicates from sample batch 5 (consensus median 0.005%^{IS}); of these only 2 laboratories detected *BCR-ABL1* in all of the triplicates, highlighting that routine detection at MR$^{4.5}$ is challenging and further optimisation is needed. When all samples were analysed, one third of the laboratories reported average *ABL1* copy numbers<10,000, with almost half reporting average *ABL1* copy numbers between 10,000 and 32,000 and 2 labs reporting average *ABL1* copy numbers >32,000. Further highlighting the need for assay/procedure optimisation.
Summary and Conclusions: This study has highlighted variability at 2 critical decision making milestones in *BCR-ABL1* monitoring, with the potential to negatively impact on patient management. Further efforts are need to reduce this inter-laboratory variability to facilitate wide spread adoption of clinical guidelines. In order to address the need to improve *BCR-ABL1* testing, specifically at 10%^{IS} and MR$^{4.5}$, Labceutics has initiated CONNECT+ for *BCR-ABL* a network of worldwide laboratories that will work collectively to improve standards in *BCR-ABL1* testing by tackling such topics as pre-analytical variables, physician education and quality indicators for diagnostic services.

PB1551

CML PATIENTS TREATED BY TYROSINE KINASE INHIBITORS IN FIRST CHRONIC PHASE: IMPACT OF MAJOR MOLECULAR RESPONSE ON OVERALL SURVIVAL AND DELAY OF ITS ACHIEVEMENT ON COMPLETE MOLECULAR RESPONSE INCIDENCE

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Background: Most patients with CML treated with Tyrosine Kinase Inhibitors (TKIs) achieve a major molecular response (MMR), that is, the absence of detectable Ph chromosomes. Even after this threshold is achieved, the disease burden continues to progressively decrease with continued treatment. Therefore, more sensitive polymerase chain reaction (PCR)–based molecular methods are required to detect and quantify levels of minimal residual disease

leading to complete molecular response (CMR), especially at long time points after TKI initiation. Low levels of minimal residual disease, as measured by real-time quantitative PCR, have been shown to be an excellent surrogate marker for long-term prognosis.

Aims: The objective of the present study is to describe at a first time the different clinical and therapeutic characteristics of chronic myeloid leukemia (CML) patients consecutively treated in first chronic phase using tyrosine-kinase inhibitors (TKI) as first line treatment, followed at our centre between years 2001 and 2011, and at a second time to assess response to TKI with the incidence of major molecular response (MMR) and then its evolution to reach complete molecular response (CMR).

Methods: We analyzed 183 CML patients, 117 males and 66 females with a median age at diagnosis of 50 years (17-81)]. Among 135 patients evaluated for Sokal score, 41 (30%) were low, 63 (47%) were intermediate and 31 (23%) were high. According to hasford score (125 evaluated), 57 (46)% were low, 61 (49%) intermediate and 7 (5%) were high. First line treatment was imatinib for 161 (88%) patients, dasatinib for 14 (8%) patients and nilotinib for 8 (4%) patients. Overall, 167 (91%) patients obtained MMR [134 (80%) after first line treatment, 26 (16%) after second line and 7 (4%) after third line treatment] within a median time of 13 months (range: 2-88); and 16 (9%) patients never reached MMR. Non-MMR patients, were as follow: 53% had high sokal score 33% intermediate and 14% low; 6% had high hasford score, 47% intermediate and 47% low; they received a median of 3 TKI-based treatment lines during a median follow-up of 37 months; 6 (37.5%) of them died after disease progression and 10 still under treatment.

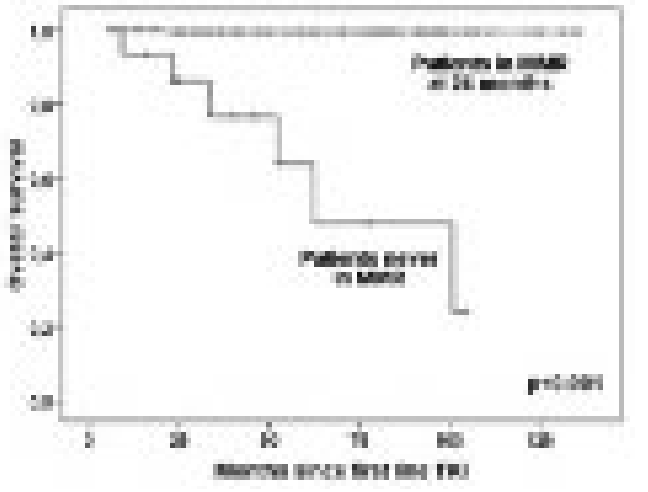

Figure 1.

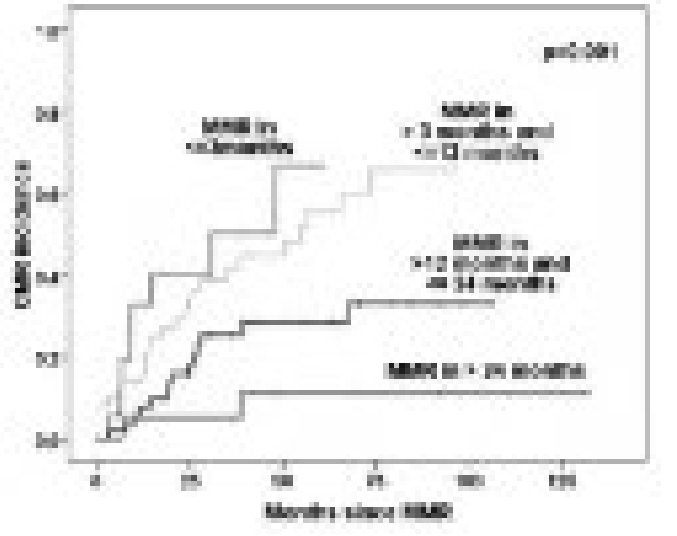

Figure 2.

Results: The median time to obtain MMR was correlated with sokal score: 9 months (range: 2-84) in patients with low score; 13 months (range: 3-78) in patients with intermediate score and 17 months (range: 3-63) in patients with high score, p=0.03, same according to hasford score with 11 months, 13 and 20 months respectively, p=0.05. Among MMR patients, 18 (11%) lost their MMR after a median time of 28 months (range: 3-65) while 57 (34%) achieved a CMR after a median time of 36 months (range: 0-73) from MMR. Interestingly, response enhancement from MMR to CMR was significantly impacted by the time to have a MMR, thus the 5 years incidence of CMR was 67% in patients who had MMR in ≤ 3 months, 56% in patients with MMR > 3 and ≤ 12 months, 28% in patients with MMR > 12 and ≤ 24 months, and 12% in patients with MMR > 24 months, p=0.01 (Figure 1). After a median follow-up of 62 months (range: 6-135), patients who were in MMR at 24 months had 5 years probability of overall survival of 99% compared to 64% for those who did not have it at that time nor later, p <0.001 (Figure 2).

Summary and Conclusions: We showed that patients with low sokal and hasford scores have significant faster time to achieve MMR. CMR incidence was significantly related to shorter time to obtain MMR. Two-years MMR is a significant factor predicting patient overall survival.

PB1552

CENTRALIZATION OF QUANTITATIVE DETECTION OF BCR-ABL1 GENE EXPRESSION FOR KOREAN CHRONIC MYELOID LEUKEMIA PATIENTS; KOREAN PATH IN CML EXPERIENCE

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Background: Molecular testing for BCR-ABL1 mRNA levels is an essential parameter in treating patients with chronic myeloid leukemia (CML). However, differences in testing methods and control genes have caused inter-laboratories variability, which makes it may limit the clinical utility and comparison of the results of the measurement. In addition, standardization process is complicated and time-consuming.

Aims: Therefore Korean representative university hospitals have agreed with an establishment of centralization of molecular assays including BCR-ABL1 gene quantitation and mutation analysis in a single laboratory.

Methods: Korean molecular test centralization (KoMoC) program has provided complimentary molecular tests including real time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and BCR-ABL1 kinase domain mutation assays, and opens to all hospitals caring CML patients. The program covers all of processes from shipping peripheral blood (PB) samples to reporting results through offline or online. We analyzed RNA quality using ABL copy number and BCR-ABL1 transcript level using a standardized international scale (IS).

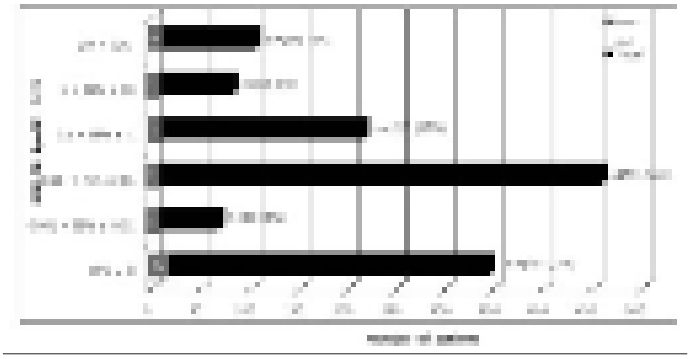

Figure 1. Best molecular response achieving various TKI therapy in KoMoC program.

Results: Between October 2012 and October 2013, a total of 1302 CML patients from 40 university affiliated hospitals have participated in the KoMoC program. Their 2169 PB samples were analyzed using qRT-PCR and 8 samples were requested for Saenger sequencing. Among 2169 PB samples, 2109 samples (98%) had ABL copy over 50,000 and only 17 (1%) samples showed low ABL copy less than 10,000. Using qRT-PCR assay, 888 (68%) out of 1302 CML patients showed BCR-ABL1 transcript<0.1% IS including 345 (27%) patients for undetectable transcript.

Summary and Conclusions: Approximately 70% of Korean CML patients treating with various TKIs are maintaining more than major molecular response and the national-wide centralization program of molecular test have been providing a reliable, high-throughput results in real clinical practice. In addition, KoMoC program can be a part of solution to overcome inter-laboratories variability from lack of standardization. Further analyses with integration of clinical information are now under contemplation.

PB1553

PROGNOSTIC VALUE OF VEGF FOR THE OVERALL SURVIVAL OF PATIENTS WITH CML TREATED WITH IMATINIB

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Background: Early prognosis of optimal response to modern treatment of chronic myeloid leukemia (CML) is among the most discussed issues within hematological community. Particularly, prognostic importance of molecular response at 3 months of treatment with tyrosine kinase inhibitors (TKI) has already been confirmed. However, very little is known about prognostic role of such cytokines as vascular endothelial growth factor (VEGF) in this subset of patients.
Aims: Purpose of this research was to establish prognostic significance of serum level of VEGF in patients with CML determined at different time-points of treatment with imatinib.
Methods: A group of 48 patients with chronic phase CML receiving imatinib either front-line (6 patients) or as subsequent treatment lines (42 patients) were studied. All patients underwent general clinical, laboratory, morphological and cytogenetic investigations. Additionally they were tested for serum levels of VEGF prior to imatinib initiation as well as at 3, 6 and 12 months of this treatment by immunoenzyme assay.
Results: This study comprised 48 patients with chronic phase CML; median observation time was 84,0 months (ranging from 9,5 to 102,2 months). Apart from the standard investigations all patients were tested for serum levels of VEGF at different stages of treatment. Patients were divided into 2 subgroups depending on the level of this cytokine: first included patients with VEGF levels exceeding healthy control group results; second – with levels comparable to the controls. According to the obtained results serum level of VEGF determined before imatinib initiation did not depend on the prior anti-leukemic treatment (median VEGF level in patients starting imatinib front-line was 333,91 pg/mL compared to 340,55 pg/mL in pretreated patients) and was three times higher than the control group. Already after 3 months of imatinib VEGF levels decreased significantly and approached normal values. Subsequently, at 6 and 12 months of treatment they remained relatively stable in the majority of patients. Analysis of the overall survival (OS) was performed in both subgroups depending on their VEGF levels before imatinib initiation and 3, 6, 12 months after its start. Obtained results showed that OS did not depend on the pre-imatinib VEGF levels whereas statistically significant OS advantage was detected for subjects with its normal levels at 3 months compared to those with increased VEGF (81,9% *vs.* 42,9% respectively). Similar but less prominent tendency was detected for VEGF levels at 6 and 12 months of imatinib.
Summary and Conclusions: Results of this study suggest that serum level of VEGF in patients with CML could be considered as an additional prognostic factor for predicting further disease course, response to treatment and overall survival of these patients. According to our data VEGF level at 3 months of imatinib treatment has the most significant potential for this aim. We conclude that after validation in bigger studies this parameter could become an additional prognostic marker for overall survival and achievement of response to treatment with imatinib. Patients with higher levels of VEGF at 3 months could be candidates for closer monitoring and possibly treatment correction especially in case of unavailable or unclear molecular response results at this time-point.

PB1554

EVALUATION OF RUNX1 EXPRESSION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) AT DIAGNOSIS AND TREATED WITH ITKS

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Background: The treatment of chronic myeloid leukemia (CML) with tyrosine kinase inhibitors (TKIs) presents satisfactory response in reducing disease cells and prolonging overall survival in the majority of patients. However, some of CML patients develop resistance to treatment presenting loss of response or disease progression. Although mechanisms underlying the resistance to treatment with TKIs or disease progression are unclear, studies show relation with genomic instability and alterations of key molecules for hematopoiesis, such as *RUNX1.* This gene is one of the subunits of the Core Binding Factor, a transcriptional regulator complex.
Aims: The aim of this study is to analyze *RUNX1* levels at diagnosis and after TKIs treatment of CML patients in all phases and its correlation with cytogenetic and molecular responses.
Methods: *RUNX1* expression was evaluated in 22 newly diagnosed CML patients (pts), 26 pts treated with imatinib 400-600mg in first line and 15 blood donors. Samples were collected from peripheral blood at diagnosis and RNA was obtained from total leucocytes. RNA samples were submitted to the synthesis of cDNA using the kit RevertAid™ HMinus First Strand cDNA Synthesis Kit (Fermentas, Life Sciences). The gene expression was evaluated by qPCR using β-Actina and GAPDH as endogenous control, the results were analyzed using $2^{-\Delta\Delta CT}$. Cytogenetic analysis was performed at diagnosis, 3, 6, 12 and 18 months after starting therapy and then every 12-24 months thereafter if CCR was achieved. BCR-ABL transcripts were measured in peripheral blood at 3-month intervals using qPCR. Results were expressed as BCR-ABL/ABL ratio, with conversion to the international scale (IS). Major molecular response (MMR) was defined as a transcript level ≤ 0.1% IS. All response analysis was based on imatinib start and any patients were classified according to criteria of the European Leukemia Net as responsive, as having a failed/resistant or sub-optimal response to TKIs. The c^2 and Mann-Whitney tests were used to define differences between groups (categorical and continuous variables respectively). $P< 0.05$ was considered significant.
Results: 48 CML pts, 55% male, average age of 52,5 years (20-85) were evaluated, 61% in chronic phase (CP), 28% in accelerated phase (AP) and 11% in blast crisis (BC). The average duration of imatinib treatment was 27 months (1-109) and 85.6% achieved complete hematological response, 69.2% complete cytogenetic response and 59% major or complete molecular response. The analysis showed that higher levels of *RUNX1* were correlated with advanced phases of disease and expression of BCR-ABL transcripts (RQ-PCR <10%) at 3 months analysis. Significant difference in expression between patients that achieved complete cytogenetic response and patients with no cytogenetic response ($p<0.0001$) was observed. Results show statistically significant differences between healthy volunteers and NoCgR patients and between CCgR and NoCgR patients. As well as, the evaluation of pts at diagnosis and pts after treatment with ITK shows that *RUNX1* expression is grown before treatment.
Summary and Conclusions: The expression of *RUNX1* gene was overexpressed in patients in advanced phase of disease when compared with patients who are responding to TKI treatment. *RUNX1* was shown promising, since the occurrence of chromosomal translocations in this gene has been associated with different types of acute leukemia – however, its association with CML is not yet clear. In addition, *RUNX1* can be used as a molecular marker of prognostic and loss of response in CML. Furthermore studies relating the level of expression of this gene with the activation of specific metabolic pathways involved in BCR-ABL can improve the state of the art at therapeutic management in resistant patients.

PB1555

EXPRESSION OF PROTEIN KI-67 BY THE HEMOPOIETIC PERIPHERAL BLOOD AND BONE MARROW CELLS IN THE CML PATIENTS WITH DIFFERENT SOCAL SCORE AND RESPONSE TO TYROSINE KINASE INHIBITORS THERAPY

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Background: Pathognomonic feature of CML is the formation of gene BCR-ABL, which products protein with strong tyrosine kinase activity, that stimulates a large number of intracellular signaling pathways and inhibites the action of protein regulators of proliferation and apoptosis. The result of these changes is the increase of the proliferation and tumor cells survival. One of the best known markers of proliferation is the intranuclear protein Ki-67. There are no clear data of expression of Ki-67 protein in patients with CML with different response to TKI therapy. The influence of Ki-67 in the formation of resistance to TKI treatment is unknown. Considering the data on the possible impact of prognostic index Sokal on the efficiency of TKI therapy, we examined the expression of Ki-67 in patients with different risk groups.
Aims: The aim of our study was to compare the proliferative activity of hemopoietic cells of peripheral blood and bone marrow which express protein Ki-67 in patients with CML, belonging to different risk groups Sokal, with the results of cytogenetic monitoring of response to TKI therapy.

Methods: We included 76 patients with CP-CML in study. Median age of patients was 43.3 years (range 19-65). Diagnostics and monitoring of CML were perfomed on the basis of cytogenetic examination of bone marrow cells by G-banding, and also molecular-genetic research of bone marrow and peripheral blood. Expression of protein Ki-67 was determined by flow cytometry in the direct immunofluorescence test using monoclonal antibodies. Socal score was asessed at time of diagnosis before commencing CML treatment by using special formula.
Results: We have found that in patients with low Socal score the number of peripheral blood and bone marrow cells expressing Ki-67 protein was equal to (2.3±0.6)% and (7.5±1.1)% respectively. In the group of patients with intermediate Socal score the number of cells expressing Ki-67 was 3 times higher in PB and twice higher in BM compared with low risk and was (7.1±1.3)% and (15.2±2.2)% respectively. In the group with a high Socal score the number of cells expressing Ki-67 in PB and BM was almost 2 times higher then the number of cells in the group with intermediate risk group and was (14.4±1.6)% and (28.4±2.2)% respectively. In patients with low-risk a little expression of Ki-67 and the optimal response to Imatinib therapy were detected, both 12 and 18 months of treatment. Even there were a some cases where the patients had initially low Socal score, a small number cells with Ki-67 in PB (about 3%) and suboptimal response to therapy after 12 months of treatment. However, at the 18 month of therapy response became optimal. In the case of patients with intermediate-risk the number of hemopoietic cells in PB with co-expression of Ki-67 was 9.5%, and the response to therapy with Imatinib at 12 months of therapy was optimal or suboptimal, but already at the 18 month treatment was ineffective. The patients with high-risk had a large number of Ki-67^{+} cells and after 12 months of Imatinib therapy inefficiency or suboptimal response was marked. However, during next cytogenetic analysis in all patients in this group inefficiency to treatment was determined.
Summary and Conclusions: Studies indicate that there is a relationship between Sokal score and the expression of Ki-67 protein in CML patients treated by Imatinib. The results of TKI therapy were the best for patients with low Socal score and low expression of intranuclear protein Ki-67.

PB1556

POPULATION BASED STUDY OF NON-PHILADELPHIA CYTOGENETIC CHANGES IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA AND IMPACT ON SURVIVAL.

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Background: The diagnosis of patients with chronic myeloid leukaemia (CML) through cytogenetics has been well established. However there are few recent large reports about additional mutations at the time of diagnosis and the impact this has on overall survival is unclear.
Aims: The Cytogenetic laboratory at this hospital provides a regional service as a single facility and 1129 patients were analysed to evaluate if cytogenetic changes should be considered in addition to established risk scoring systems like the Hasford index.
Methods: As this was a population based study, details about the haematological parameters and treatments offered to individual patients were not available. Patients were classified as complex-Philadelphia (Ph) if they had t(9;22)(q34;q11) with additional chromosomal abnormalities. Impact of individual additional abnormalities were analysed and then the effect was stratified according to presence of chromosomal gains, deletions or translocations. There were a few cases with normal cytogenetics on their first sample to this laboratory because they were initially treated elsewhere.
Results: 1129 patients were diagnosed with CML between 1985 and 2013, with 4760 samples analysed. The median age was 52.4 years (4.3-103) with 602 males, 511 females and in 16 gender was not listed. We used 10 year overall survival(OS) as the analysis endpoint. Median follow up was 6.4 years (range: 0-26.8 years; 725/1129(64%) had follow-up more than 10 years). 194/1129 (17.2%) had complex-Ph at diagnosis, 759/1129 (67.2%) had standard Ph, 77/1129 (6.8%) had negative cytogenetics and in 34/1129 (3%) cytogenetic analysis failed at diagnosis. Patients with standard Ph translocation had significantly higher chance of achieving cytogenetic CR than those with complex-Ph (23.4% *vs.* 13.4%, p<0.001). OS was significantly better in patients below the age of 45 (65% *vs* 25% p <0.0001). OS was also better in patients diagnosed after 2000 (67% *vs* 40%, p<0.0001). In univariate analysis, OS was significantly lower with trisomy 8 (10% *vs* 50%, p<0.0001), del(5q) (20% *vs* 50%, p=0.001), other deletions (12% *vs* 48%, p=0.0005), del(17p) (48% *vs.* 0%, p<0.0001), add(21q) (50% *vs.* 0%, p<0.0001), any translocations (50% *vs.* 22%, p<0.0001), additional der(22q) (Ph) or isochromosome 17q (48% *vs.* 10%, p<0.0001), any deletions (50% *vs.* 20%, p<0.0001) and variant Ph translocations (50% *vs* 22%, p=0.004). In multivariate analysis, excluding year of diagnosis, age group (HR 1.93, 95% CI:1.6-2.4 P=<0.0001), complex t(9;22;v) (HR 1.8, 95% CI:1.0-3.1, P=0.035), del(17p) (HR 3.8, 95% CI:1.1-12.6, P=0.033), additional translocations (HR:1.6, 95% CI: 1.1-2.25, p=0.013), trisomy 8 (HR: 1.76, 95% CI: 1.19-2.65, p=0.005), add(21q) (HR: 3.21, 95% CI: 1.65-6.25, p=0.001) and +der(22q) or iso(17q) (HR: 1.57, 95% CI: 1.1-2.25, p=0.013) were independently associated with inferior OS. Number of risk factors in individual patients was used to design a score to evaluate the impact on OS. Patients with 0 risk factors (70% OS at 10 years), 1 risk factor (40% OS at 10 years), 2 risk factors (22% OS at 10 years) or more than 3 risk factors (12% OS at 10 years) had incrementally reduced OS. This was true of patients diagnosed before and after 2000. In the TKI era post year 2000, maimum benefit is seen in patients with standard Ph translocation.
Summary and Conclusions: To our knowledge this is one of the largest population based studies about cytogenetics in CML patients. This analysis suggests that nature of karyotype at diagnosis may add to established risk scoring systems. However these data need to be analysed with a larger patient population including all established risk factors and the effect of therapeutic measures.

PB1557

STANDARD AND HIGH SENSITIVITY MOLECULAR ANALYSIS IN CHRONIC MYELOID LEUKEMIA PATIENTS CANDIDATE TO DISCONTINUATION OF TYROSINE KINASE INHIBITORS

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Background: The introduction of Imatinib and 2nd generation Tyrosine Kinase Inhibitors (TKI) nilotinib and dasatinib has dramatically changed the prognosis of chronic myeloid leukemia (CML). For patients who maintain deep molecular remission for many years, it is now under discussion the possibility to discontinue TKI therapy.
Aims: To identify a subset of patients who achieved deep and durable molecular remission under TKI therapy potentially suitable for future TKI withdrawal, and to assess the real depth of molecular response by Replicate-PCR, a higher sensitivity PCR (up to 10^{-6}) in selected patients willing to discontinue TKI therapy.
Methods: We reviewed molecular data from all chronic phase CML patients (102) followed in our Center in order to identify patients showing either undetectable BCR/ABL transcript (at least MR4) or detectable transcript <0.0032% (MR 4.5) during the last two years (here defined as Complete Molecular Response, CMR). Minimal residual disease was also assessed with a new strategy of Replicate RQ-PCR by using a 82 well plate, each performing an amplification reaction, that demonstrated approximately a 2 log improvement in detection sensitivity limit compared to conventional RQ-PCR (Goh UG et al, Leukemia & Lymphoma 2011). Results from patient samples were compared with 12 normal controls, which resulted in positive amplification in 6 out of 983 total wells with a mean number of positive wells of 0.5 (SD 1.0).
Results: Among the 102 patients we selected a group of 76 patients who were diagnosed after the year 2000 and were treated with TKIs, with a minimum follow-up time of 2 years. At last observation, 43 were treated with Imatinib, 16 with Nilotinib, 14 with Dasatinib, 1 with Bosutinib and 2 were off-treatment. We found that 23 (30.2%) patients met the potential discontinuation criteria we previously described; out of 23 patients, mean age at diagnosis was 52 (28-73), mean time from start of last treatment to CMR was 25 months (3-94), median duration of CMR was 70 months (24-139). 15 patients (65.2%) were receiving Imatinib, 8 (34.8%) were receiving 2nd generation TKIs, in particular 5 Nilotinib (4 as first-line treatment) (21.7%), 2 Dasatinib (1 as first-line treatment) (8.7%) and 1 Bosutinib (4.3%). Among these patients 2 were taking second line therapy and 1 was under third line treatment due to toxicity or resistance. 26% of our selected group had been treated with IFN. Mean time to CMR was 29 mos (4-94) in patients under frontline Imatinib, and 13 mos (6-24) in patients under frontline Nilotinib. Replicate-PCR was performed on 7 of these patients (including 2 patients off-treatment). Samples from 2 other patients already off-treatment who had started therapy before year 2000 were also analyzed. BCR/ABL was undetectable or had a low-level positivity (respectively 1 and 2 positive wells) below the range of normal controls (2.5 = mean +2SD) in 5 patients (2 off and 3 on treatment); in 4 patients (including 2 off-treatment) positive wells were > 2.5 at least in one sample. With these criteria, the 9 patients could be classified as negative (5 cases) or positive (4 cases) at replicate PCR analysis. We are currently expanding the analysis to the other patients.
Summary and Conclusions: In a single center experience, we confirm that a significant percentage of patients treated with TKs (around 30%) may eventually reach criteria for discontinuation. High sensitivity molecular analysis demonstrate that residual transcript is evident in some patients who remain in stable MR4.5, thus indicating that this population of patients is still heterogeneous. The role of replicate PCR (or other highly sensitive molecular techniques) deserve further evaluation.

PB1558

RESULTS OF "REAL-LIFE" FRONTLINE DASATINIB TREATMENT IN UNSELECTED ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Dasatinib has been very recently licensed for first line treatment of patients with chronic myeloid leukemia (CML). However, there are still no data available as to toxicity and efficacy of dasatinib in elderly unselected CML patients.
Aims: To address this issue, we revised a "real-life" cohort of 29 CML patients in chronic phase aged > 65 years treated with frontline dasatinib in 14 Italian Centers from 6/2012 to 1/2014 as concern toxicity and efficacy data.
Methods: The main clinical features of the patients at diagnosis were as follows: M/F 14/15 (48.3%/51.7%), median age 74.1 years [interquartile range (IQR) 69.3 – 78.1), median Hb 12.2 g/dl (IQR 11.2 – 13.5), median WBC 51.1 x 10^9/l (IQR 27.4 – 83.4), median PLTS 470 x 10^9/l (IQR 243 – 692). According to Sokal risk classification, 1 patient (3.4%) was at low risk, 17 patients (58.6%) were at intermediate risk, 6 (20.7%) at high risk and 5 (17.2%) were not classificable. 13/29 patients (44.8%) had ≥ 2 comorbidities requiring concomitant therapies: according to ECOG scale, performance status at baseline was 0 – 1 in 22 patients (75.9%) and 2 in 7 patients (24.1%).
Results: Median interval from diagnosis to dasatinib start was 23 days (IQR 17 – 33). Starting dose of dasatinib was 140 mg/day in 1 patient (3.4%), 100 mg/day in 21 patients (72.4%) and<100 mg/day in 7 patients (24.1%), respectively. After a median period of treatment of 6.6 months (IQR 2.5 – 14.9) all patients were evaluable for toxicity; on the whole, grade 3 – 4 hematological and extra-hematological toxicities were reported in 3 (10.3%) and 4 (13.8%) patients, respectively. Overall, 6 patients (20.6%) permanently discontinued dasatinib due to toxicity (2 patients in the first 3-month period of treatment and 4 beyond that period). Pleural effusions of all WHO grades occurred in 6 patients (20.6%): in 2 of them the pleural effusion occurred during the first 3-month period of treatment. As to treatment efficacy, 6 patients were considered too early (< 3 months of treatment) and 23 were evaluable for cumulative response; on the whole, 19/23 patients (82.6%) achieved complete cytogenetic response and 14/23 (60.8%) also a major molecular response.
Summary and Conclusions: Present data shows that dasatinib could have a major role in the treatment of unselected patients aged > 65 years; in particular, dasatinib seems very effective and has a favourable safety profile also in elderly subjects with severe comorbidities.

PB1559

EARLY SWITCH THERAPY APPROACH FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE

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Background: New guidelines for chronic myeloid leukemia (CML) 2013 developed by National Society for Hematology in Russian Federation declare prevention of disease progression by early therapeutic interventions at 1st year of therapy, opportunity to use 2nd generation tyrosine kinase inhibitors (TKI2) as 1st line and switch of TKI from 3rd month of therapy in case of failure.
Aims: To evaluate early treatment interventions in CML patients with chronic phase (CP) CML including TKI switch from 3rd month of therapy.
Methods: From August 2012 till February 2014 we included 36 adult patients(pts), 18 men and 18 women, with CP CML approved by cytogenetic and/or molecular analyses. No pretreatment except hydroxyurea was allowed. Median age was 47(19-76) years. Median observation time after starting TKI therapy was 9(0-19) months. Low, intermediate and high Sokal risk score were in 25(69%), 7(19%) and 4(12%) of pts correspondingly. Standard cytogenetic and molecular analyses were done at 3rd, 6th and 12th month of therapy. Cytogenetic analyses were done until complete cytogenetic response (CCyR). FiSH was used in case of non-informative standard cytogenetics. *The optimal response* was considered in case of BCR-ABL≤10% at 3 months, <1% at 6 months and ≤0,1% at 12 months and/or Ph+≤35% at 3 months, Ph+0% since 6th months. The *treatment failure* was if BCR-ABL>10%, Ph+>65% and/or no hematologic response at 3 months; BCR-ABL≥10%, Ph+>35% at 6 months; BCR-ABL≥1%, Ph+>0% at 12 months. The intermediate values were defined as *warning*. For *failure* the therapy was changed, in case of *warning* dose increasing or switching to another TKI was allowed. Assessment of BCR-ABL mutations before switching was performed.
Results: Therapy of 1st line was imatinib 400 mg in 31(86%), nilotinib 600 mg in 4(11%) and dasatinib 100 mg in 1(3%) of newly diagnosed pts. A 3 months evaluation have been performed for 27(75%) pts. *Optimal response* at 3 months was achieved in 21(78%): 16 on imatinib, 4 on nilotinib, 1 on dasatinib. No pts were in *warning* cathegory. *Treatment failure* was in 6(22%) of pts, all on imatinib. CML progression to blast crisis (BC) and death was in 1 of 6 pts. Three pts were switched to nilotinib 800 mg, 2 to dasatinib 100 mg. No BCR-ABL mutations were detected before switching. *Optimal response* at 6 months of therapy was achieved in 16(73%) of 22 evaluated pts: 9 on imatinib, 6 on nilotinib, 1 on dasatinib. *Warning* was applicable for 5(22,5%) of pts. *Treatment failure* still was in 1(4.5%) of pts previously switched to nilotinib 2nd line, it was decided to continue nilotinib for 3 month more. At 12 months of therapy optimal response was marked in 8(57%) of 14 pts who reached this term, 6 (43%) of pts remained in *warning* zone. No *treatment failure* at 12 months was observed, the patient with failure at 6th month moved to *warning*. All 14(100%) of pts had CCyR, 8 of 14 have no less than MMR. Of those 5 pts with treatment failure at 3 months after switching to TKI2 from imatinib 2 of 5 achieved optimal response, 3 of 5 moved to *warning* category during further observation.
Summary and Conclusions: Regular monitoring at the 1st year of treatment of CML CP pts has allocated the groups of pts with optimal response, warning and treatment failure. Switching pts with *treatment failure* at 3 months to more potent TKI allowed to improve therapy results and to overcome the primary resistance. Early treatment intervention approach is a promising strategy of prevention of disease progression in CML. A longer period of observation and more pts should be validated.

PB1560

LONG-TERM SURVIVAL OF PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA RECEIVING TYROSINE KINASE INHIBITORS THERAPY AFTER INTERFERON-ALFA FAILURE

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Background: Treatment of patients (pts) with chronic myeloid leukemia (CML) in chronic phase (CP) with very durable disease anamnesis now continues in a routine clinical practice. Those pts in late CP have started tyrosine kinase inhibitors (TKI) therapy after IFN-α and subsequently underwent treatment by various TKI (imatinib, nilotinib, dasatinib, bosutinib). Information about the therapy approaches and effectiveness of TKI in CML CP pts with follow-up over 13 years has not yet been presented.
Aims: To evaluate long term results of treatment in pts in late CP CML including 12-years overall survival (OS) and progression free survival (PFS) and to characterize their current treatment.
Methods: Since July2000- Sept2001 79 pts with Ph-positive CML in CP resistant/intolerant to IFN-α therapy have been included into this non-randomized, open-label study and started therapy by imatinib(IM) 400mg daily. Male/female ratio 41:38, median (Me) age at diagnosis 39 (15-64) years. The Sokal risk groups ratio: 54%/29%/17% for low, intermediate and high score correspondingly. Me time pretreatment by IFN-α 26 (0,5-156) month (mo). Me time from diagnosis to treatment by IM was 35,5 (3-157)mo. Cytogenetic monitoring was done every 6mo at 1st year, after that - as decided by clinician. Molecular monitoring by Real-time PCR was performed since 2005 2-3 times a year. Therapy changes were made according to currently used recommendations and availability of TKI2. Statistical analysis was performed using the SAS 9.3 program.
Results: The 12-year OS of pts receiving TKIs after IFN-α failure was 68%, PFS was 66% (fig1). Me time of life from the start of TKI treatment was 12.2 (0.3-13.5)years. Me follow-up from diagnosis till Feb2014 was 173 (13- 310)mo. Structure of mortality in 25 died pts: 1) progression of CML in 20pts: CP- 3pts; accelerated phase (AP)/ blast crisis (BC) - 17pts 2) comorbidities in3pts 3) unknown reason in 2pts. At the moment of analysis 54pts were alive, 52 of 54 in

CP CML, 2 of 54 in AP. IM therapy continued in 23pts. Me duration of IM therapy was 148(102- 155)mo. Doses of IM: 400mg- 17pts with stable Major Molecular Response (MMR), 600mg-3pts, 800mg-3pts. Totally with IM, complete cytogenetic response (CCyR) and MMR was achieved in 44(55%) and 25(31%)pts, respectively. Me time to CCyR and MMR was 30 (4-91)mo and 87 (48-141)mo, respectively. One patient received IM as a 4th line after the failure of two TKI2. TKI2 received 24 alive pts: nilotinib-15, dasatinib-8, bosutinib-1. Treatment by more than two TKI was in 11pts. Me duration of TKI-2 therapy was 57 (1,5- 95)mo. Me time of switching to the TKI2 was 66,6mo. Active stop of TKI was safely performed in 4pts with complete molecular response (BCR-ABL<0,01%): 2pts- due to toxicity, 2pts- by their own desire. In 2 more pts who stopped therapy progression occurred; among pts who have died due to progression, 3 pts also stopped treatment by themselves. We cannot exclude non-adherence in patients receiving TKI who lost previously achieved CCyR and MMR.

Summary and Conclusions: In 37,5% of CML CP pts with a well performed follow-up for more than 12 years IM therapy continues within persisting MMR. Changing of the TKI in this group was performed mostly at late terms - in 35-84mo after initiation of IM therapy and that was associated with the appearance of TKI2 in clinical practice. It is illustrated that uncontrolled treatment discontinuation can be unsafe for the patients and can lead to disease progression until death.

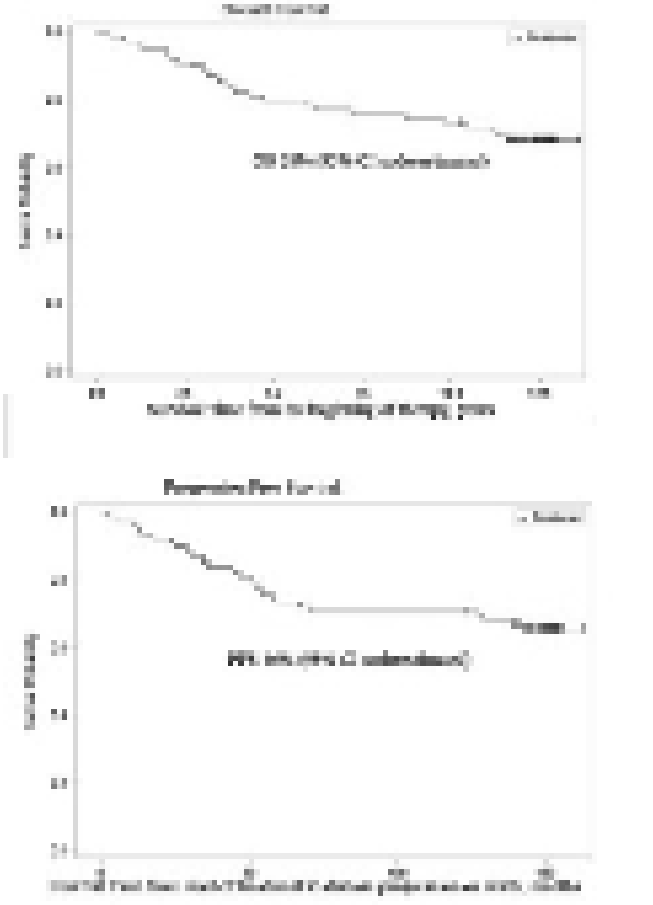

Figure 1.

PB1561

FERTILITY IN MALE PATIENTS WITH (CHRONIC PHASE) CHRONIC MYELOID LEUKEMIA RECEIVING TYROSINE KINASE INHIBITORS AS FIRST LINE OF THERAPY.

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Background: The introduction of TKIs for the treatment of CML raises the question of whether male fertility is affected and the degree of this affection.
Aims: to evaluate semen parameters and pituitary gonadal function before and 4 months after starting TKIs in patients with CP CML

Methods: We studied prospectively the effect of TKIs' on semen parameters, endocrine functions in 20 euogonadal male patients with CML aged from 35 to 51 years ,were receiving either Imatinib, Dasatinib or Nilotinib as upfront therapy. We studied LH and FSH and testosterone (T) and evaluated sperm parameters before and after four months of using these TKIs.
Results: Four months after starting TKIs there were significant decreases in serum testosterone, LH, FSH concentrations. The total sperm count, total and rapid progressive sperm motility, and% sperms with normal morphology decreased significantly *versus* before treatment. After 4 months of therapy, Dasatinib effects on sperm count (SC), volume(SV), all sperm motilities and% of sperms with normal morphology were significantly less harmful compared to Imatinib and Nilotinib.
Summary and Conclusions: TKIs are associated with significant decrease of sperm parameters and decreased concentrations of serum T, LH, FSH. These potentially toxic effects on spermatogenesis are less prominent in patients treated with Dasatinib compared to Imatinib and Nilotinib. The mechanisms and pathways for these effects need further human and/or experimental studies.

PB1562

LOWER DOSAGE OF IMATINIB (IM) IS SUFFICIENT TO MAINTAIN COMPLETE MOLECULAR RESPONSE (CMR) IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WITH LONG-TERM TOXICITY OF THE TREATMENT

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Background: Continuation with imatinib (IM) treatment is currently the recommended strategy in all patients with CML and optimal treatment response. However, there is evidence that about 30% of patients may have problems with long-term side-effects symptoms burden. Information about the response in patients with molecularly undetectable leukemia (complete molecular response) CMR after IM in whom the dose should have been reduced in an effort to relieve from long-term toxicity is insufficient.
Aims: Safety and efficacy of IM dose reduction in patients with long-term toxicity and CMR after standard IM dosage was assessed in a retrospective fashion.
Methods: Files of all patients with CML treated with IM at our center were retrospectively analyzed for CMR, toxicity and dosage. 61 (29.8%) of 205 patients treated with first or second line IM with median follow-up 54 and 105 months respectively have achieved CMR. In 19 of them (31.2%; 13 females and 6 males aged 20-71; average m=47 years at diagnosis) the dose of IM was reduced due to toxicity. Sokal score was low in 9, intermediate and high in 4 each and in 2 patients unknown. Patients achieved MMR and CMR after average 13.3 and 27.7 months respectively, 12 of them were pretreated with interferon-alpha. Duration of CMR before dose reduction was 16-123 (m=56.7) months. Reasons for the dose reduction comprised fluid retention, eye-lid swelling, hemorrhage into conjunctiva, hematologic toxicity, muscle cramps, dyspepsia and complications after surgery. Dose reduction was strictly individualized: in 3 patients IM was interrupted, 9 patients received 400mg once to five times a week, 6 patients 300mg three times weekly to daily and one patient was treated with 200mg of IM daily.
Results: After average observation period of 37 (9-84) months CMR has been maintained in 13 (68.4%) patients with lower dose of IM: in three of them with single elevations of BCR-ABL level to MMR. In two other patients the response fluctuates between CMR and MMR while continuation with lower dose. Three patients have lost CMR: one regained it on full-dose IM, two maintain MMR on standard or lower dosage. Only one patient has lost MMR after interruption of treatment: CMR was gained after standard dose of IM and is maintained on lower dose. In all patient, toxicity of the therapy was ameliorated during lower dosage. Even during intermittent treatment patients were able to achieve comparable plasma levels with those achieved on previous standard therapy.
Summary and Conclusions: CMR has been safely and efficiently maintained in 16 (84%) of our patients with CML suffering from long-term toxicity of IM using lower dosage regimens. One patient maintained CMR with full dose and 2 remaining patients have maintained MMR on lower IM dose. Lower or intermittent maintenance treatment with IM have lead to significant reduction of side-effects and cost burden and may serve as an option for the patients who relapse after stopping of IM in CMR. Larger prospective trials are necessary in order to confirm efficacy and safety of this strategy.
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PB1563

DIABETES MELLITUS AND TKIS TREATMENT IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterised by a pathognomonic chromosomal translocation t(9;22)(q34;q11.2) which results in the oncogenic BCR-ABL1 fusion gene, encoding for a mutant, continuously activated tyrosine kinase. Specific Tyrosin Kinase Inhibitors (TKIs) therapy, having this translocation as a target, dramatically improved life expectancy of CML patients. First (imatinib) and second generation TKIs (dasatinib and nilotinib) act on different molecular targets, leading to varying side effects; several studies have revealed that nilotinib induces hyperglycemia in a subset of non diabetic and diabetic CML patients, whereas imatinib and dasatinib usually do not.
Aims: To evaluate the glico-metabolic assessment of CML patients treated with TKIs.
Methods: A total of 90 unselected CML patients treated with TKIs and referred to our Hospital were included. Blood samples for fasting glycemia, insulin, c-peptide and other clinical variables as anthropometric parameters were collected for each patient. We studied Beta-cell function assessed by HOMA-IR and HOMA-beta with the aim to determine insulin-resistance and insulin-secretion, respectively. The diagnosis of diabetes was performed using criteria (updated 2010) from the American Diabetes Association (ADA).
Results: In our population, DM was diagnosed in 12 patients (13.5%), whereas impaired fasting glucose (IFG) was reported in 22 patients (24.4%). Among the three TKIs, we have not found a significant difference in hyperglycemia and DM presence, although they were both higher in the nilotinib treated group. The same group showed higher basal insulin and c-peptide levels and higher HOMA-IR ($p<0.05$) than the others, without a significant difference in HOMA-beta. In all groups, diabetic patients showed higher HOMA-IR ($p<0.05$), BMI ($p<0.01$), weight ($p<0.01$), age ($p<0.05$) and triglycerids levels ($p<0.01$) than non DM, while HDL cholesterol levels were lower ($p<0.05$).
Summary and Conclusions: Our data confirm the few reports in literature that show higher prevalence of IFG and DM in CML patients. The underlying mechanism is not clear but in our population, diabetic patients showed higher insulin-resistance and BMI, whereas insulin secretion was not reduced.

PB1564

IMATINIB DISCOTINUATION IN CHRONIC MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS ON 28 UNSELECTED PATIENTS

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Background: Tyrosine kinase inhibitors (TKI) have radically changed the prognosis of chronic myeloid leukemia (CML) patients but current guidelines still recommend to continue a successful therapy indefinitely. However some recent studies proved the safety of imatinib discontinuation and showed the durability of a sustained molecular response in around 40 per cent of the patients without any further therapy.
Aims: The aims of this analysis is to present our experience with imatinib discontinuation.
Methods: We retrospectively analyzed the outcome of patients treated in the Hematology Divisions of Turin and Orbassano (TO) who discontinued imatinib. After discontinuation patients were monitored every 1.5-2 months in the first year and every 3 months thereafter. In case of loss of MMR in one test or loss of MR4 in 2 consecutive analysis, imatinib therapy was restarted immediately.
Results: Twenty-eight patients with chronic phase CML discontinued imatinib. Twenty-two patients (78.6%) had an Undetectable Molecular Disease (UMD) Molecular Response 4.0 log (MR4) at the time of imatinib cessation: in most cases they had an elective discontinuation due to persistent deep molecular response. Three patients had detectable transcript MR4 and 3 were in MMR only: they discontinued because of intolerance or strong patient's desire. Twelve patients had been treated with interferon +/- ARA-C before starting imatinib. Median duration of imatinib treatment was 68 months (range 16-121 months). Median follow up after treatment cessation was 41 months (range 6-104 months). Median duration of MR4 for the 22 patients with UMD at discontinuation was 38 months (range 30-90 months). After discontinuation 17 of them showed a detectable BCR-ABL transcript in at least one occasion (77%,) and 11 in at least 2 consecutive tests (50%). However, only 8 (36%) lost the MR4 and had to restart therapy after a median time from imatinib cessation of 5 months (range 3-11 months). In particular, the reasons for therapy restart were loss of CCYR in 2 cases, loss of MMR for 3 patients and confirmed loss of MR4 in the other 3 cases. All the 6 evaluable patients (with a follow-up of at least 3 months) regained UMD after restarting imatinib, or nilotinib in one case intolerant to imatinib, after a median time of 3 months (range 3-14 months). Patients who received more than 1 year of interferon before stating imatinib treatment were more likely to remain off therapy compared to patients who did not (86% vs 53% p=0.038). Among the 6 patients without UMD at the time of imatinib discontinuation, 2 had to restart treatment (33%) after 3 and 30 months respectively and all regained their previous level of response. On the whole 36% of the patients (10/28) had to restart therapy while 18 patients (64%) remained off treatment, for a median time of 38 months (range 7-104 months). At the last follow up 3 patients had died, all for causes not related to CML and no progression to accelerated or blastic crisis was observed.
Summary and Conclusions: Our experience confirms that treatment discontinuation is feasible and safe for CML patients treated with imatinib, but a close molecular monitoring and a careful selection of candidates is required. Several clinical trials are ongoing to explore the feasibility of treatment discontinuation, also for patients treated with second generation TKI.

PB1565

EPIDEMIOLOGICAL CROSS-SECTIONNAL SURVEY OF FIRST LINE TREATED PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN ROUTINE CONSULTATIONS DURING A ONE-MONTH PERIOD: EPIDOR FRENCH SURVEY

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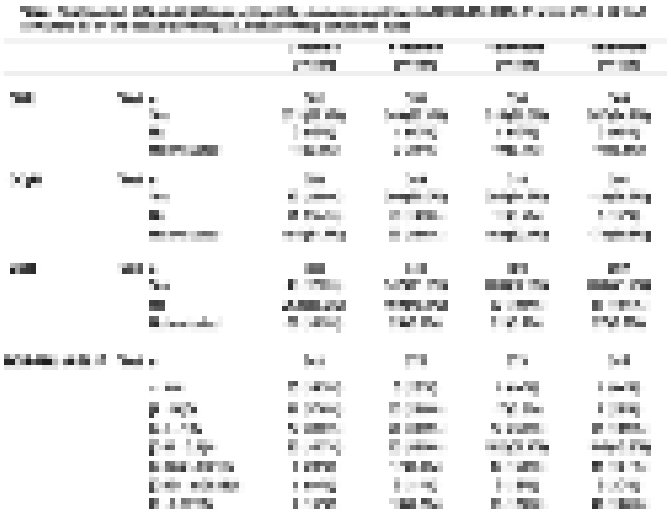
Background: 2nd generation TKIs availability and numerous clinical trials assessing their effects, alone or in combination, have extended the range of 1st line therapy options for newly diagnosed CML-CP patients.
Aims: To describe the 1st line therapeutic choices for patients with CML-CP in France and response kinetics.
Methods: Multicentric, national, descriptive, observational, cross-sectional epidemiological survey. 145 physicians were randomly contacted from a list of 1200 specialists; 45 agreed to participate and included all CML-CP pts seen during outpatient routine visits over a rolling 30-day period (Nov-Dec 2013). Primary objective: to describe type of treatment for pts currently receiving 1st line treatment (1st LT). Secondary objectives: to evaluate the proportion of pts still receiving 1st LT and to describe their clinical profile, kinetics of hematologic, cytogenetic and molecular responses at 3, 6, 12 and 18 months (mos), and quality of responses at last available assessment.
Results: 702 CML-CP pts were enrolled, 697 pts (98.9%) analyzable. 62.8% (438) of pts on 1st LT, 37.2% (259) on ≥ 2nd line treatment. Whole population (n=697): Sex ratio M/F: 1.29; Median age was 62 yrs (IQR: 50-72). Median CML history duration was 4 yrs (IQR: 2-9): duration of CML<1yr for 78 pts (11.2%), [1-5[yrs for 271 pts (38.9%); [5-9[yrs for 163 pts (23.4%); [9-14[yrs for 124 pts (17.8%); and ≥14 yrs for 61 pts (8.8%). 1st line population (n=438): Sex ratio M/F: 1.33; Median age was 61 yrs (IQR: 50-72). Median CML history duration was 4 yrs (IQR: 1-9): duration<1yr for 72 pts (16.4%), [1-5[yrs for 195 pts (44.5%); [5-9[yrs for 111 pts (25.3%); [9-14[yrs for 56 pts (12.8%); and ≥14 yrs for 4 pts (0,9%). Sokal score was determined for 78.3% of pts: as assessed by investigators, 42.3% of pts (145/343) had low; 39.7% (136/343) intermediate and 18.1% (62/343) high Sokal score. Bone marrow karyotyping analysis and BCR-ABL1 assessment at diagnosis were performed respectively for 97.5% and 93.1% of pts. Treatment description in 1st line population: 1st LT were (alone or in combination): imatinib (IM) 73.3% (n=321), dasatinib (DAS) 11.9% (n=52), nilotinib (NIL) 11.4% (n=50), interferon (IFN) 4.8% (n=21), ponatinib 0.7% (n=3), bosutinib 0%, others 1.6% (n=7). 29.9% of pts received 1st LT as part of an interventional clinical trial. Treatment combinations consisted in a TKI combined with IFN in 18 pts (4%), the TKI was IM for 11 pts, NIL for 5 and DAS for 2 pts, most often (15/18) as part of clinical trials. Response kinetics in 1st line population: Incidence of CHR, CCyR and MMR at 3, 6, 12 and 18 mos and BCR-ABL1/ABL1^{IS} levels are detailed in the table. At the 12th month assessment, 30.9% (99/320) of patients were not in MMR, and 6.6% (18/273)

had BCR-ABL1/ABL1^{IS} > 1% (respectively new warning and failure definitions according to 2013 ELN guidelines). Quality of response at last available assessment: Described for pts currently receiving 1st LT (median CML history of 4 yrs). Among pts with duration of 1st LT ≥ 12 mos and available data for MMR and Deep Molecular Response (DMR: MR$^{4.0}$ or MR$^{4.5}$): 91.5% (313/342) have achieved MMR, 66.5% (214/322) have achieved DMR.
Summary and Conclusions: Among this unselected real-life population of CML-CP pts seen in a one-month consultations period, 62.8% were still in first line therapy. Focus on pts on 1st line treatment suggests that TKIs induce good molecular response, even outside of clinical trials.



PB1566

IMPORTANCE OF EARLY MOLECULAR RESPONSE IN CHRONIC MIELOID LEUKIMIA (CML) PATIENTS IN CHRONIC PHASE TREATED WITH TIROSINKINASE INHIBITORS (TKI) IN ARGENTINA

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Background: Early cytogenetic and molecular response are associated with better outcome in CML patients treated with imatinib (IM), dasatinib (DA) or nilotinib (NI). BCR/ABL transcript leve ≤10%IS at 3 months of treatment is associated with higher rates of complete cytogenetic response (CCR), mayor molecular response (MMR), molecular response (MR) 4.5, higher event- free survival (EFS) and progression- free survival (PFS). Evaluation of transcript levels at 3 month is useful to early identify patients with less probability to achieve optimal response.
Aims: To describe clinical cytogenetic and molecular outcome of CML patients treated with TKI in Argentina according to the BCR/ABL transcript levels after 3 months of treatment.

Table 1.

Methods: Cohort observational study . Data of CML patients treated with TKI that had BCR/ABL IS transcript levels measured at 3 was collected retrospectively in 7 centers in Argentina. Frequency of CCR at 6 months, MMR at 12 and 18 months, even free survival, progression free survival and overall survival was calculated in the group with transcripts ≤10%IS and >10% at 3 months.
Results: 76 patients were included with a median follow up of 46 months (IQR: 18-61). Average age was 48 years old (22-74), male 45%(34/76). Sokal risk distribution was: low risk 37%(28/76), intermediate 36%(27/76), high 28%(21/76).Patients with Imatinib treatment 45%(37/76), nilotinib 22% (17/76) and dasatinib 29% (22/76). BCR/ABL transcripts level ≤10% IS at 3 months treatment was found in 86% (65/76) and > 10%IS in 14% (11/76). Regarding the different TKI, the proportion of patients with transcript level ≤10% in treatment with IM was 83% (31/37), with NI 88% (15/17) and with DA 86% (19/22). Characteristics and patients evolution according MR at 3 months were asses. See Table 1.
Summary and Conclusions: Frequency of optimal response at 3 month is high with IM, NI and DA. In the group with BCR/ABL >10%IS, high risk Sokal Score, lower ratio of CCR and MMR was observed. The frequency of events, progression and death was also higher in this group. The analysis is limited by the difference in number of patients in each group, but the results confirm the value of early molecular response assessment to predict outcome in this population.

PB1567

ANALYSIS OF TRANSCRIPTOME IN CHRONIC MYELOID LEUKEMIA REVEALS GENES AND PATHWAYS POTENTIALLY INVOLVED IN THE RESISTANCE TO TKI THERAPY

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Background: High efficiency of targeted therapy of chronic myeloid leukemia (CML) by tyrosine kinases inhibitors (TKI) has been confirmed by multiple long term observation studies. However these studies also demonstrate that primary resistance to TKI may develop in part of CML patients. Whole transcriptome expression analysis of cancer cells in CML is thought to detect genes and pathways involved in molecular mechanisms of TKI-resistance. These findings may help to find more effective strategies of CML treatment.
Aims: Our aim was to find particular expression patterns in cancer cells of primary CML patients, who demonstrated sensitivity or resistance to TKI.
Methods: Gene expression profiles were analyzed using Illumina HT-12 Expression Bead Chip. These chips quantitate expression levels of more than 47000 transcripts. According to European Leukemia Net (2013) criteria patients were divided into resistant to TKI therapy – molecular response >10% in 6 months of therapy; and optimal responders with molecular response <1% in 6 months of therapy.
Results: Comparison of expression data with Partek Genome Suite (Partek) revealed 2672 genes differently expressed in responders and non-responders. Selected genes were used for pathway enrichment analysis which identified several molecular networks influenced by these genes (p<0,05): HTLV-1 infection (*FZD10, ADCY1, MYB*); PPAR signaling pathway (*SCD-1, OLR1*); Transcriptional misregulation in cancer (*MPO, CEBPE, ELANE*); Melanogenesis (*ADCY1, FZD10*). Detailed analysis demonstrated that all of the selected genes are overexpressed in cancer cells of patients sensitive to TKI therapy.
Summary and Conclusions: Targeted expression analysis of the selected pathways may help to find other genes involved in clinical features of CML and its susceptibility to TKI treatment. Identified genes may be found as useful molecular markers for prognosis of the TKI therapy efficacy and treatment optimization of CML.

PB1568

A CLINICAL AND LABORATORY STUDY OF CHRONIC MYELOID LEUKEMIA WITH ATYPICAL BCR- ABL FUSION GENE SUBTYPES

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Background: The clinical and laboratory features of f chronic myeloid leukemia (CML) with atypical transcripts are unclear.
Aims: To explore the clinical and laboratory features of CML with atypical e14a3and e19a2BCR- ABL fusion gene subtypes.
Methods: We retrospectively analyzed a cohort of CML patients with Ph chromosome positive confirmed by cytogenetic and FISH but classical e13a3(b2a2) e14a2 (b3a2) and e1a2fusion transcripts negative confirmed by conventional realtime quantification RT- PCR RQ- PCR. Further RQ- PCR was done with the forward primer and reverse primer designed to detect rare atypical BCR- ABL fusion genes including e14a3and e19a2transcripts. Direct sequencing analysis was performed on the PCR products and mutations in the BCR- ABL kinase domain were detected. The clinical data of patients were retrospectively analyzed.

Results: Six CML patients were found to have t□9;22□abnormality and BCR- ABL rearrangement confirmed by FISH but classical BCR-ABL fusion genes negative detected by RQ-PCR. Further RQ-PCR and sequencing analysis confirmed the fusion of BCR exon 14 and ABL exon 3 in five CML patients□case1-5□and the fusion of BCR exon19and ABL exon 2 in one CML patient□case6□. E255K and I293T IM-resistant mutation were detected in case1and 2, respectively. Among five cases with e14a3 transcripts, four were CML- CP, one CML-AP. Four patients were male and one was female. The median age was 48 years. The patient (case6) with e19a2 transcripts was 40-year-old female with a diagnosis of CML-CP and PLT count was more than 1000×10^9/L. Imatinib (IM) therapy was started in case 1,2,3,4and hematopoietic stem cell transplantation (HSCT) was undergone in case5after hydroxyurea (Hu) or interferon failure. Case1who had E255K IM resistant mutation, responded poorly to IM and obtained a complete cytogenetic remission (CCyR) after converting IM to dasatinib. Case 2 and 3 achieved CCyR 6 months later after IM treatment and had been maintained well with IM despite I293T mutation in case 2. Case 4 attained CCyR 3 months later after IM treatment but relapsed and died soon. Case 5was still in CCyR after HSCT. Case 6 with e19a2 transcripts got complete hematologic response after Hu treatment. Thereafter, IM was given and CCyR was achieved soon.
Summary and Conclusions: Incidence of CML with atypical transcripts is extremely low. They could benefit from tyrosine kinase inhibitors or HSCT. Rare and atypical BCR-ABL fusion gene subtypes could be missed by conventional RQ-PCR.

PB1569

CLINICO-HEMATOLOGICAL PRESENTATION AND SURVIVAL ANALYSIS IN PEDIATRIC CHRONIC MYELOID LEUKEMIA: A TERTIARY CARE HOSPITAL EXPERIENCE

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Background: CML comprises 3% of all pediatric leukemias, with an annual incidence of 1 per million children.
Aims: To highlight the clinical and hematological presenting features and outcome in pediatric CML cases.
Methods: 17 pediatric CML cases (0-12 years), diagnosed over last three years (2010-2013) were selected and clinical and follow up data analyzed from record files.
Results: Male: Female ratio was 1:0.5. All (100%) had massive spleenomegaly and 8 (47%) mild hepatomegaly. 13/17 (76%) were in chronic phase, 3/17 (18%) accelerated phase and 1 (6%) in blast crisis. All had high TLC, with 15/17 (88%) cases having a WBC count more than 100 x 10^9/L. 8/17 (47%) had high platelet count (>450 x 10^9/L) and 2/17 (12%) had low platelet count. High absolute basophil count (> 0.5 x 10^9/L) was seen in 16/17 (94%) cases. 12/17 (70.5%) cases had a low LAP score (<15; normal range 24-120). Cytogenetic analysis revealed t (9; 22) in 14/17 (82%) cases. All (100%) were positive for BCR-ABL transcript (p210 type) by RT-PCR analysis. 15/17 (88%) cases received imatinib treatment, 2/15 (13%) developed relapse, while 1/15 (6%) died and 1/15 (6%) did not respond to treatment. The median duration of remission free interval was 26 months, while the median survival duration for responders was 29 months (Figure 1a & b).
Summary and Conclusions: Our study highlights that pediatric CML presents with high WBC count and massive spleenomegaly and has an improved outcome with imatinib. Pediatric CML though rare, needs to be considered in cases with high TLC and basophil count. BCR-ABL studies are necessary to confirm diagnosis and imatinib is an effective first line treatment modality.

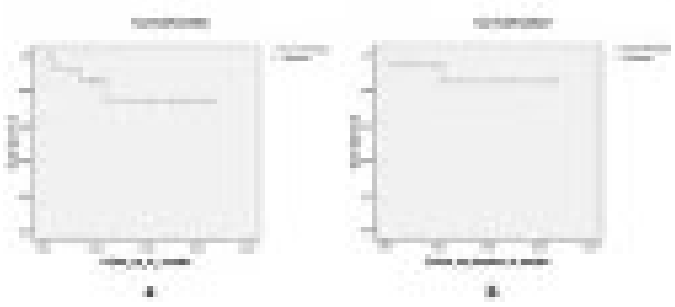

Figure 1. a) Shows remission free interval in months and b) shows over-all survival in rsponders in months.

PB1570

IMMUNOPROFILING OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN TREATMENT WITH TYROSINE KINASI INHIBITOR

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Background: For most patients with chronic myeloid leukemia (CML), the advent of ABL1 tyrosine kinase inhibitor (TKI) therapy has turned their chronic leukemia into a truly indolent medical condition, although only in a small subset of patients may discontinue treatment. In those last patients, it was hypothesized immunogical control of the leukaemia clone. In addition to target BCR-ABL oncoprotein, the TKIs inhibit off-target kinases (such as c-KIT, TEC, SRC), some of which have physiological functions in immune responses. Several studies have documented the influence of TKIs on immunological system, not always clean, some show inhibitions others stimulation.
Aims: The aim of this study was to evaluate the existence of correlation between immunoprofiling and the depth of molecular response.
Methods: Were evaluated patients with CML in chronic phase treated in our institution with TKIs. It were excluded those with less than one year of treatment and/or undergone at allogeneic stem cell transplantation.
We valued on peripheral blood (PB) immunoglobulin levels, electrophoresis and lymphocyte count. Molecular responses were evaluated with RT-Q-PCR in PB. The patients were divided into two groups, “deep molecular” response ($BCR\text{-}ABL^{IS} \leq 0.0032$) and “superficial molecular” response ($BCR.ABL^{IS} > 0.0032$).
Results: 48 patients were considered. The median age of patients was 61 years (range 22-80 years) and 29 (60%) were male. Sixteen patients were in “deep molecular” response (dMR) and thirty-two were in “superficial molecular” response (sMR). A mild hypogammaglobulinemia (IgG > 450 mg/dl) was seen in both groups (56% *vs* 43%) and an increased CD4/CD8 ratio were reported (56% *vs* 44%), without difference between the two groups. (table1 a,b).
Summary and Conclusions: In our study the increase in the CD4/CD8 ratio had demonstrated the activation of immune system,that does not correlate with the a depth of molecular response. To confirm this observation is necessary a higher number of patients.

PB1571

PREDICTIVE VALUE OF PRETREATMENT BCR-ABLIS TRANSCRIPT LEVEL ON RESPONSE TO IMATINIB THERAPY IN EGYPTIAN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CPCML)

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Background: A wide range of responses of patients with CPCML to IM has been reported. Several factors were proposed to predict response including molecular response at 3 and 6 months
Aims: To study the impact of pretreatment BCR -ABL transcript level on molecular response to IM, and to assess the value of the milestone ≤10% transcript at 3 months on PFS and OS.
Methods: Fifty five adult CP-CML patients receiving daily dose of 400 mg IM were subjected to molecular and cytogenetic analysis at diagnosis and at regular time intervals. Median follow up period was 36 months (15-48). Hematologic, cytogenetic, and molecular responses were rated according to ELN.
Results: Two Patient groups were distinguished regarding response to IM therapy. A group of 22/55 patients (40%) having pretreatment BCR-ABLIS level ≤200% and a second patient group 33/55 (60%) having transcript level >200%. The ≤10% milestone was achieved by 15/22 patients (68%) *versus* 7/33 patients (21%) (p=0.04) in favor of the first group. Optimal responders in first group were 14/22 (64%) compared to 13/33 (39%) in second group (p=0.02). Achievement of 10% transcript level significantly correlated with longer PFS. The median BCR-ABLIS transcripts levels in optimal responders at 3, 6 and 18 months was 10%, 2% and 0.1%, respectively compared to 100%, 65% and 10%, in suboptimal/resistant patients p=0.001. Resistance in 11 patients was correlated with identifiable ABL Kinase mutations.
Summary and Conclusions: The Pretreatment 200% cutoff and the 3 month BCR-ABLIS ≤10% transcript levels proved strong predictors of response to IM and significantly correlated with probability of CCyR, MMR and PFS.

Myelodysplastic syndromes - Biology

PB1572

MOLECULAR PREDICTORS OF RESPONSE TO DECITABINE IN CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: The hypomethylating agent 5-aza-2'-deoxycytidine (decitabine, DAC) has been demonstrated to have efficacy in the therapy of chronic myelomonocytic leukemia (CMML). However,response is quite heterogenous with overall response rate ranging from 25-70%(Braun,Blood 2011).Given that no other specific therapy has been identified for CMML,there is a need to identify molecular markers that could support decision making when choosing DAC therapy.DAC is a pyrimidine nucleoside analogue of cytidine,and different studies have demonstrated the importance of the levels of expression of the enzymes involved in nucleoside metabolism and consequently in the resistance/response to this kind of drugs(ie gemcitabine,cytosine arabinoside).In addition,mutations in several genes have been demonstrated to impact survival of CMML patients.
Aims: In this study,we(1)measured expression levels of genes involved in the metabolism of DAC and(2)performed mutational analysis for genes recurrently mutated in CMML in pretreatment samples from CMML patients prospectively treated with DAC as part of a clinical trial.
Methods: DNA and RNA were extracted from marrow mononuclear cells of 39 patients treated with at least six cycles of DAC iv 20mg/m^2/5 days every 28 days. Quantitative real-time PCR was then performed in 22/39 for *hENT1,hENT2,DCTD,hCNT3,CN-II,DCK and CDA*.
Results: The expression of all genes analyzed was compared between responders and non-responders. In addition, samples were sequenced for the 15 genes known to be recurrently mutated in CMML at a mean depth of coverage of 520X(range 169–714x).Of the 39 patients,16 patients were responders and 20 non-responders to DAC,according to the IWG 2006 criteria(3 had response which was non-evaluable).Individually,none of these genes was differentially expressed in responders *versus* non-responders. The mutational frequencies in this cohort were:48.7%SRSF2,43.6% *ASXL1,* 17.9% *NRAS,*12.8% *TET2,*10.3% *RUNX1,* 10.3% *DNMT3A*,*U2AF1* 7.7%,7.7% *TP53,*7.7% *KRAS,*5.1% *JAK2,*2.6% *EZH2,*2.6% *IDH1,*2.6% *IDH2,*2.6% *SF3B1,*2.6% *KIT.*No single genetic alteration was significantly associated with adverse overall survival or resistance to decitabine in this cohort.
Summary and Conclusions: Although the main limit of the study is the small number of cases,our results seem to show that the absence of clinical response to DAC of CMML patients is not strictly correlated with the expression of genes involved in the DAC metabolism or to specific genetic alterations.This finding is not in line with what our group recently demonstrated in MDS cases treated with the nucleoside analog azacitidine,for whom UCK1 expression levels correlated with response.Further studies on larger series of CMML cases are needed to validate these results.

PB1573

CSF3R POINT MUTATIONS IN A COHORT OF PATIENTS WITH MYELODISPLASTIC SYNDROMES AND NEUTROPENIA AT DIAGNOSIS

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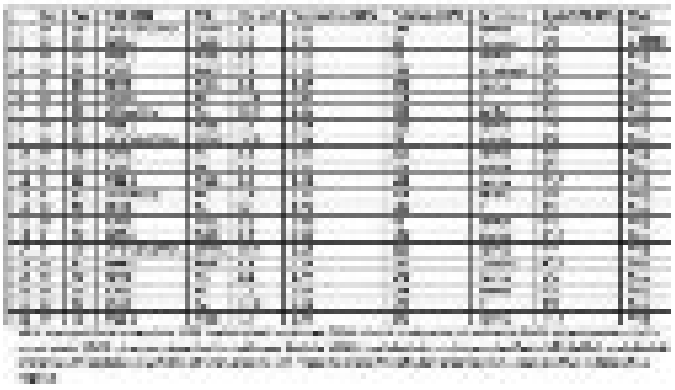
Background: Granulocyte colony-stimulating factor (GCSF) receptor, also known as CSF3R, plays an essential role in the proliferation and granulocyte differentiation. Two types of *CSF3R* mutations have been described, mutations causing truncations of the cytoplasmatic domain and membrane-proximal point mutations. *CSF3R* truncation mutations have been observed in patients with severe congenital neutropenia (SCN) chronically treated with pharmacologic doses of GCSF. Membrane proximal mutations confer ligand-independent-growth and are the mutations most frequently reported in chronic neutrophilic leukemia (CNL) and atypical chronic myeloid leukemia. Myelodysplastic syndromes (MDS) are acquired clonal hematopoietic disorders, characterized by cytopenias, dysplasia in one or more of the myeloid cell lines and an increased risk of development of acute myeloid leukemia. To our knowledge there is limited information about the incidence of *CSF3R* mutations in MDS. Abnormalities in the signal-transduction pathways for CSF3R may play a role in the pathogenesis and the development of neutropenia in MDS
Aims: The aim of this study was to investigate the presence of *CSF3R* point mutations in patients diagnosed with MDS that presented neutropenia.
Methods: Mutational analysis of the *CSF3R* gene (exons 14-17) was performed by Sanger sequencing using DNA obtained from bone marrow or peripheral blood samples of 22 MDS patients who presented neutropenia <1800/mm^3 at diagnosis. Diagnosis of MDS was made according to the FAB classification. Three out of the 22 patients were diagnosed of chronic myelomonocytic leukaemia according to the 2008 WHO classification. Samples were collected at the time of diagnosis and without any prior therapy.
Results: Median age at diagnosis was 76 years (range 63–86). Fifteen out of the 22 patients (68%) were males and 90% were older than 70 years. Baseline characteristics are shown in Table I. Median overall survival was 47 months (95% CI: 24-70). We identified a *CSF3R* mutation in 1 out of the 22 patients. The mutational analysis showed one missense variant resulting in the substitution of a glutamic acid to a lysine at amino acid position 808 (p.E808K) located at exon 17 of the *CSF3R* gene (G>A coding location 2422, NM_000760.3). This mutation has previously been described in one case of chronic myelomonocytic leukaemia and in one case of CNL, this last one coexisting with the P.T618I mutation. Our patient (#2) was a 73-year old man who was referred to our service for pancytopenia (neutrophils count 1.72 x10^9/L, hemoglobin level 9.6 g/dL, platelets count 69 x10^9/L). Bone marrow was hypercellular with dysplasia involving the three myeloid lineages and 6% of blasts. Conventional cytogenetic analysis on bone marrow cells revealed the following karyotype: 46,XY,-5,del(7)(q11q35),+8,der(17)t(5;17)(p11;p11)[20]. Diagnosis of RAEB-1 was established. The patient developed an acute myeloid leukaemia 16 months later from diagnosis. Overall survival for this patient was 18 months, clearly lower compared to the rest of the patients.
Summary and Conclusions: In our experience, mutations affecting the *CSF3R* gene are an uncommon event in MDS patients. Here we report a rare variant located at exon 17 (p.E808K) in a patient with MDS and neutropenia. The biological and the clinical relevance of this mutation remains unknown. More studies in larger series are required to determine the incidence and role of *CSF3R* mutations in MDS.

Table 1.



PB1574

SIGNIFICANCE OF P53 OVEREXPRESSION IN BONE MARROW BIOPSIES FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) AND NON-NEOPLASTIC CONDITIONS ASSOCIATED WITH MYELODYSPLASIA

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Background: Myelodysplastic syndromes (MDS) are clonal diseases of the bone marrow, characterized by ineffective hematopoiesis causing peripheral blood cytopenias. Apoptosis is suggested in the pathogenesis of MDS. An important controller of this process is p53 tumor supressor gene. Although p53 mutations commonly found in many cancer types, the results of the role of the p53 in MDS are variable in the literatüre.
Aims: To investigate the role of p53 overexpression in the development of Myelodysplastic syndrome (MDS) and to assess their impact on differential diagnosis with myelodysplasia associated with non-neoplastic conditions.
Methods: Bone marrow biopsy samples from 72 patients admitted to the

hospital with cytopenias with preliminary diagnosis of MDS were obtained from archives of Marmara University Pathology Laboratory. Fiftyfour patients were diagnosed as MDS (24 female , 30 male), 18 patients diagnosed as reactive conditions associted with mild dysplasia (10 female, 8 male). MDS group was classified as Refractory anemia (MDS- RA)(n = 10), Refractory anemia with ring sideroblasts (MDS- RARS) (n = 10), Refractory cytopenia with multilineage dysplasia) (MDS- RCMLD) (n = 10),Refractory anemia with excess blasts-I (RAEB-I) (n = 10) , Refractory anemia with excess blast - II(RAEB-II) (n =10), MDS associated with isolated 5q deletion (MDS 5q-) (n = 4) according to the WHO 2008 classification. Patients having preliminary diagnosis as MDS were classified in reactive group composed of patients having mild dysplasia (<%10 dysplasia in myeloid cells) founded in bone marrow biopsies with no detected cytogenetic abnormality, and patients who were evaluated as Paroxysmal nocturnal hemoglobinuria , Idiopathic thrombocytopenic purpura , collagen vascular disease , vitamin B12 deficiency , anemia of chronic disease with subsequent follow-up. The p53 (Leica, concantrated 1/100) staining was evaluated by counting nuclear positivity in all cells at x1000 magnification in "hot spot" area.

Results: In reactive group, no positivity of p53 was detected in their marrow. The p53 positivity was detected in 37(68%) of all patients with MDS . The p53 staining was detected in 3 patients with mean staining 0.3% (% 0-2.9) in MDS-RA group , 8 patients had a mean of 1.75% (% 0-5.3) in RARS group, 6 patients had a mean of 1.9% (% 0-6.1) in RCMLD group , 7 patients had a mean of 7.4% (0-22.4%) RAEB -I group, 10 patients had a mean of 20.7% (6.6-50%)in RAEB -II group, 3 patients had a mean 10.4%(0-23%), in MDS 5q (-) group. The p53 values were found to be statistically significantly between reactive and myelodysplasia groups .(p <0.001) (Kruskal-Wallis test)

The p53 values were found to be statistically significant between the RA group that had the lowest p53 immunoreactivity compared with the reactive group. (p <0.05) (Mann-Whitney test).

Summary and Conclusions: The detection of no expression for p53 immunhistochemistry in bone marrow biopsy from clinically suspicious MDS patients having limited dysplasia in the bone marrow, can favor the reactive condition. The p53 positivity in bone marrow biopsy is a valuable tool in the differential diagnosis of myelodysplaia assaciated with MDS and myelodysplasia associated with non-neoplastic conditions. p53 may also have prognostic importance in MDS patients due to the a higher rate of detection of p53 especially in high risk MDS group (RAEB-I/RAEB-II). The detection of high p53 positivity in patients with MDS with isolated 5Q(-) sydnrome , suggest that p53 activation plays an important role in the development of MDS in Isolated 5Q (-) syndrome

PB1575

POLYMORPHISM OF FOLATE AND METHIONINE METABOLISM GENES IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

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Background: Epigenetic mechanisms, including aberrant methylation of CpG islands, have an important role in pathogenesis of human neoplasias and are often seen in patients with myelodysplastic syndrome (MDS). Polymorphism of folate and methionine metabolism (FMM) related genes can influence the methylation processes. The role of gene variations in FMM genes in predisposition to MDS is not well known.

Aims: The aim of this study was to assess the peculiarities of alleles and genotypes distributions of the FMM genes in MDS patients.

Methods: Twenty-nine patients with MDS (10 men and 19 women, mean age 63.7 yrs) and 121 healthy controls (HC) were genotyped for the MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G and MTHFD G1958A polymorphisms by PCR-RFLP technique. The differences in allele and genotype frequencies between patient and HC groups were assessed by Fisher's exact test with computation of odds ratios (OR), their 95% confidence intervals (CI) and p-values.

Results: For all studied genes, the alleles and genotypes distributions in patients were not statistically different from those in HC. The frequency of the MTFR 677CC genotype was slightly decreased in MDS patients when compared to HC group (41.4% *vs.* 52.1%, respectively, p=0.4). At the same time, the MTRR 66GG variant was present in 10 (34.5%) patients and 28 (23.1%) controls (p=0.24). The simultaneous presence of the MTHFR 677T allele and MTRR 66GG genotype was more often detected in MDS group (24.1% *vs.* 9.1%, OR=3.2, 95%CI: 1.1-9.1, p=0.049). Moreover, the frequency of the MTHFD 1958GG genotype was increased in patients (41.4% *vs.* 24.8%, OR=2.1, 95%CI: 0.9-5.0, p=0.1).

Summary and Conclusions: We conclude that polymorphism of the FMM genes could have an important role in pathogenesis of MDS. The simultaneous presence of the MTHFR 677T allele and MTRR 66GG genotype is a significant risk factor for MDS development.

PB1576

INTRACHROMOSOMAL AMPLIFICATION OF RUNX1 GENE (IAMP21) IN A PATIENT WITH HYPOPLASTIC MYELODYSPLASTIC SYNDROME (MDS): FIRST OBSERVATION

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Background: Most recurrent cytogenetic abnormalities in MDS are unbalanced, with loss of or deletion of parts of chromosome. The intrachromosomal amplification of *RUNX1* gene (iAMP21), defined as three or more extra copies of *RUNX1* gene on a single abnormal chromosome 21 is a distinct clinical entity reported in pediatric acute lymphoblastic leukemia (ALL). Although *RUNX1* mutations are known to occur in MDS, iAMP21 has never been described in MDS. Here, we report a case of iAMP21 associated with intrachromosomal amplification of the MLL gene (iAMPMLL) in a 28 year old patient with hypoplastic MDS and history of chronic exposure to cytotoxic chemicals.

Aims: To document the presence of iAMP21 in hypoplastic MDS highlighting the first ever report of its correlation with cytotoxic exposure.

Methods: Karyotyping and FISH using *ETV6*/*RUNX1* fusion and *MLL* breakapart probes were carried out on bone marrow aspirates cultured overnight using standard protocols.

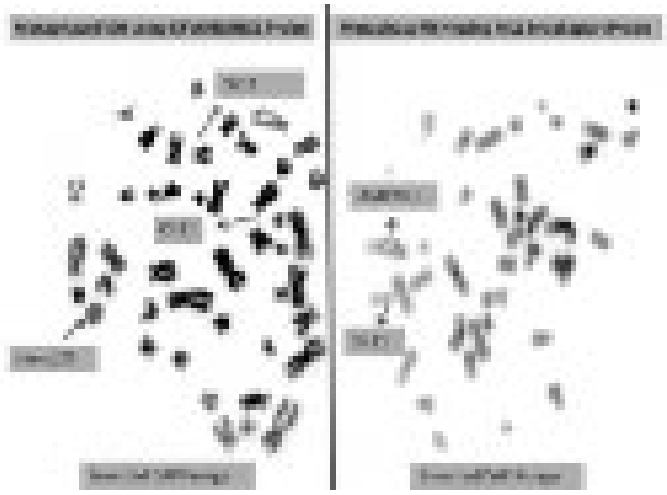

Figure 1.

Results: A 28 year old male presented with history of weakness for two months. He had worked for many years in the leather industry and had nearly 2 decades of exposure to organic solvents, polyurethane and polycholoropene compounds. Clinical examination showed pallor with no other significant findings. Blood counts revealed pancytopenia (Hb 6.6g/dL; total leucocyte count 2.2 × 10^9/L; platelet 5 × 10^9/L) with features of dysgranulopoiesis on peripheral blood smear. Marrow studies indicated hypocellularity (30% cellularity) and florid trilineage dysplasia with 7% blasts and no significant marrow fibrosis. Cytogenetic analysis revealed a complex hypodiploid karyotype with multiple subclones. A deletion 7q and a derivative chromosome 21 were observed in all the cells. In 16 metaphases, a derivative chromosome 15 with an unbalanced translocation was seen. The modal karyotype was 44~46,XY, del(1)(p34.3), t(1;3)(p31;p25), -4, del(5)(q21q33), del(7) (q11.2), add(8)(q24), -9, der(10)t(10;?)(q26;?), -11, ?i(11)(p11), dic(11;12)(q25;p13), der(15)t(15;?)(p11.2;?), -18, +der21?iamp(21), der(21)?iamp(21) [CP20]. A metaphase FISH using the ETV6/RUNX1 fusion probe confirmed the presence of iAMP21.Metaphase FISH using the MLL breakapart probe showed iAMPMLL on the derivative chromosome 15. The patient is planned for a haploidentical allogeneic bone marrow transplant.

Summary and Conclusions: We report a patient with hypoplastic MDS, probably arising from chronic exposure to hazardous chemicals. Cytogenetic analysis showed a complex karyotype with iAMP21 as well as iAMPMLL. iAMP21 is a recognised cytogenetic abnormality in precursor-B ALL and the observation of this abnormality in MDS is unusual. Its co-existence with iAMPMLL suggests a common mechanism of chromosome instability associated with the breakage-fusion-bridge cycle, resulting in amplification and formation of dicentric chromosomes. Since the iAMPMLL was seen in only a subpopulation of iAMP21 positive cells, it is likely that the iAMPMLL arose as a secondary event in this patient. A more detailed molecular study would uncover molecular and cellular mechanisms underlying this clinical phenotype.

PB1577

SRSF2 IN CMML: CORRELATION BETWEEN CLINICAL AND LABORATORY FINDINGS

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Background: Chronic Myelomonocytic leukemia (CMML) is a clonal blood disorder that belongs to the Myelodysplastic Syndrome/ Myeloproliferative Neoplasm (MDS / MPN) group of the World Health Organization classification of 2008 (WHO), because it shares clinical and biological data of both entities. Taking in reference the WHO classification, CMML is divided in two groups depending on morphologic features: CMML-1 with fewer than 5% blasts in peripheral blood (PB) or 10 in bone marrow (BM), and CMML-2 with 5-19% blasts in PB or 10-19% in BM. The French-American-British (FAB) classification considered 2 groups of this pathology, dysplastic and proliferative variants, with the cut-off of 13000 leucocytes in PB. At present, the only specific molecular criterion for the diagnosis of this entity is the absence of BCR-ABL1 fusion transcript, but there are not specific cytogenetic or molecular abnormalities described. Although other groups have studied the association of some common mutations in MDS, like the mutation that affects the splicing gene SRSF2. The SRSF2 gene, located on chromosome 17q25.1, encodes the splicing factor 2, rich in serine /arginine, important in this process in which the pre-mRNA into mature mRNA is transformed, essential for the production of proteins. Current literature shows a prevalence of the SRSF2 mutation between 40-50% of patients diagnosed of CMML. These findings make it as a possible factor in the diagnosis of this entity.
Aims: The aim of this study is to analyze the SRSF2 mutation in a series of patients diagnosed of CMML. We included also patients affected of other pathologies to have a relation of the association of this mutation with myeloid pathology in general.
Methods: The study included 40 patients with the following diagnoses: CMML (9) , MDS (4) , MDS / MPN (2) , Acute myeloid leukemia (AML) (10) ,Chronic lymphatic leukemia (CLL- B) (3), monocytosis (1) , metastatic carcinoid tumor in BM (1), immune thrombocytopenic purpura (1), mastocytosis (1).
The method of analysis used is the real-time PCR, obtaining results through High resolution Melting (HRM) with two possibilities (mutated or not mutated). In a second step, positive results have been checked by sequencing.
Results: The results obtained are shown in the Table 1.

Table 1.

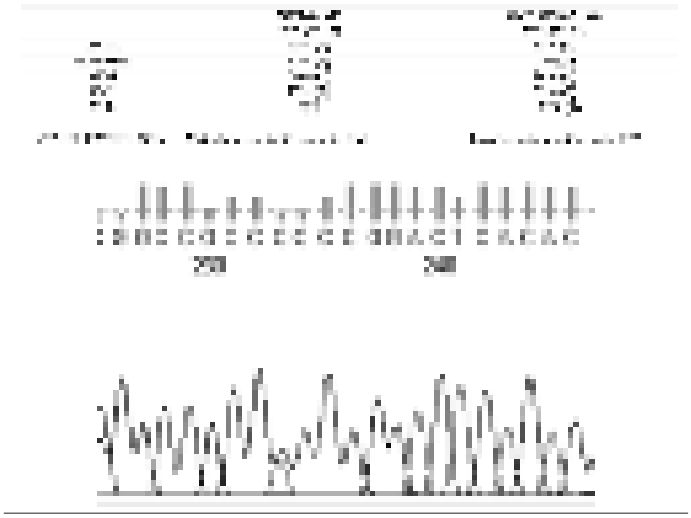

Figure 1.

Summary and Conclusions: The analysis showed a prevalence of 65% of the SRSF2 mutation in patients with a diagnosis of CMML. Although the overall sample size for other diseases is insufficient, we observed that patients with different myeloid pathologies may also have the mutation. We did not find association of the SRSF2 mutation with sex or age. At the beginning of the study we found a relation between the dysplastic variant of the FAB classification and the SRSF2 mutation, but when we expand the sample size, we observed that it was a chance finding. In conclusion, we did not find any relation between the variants of the WHO and FAB classification with the presence or absence of the mutation. More than a half of patients with CMML showed the SRSF2 mutation, for this reason, we concluded, that it could be an important finding to help in the diagnosis of this heterogeneous patholoy.

PB1578

DISTRIBUTION OF CYTOGENETIC ABNORMALITIES IN MYELODYSPLASTIC SYNDROMES IN A SINGLE CENTER IN ALGERIA

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Background: The myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases which reflect a clonal hematopoietic stem cell achievement. SBDS are associated with a significant risk of transformation into acute myeloid leukemia (secondary leukemia).Cytogenetics (Karyotype and FISH) became an essential consideration in the management of Myelodysplastic syndromes, as much for the diagnosis, the prognosis and the monitoring of residual disease.The 2008 who classification takes into account the cytogenetic study in order to stratify on the prognostic map myelodysplastic syndromes.Main cytogenetic abnormalities observed in myelodysplastic syndromes are highlighted by the karyotype. Hybridization *in situ* because its sensitivity found a framework of choice for the revelation of targeted abnormalities.
Aims: Our presentation is designed to report the main cytogenetic abnormalities observed in our practice, and their contribution to the diagnosis and prognostic classification
Methods: The study involved 40 patients of average age = 60 years (25-84), sex ratio = 1, 1 .Karyotype and hybridization *in situ* have been made systematically on a bone marrow sample.A culture of 24 hours with synchronization was performed, R banding was adopted with an analysis of at least 20 mitoses.The reading was made by microscope coupled with analysis type Cytovision for software; the FISH probes targeting the most common abnormalities have been used: probe 5 q - (5q31.2; 5q33.2); Probe 5q 33 break; Probe 7 q - (7q 22 q 7, 35); Probe 20 q - (PTPRT20q12 / 20q 11); Probe P53 (17 p 13/SE17)
Results: On 40 completed karyotypes, 30 were informative. Chess are represented by 7 failures of culture because of poor sampling (hypoplastic forms) and 3 failures of karyotyping: difficulty of banding.On 30 informative karyotypes: no abnormality was detected in 17 cases (56.7%) Karyotype with abnormalies in 13 cases (43.3%): A single anomaly in 8 cases, two anomalies in 1 case, ≥ 3 abnormalities in 4 cases.The karyotype abnormalities: Del 5 q / monosomy 5: 4 cases (13.3%), isolated in 2 cases (5%); associated with a complex karyotype in 2 cases; Del7q / monosomy 7: 4 cases (13.3%), isolated in 1 case (2.5%), associated with a complex karyotype in 3 cases; Del 20q: 2 isolated cases (5%). Del 13q (iso 17q): 1 case (2.5%) insulated; Tri 8: 4 cases: secluded in 2 cases, associated in 2 cases .Translocations unbalanced in complex karyotype abnormalities: 4 cases (10%).On 40 FISH carried out there: without abnormalities: 24 (60%); with anomalies: 16 (40%): del 5q : 6 cases: 2 isolated, 4 associated ; del 7q : 5 cases : 1 isolated, 4 associated ; del 20q: 2 cases: all isolated ; del P53: 5 cases : 4 isolated, 1 complex ; other anomalies : duplication of the 20q, the 7 q , the 5q ; t (7; ?); + 17.
Summary and Conclusions: The combination of the Karyotype and FISH allowed to detect abnormalities in 18 patients (45%) and the absence of anomalies in 22 patients (55%).In 10 cases of failure of the karyotype, the FISH has identified 3 abnormalities. In cases where the karyotype was normal, the FISH found targeted anomalies. In 15 cases where the karyotype was normal, the FISH did not reveal targeted anomalies. In 9 cases the abnormalities found in the karyotype were confirmed by the FISH. The karyotype revealed Trisomy 8 isolated or associated in 4 cases, abnormality not targeted by the FISH. The karyotype is the only procedure which revealed complex abnormalities. These data are as those identified in the literature; They also attest to the complementarity of these two procedures.

Myelodysplastic syndromes - Clinical

PB1579

DECITABINE AS COMPARED TO AZACITIDINE THERAPY MAY RESULT IN BETTER OVERALL SURVIVAL BUT NOT QUALITY OF LIFE IN PATIENTS DIAGNOSED WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): A RETROSPECTIVE, SINGLE I

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Background: CMML is one of the clonal hematopoietic disorders classified under the myelodysplastic/ myeloproliferative overlap category. Hypomethylating agents (HA) (azacitidine [AZA] and decitabine [DAC]) have been approved by the FDA for the treatment of myelodysplastic syndromes (MDS) and CMML, based on MDS studies which included few patients (pts) who received AZA and DAC. To our knowledge, these two therapies have not been compared in either a prospective or retrospective study for the treatment of CMML.
Aims: To compare the clinical and quality of life outcomes in pts on HA for CMML.
Methods: We performed a retrospective chart review of pts diagnosed with CMML at the Mayo Clinic between 1994 to 2011. Overall response (OR) was categorized as complete remission (CR), partial remission (PR), marrow remission (MR) or hematological improvement (HI), based on the modified International Working group (IWG) criteria in MDS (Cheson B et al Blood 2006). IRB approval was obtained in accordance with the Helsinki declaration. Comparison between the two groups was done using the Wilcoxon test, while survival estimates were calculated using Kaplan-Meier estimates via JMP software v.9. For the cost-effectiveness analysis, Incremental cost-effectiveness ratios (ICER) were calculated (cost difference /quality adjusted life years [QALY]). Only direct costs were included due to absence of the indirect costs, and the drug prices for both AZA and DAC were obtained from the Pharmacy Red Book 2010.

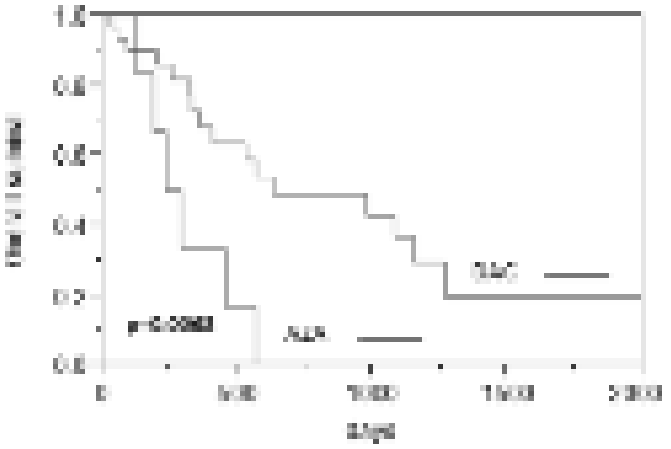

Figure 1.

Results: A total 268 pts with CMML were identified and of these, 36 pts (13%) were treated with HA: 29 (81%) received DAC (group, (Gp 1)) and 7 (19%) received AZA (Gp 2). No significant baseline differences in median age (70 *vs* 73 years), hemoglobin (11 *vs* 10 g/dl), WBC (14 *vs* 9 x10^9/L), platelets (74 *vs* 40 x10^9/L), monocyte (18% *vs* 26%), Peripheral blood (PB) or BM blast (0% and 4%) was found between Gp 1 and Gp 2, respectively. In Gp 1 cytogenetics were diploid in 62% *vs* 100% in Gp 2 (p=0.016). There was no significant difference in the median number of cycles (5 [range 1-22] cycles of DAC compared to 3 [range of 1-9] of AZA) and median follow up in days between both groups (409 for Gp 1 *vs* 239 for Gp 2). Differences in OR for Gp 1 *vs* Gp 2 were not significant: OR 41% *vs* 29% (p=0.5), CR 20% *vs* 0 (p=0.09), MR 17% *vs* 0 (p=0.13), HI 3 *vs* 29% (p=0.059) as was transformation to AML, 28% *vs* 14% (p=0.4). Median overall survival (OS) was significantly higher for Gp1 *vs* Gp 2 (634 *vs* 266 days, respectively, (p=0.0068)). When limiting analysis to pts with BM blast >4%, median OS was 531 *vs* 292 days, respectively (p=0.13) compared to pts with BM blast <5%, 979 *vs* 266 days, respectively (p=0.023). On multivariate analysis, platelets (p=0.04) and HA type of therapy (DAC *vs* AZA) (p=0.048) were significant for OS, but not age, hemoglobin, WBC, monocyte count, PB or BM blasts. For ICER, a difference of US$4727 for dominance of DAC was found, which is insufficient to recommend a meaningful difference between the two drugs from a societal or a third party payor perspective as far as QALY's is concerned.
Summary and Conclusions: In a single institution retrospective study DAC was used more frequently compared to AZA for CMML. OS was significantly better for patients on DAC (in particular if BM blast <5%). Only type of HA therapy and platelet count remained significant on multivariate analysis. Our findings suggest the DAC as compared to AZA may result in better OS in CMML and require confirmation in prospective studies.

PB1580

A PHASE 4, OPEN-LABEL, SINGLE-ARM STUDY TO EVALUATE THE EFFICACY, SAFETY, AND PHARMACOKINETICS OF SUBCUTANEOUS AZACITIDINE IN ADULT TAIWANESE SUBJECTS WITH HIGHER-RISK MYELODYSPLASTIC SYNDROMES

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Background: Subcutaneous (SC) azacitidine (AZA) significantly improved overall survival (OS) and hematologic improvement (HI) in Western patients (pts) with higher-risk MDS (HR-MDS) (Fenaux *et al*, 2009). Hematologic response with AZA was evaluated in small studies in Japanese and Korean pts with HR-MDS (Kim *et al*, 2011; Uchida *et al*, 2011). Variation in gene polymorphisms among Asian populations (Kurose *et al*, 2012) may influence AZA clinical outcomes. The PK parameters of SC AZA in Taiwanese pts (n=12) from this study were comparable to those in North American pts (Laille *et al*, 2013; Garcia-Manero *et al*, 2011). To our knowledge, the treatment effects of AZA in Taiwanese pts with HR-MDS have not previously been evaluated.
Aims: This study evaluated the safety and efficacy of AZA in Taiwanese pts with HR-MDS.
Methods: This multicenter open-label, single-arm, phase 4 study enrolled Taiwanese pts aged ≥18 years, with IPSS HR-MDS (Intermediate-2 [Int-2] or High risk), a diagnosis of RAEB, RAEB-T, or CMML (10-29% bone marrow [BM] blasts), and an ECOG PS of 0-2. All pts received SC AZA 75 mg/m^2/day x 7 days/28-day cycle for up to 6 cycles. BM aspirates were assessed by central review. Investigator-determined complete and partial remission (CR, PR), and HI and transfusion independence (TI), were defined per IWG 2000 criteria. Pts must have had a BM assessment at baseline (BL) and at cycle 6 to be evaluable for CR or PR. TI was assessed in pts who were transfusion-dependent (TD) at BL (≥1 RBC/platelet transfusion over 56 days before receiving AZA). Safety was assessed by frequency and severity of treatment-emergent adverse events (TEAEs) occurring between the first AZA dose and 28 days after last AZA dose (NCI-CTCAE v.4.0).
Results: In all, 44 pts had a median (range) age of 64 (36-90) years. Most were male (n=27, 61%). Forty-two pts were classified as having IPSS Int-2 (n=16, 36%) or High (n=26, 59%) risk MDS. Two pts (5%) had IPSS Int-1 risk MDS. Most pts had RAEB (n=32, 73%) or RAEB-T (n=9, 20.5%) disease, 2 had CMML (5%), and 1 had AML (2%). Median number of treatment cycles was 6 (range 1-6), with 68% (n=30) receiving ≥4 cycles. Eleven pts were not evaluable for CR or PR. Reasons for discontinuation were TEAEs (n=6, 14%); death (n=4, 9%); disease progression (n=3, 7%), withdrawal of consent (n=3, 7%), or other reasons (n=3, 7%). No evaluable pt had a CR or PR but 64% (n=28) of pts maintained stable disease. Additionally, 50% (n=22) of pts achieved an HI (HI-E n=14, HI-P n=14, HI-N n=2). Of BL TD pts, 38% (12/32) achieved RBC TI, and 39% (7/18) achieved platelet TI. The most common Grade 3/4 TEAEs were neutropenia (52%); leukopenia (39%); and anemia, febrile neutropenia, and thrombocytopenia (36% each). One or more serious TEAEs were reported by 14 pts, including febrile neutropenia (16%); neutropenia (7%); and pneumonia, pyrexia, and thrombocytopenia (5% each). Three TEAEs resulted in death (pneumonia n=2, hepatic/respiratory failure) that were suspected to be related to study drug.
Summary and Conclusions: These data confirm the efficacy and safety of SC AZA in Taiwanese pts with HR-MDS. AZA reduced transfusion burden in more than one-third of treated pts and 50% achieved HI. HI has been associated with improved OS in HR-MDS (Gore *et al*, 2013). Outcomes in Taiwanese pts were comparable to those reported in a primarily Caucasian population in the pivotal phase 3 AZA-001 study (Fenaux *et al*, 2009). The safety of AZA in Taiwanese pts was consistent with the known safety profile of AZA in Western pts.

PB1581

CLINICAL EVOLUTION OF PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOMONOCYTIC LEUKEMIA DEPENDING ON INITIAL TREATMENT STRATEGY. A REPORT FROM ERASME STUDY

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder characterized by a heterogeneous clinical and morphological expression that shares features of both myelodysplastic syndromes (MDS) and chronic myeloproliferative disorders. In the last years some new treatments have been included in the clinical practice, but data from the general population on how these treatments are being used and their impact on the evolution remain scarce.
Aims: The aim of the study is to describe the "real world" management of CMML patients and to analyze the impact of the time to therapy initiation from diagnosis and the therapeutic strategy chosen on the clinical outcomes.
Methods: The ERASME (CEL-SMD-2012-01) study is an observational, post-authorization, prospective, multicentre study that will include a total of 600 newly diagnosed MDS and CMML patients and follow them during three years or until death. We present here the results of an intermediate analysis with data of the first 21 CMML patients enrolled into the ERASME study. The primary endpoint of this study is to describe the disease progression in routine clinical practice, analyzing the impact of time to therapy initiation from diagnosis and the initial therapeutic strategy chosen. The initial management of the patients is classified in three groups: Observation & support (SP) (including growth factors), active therapy (AT) (chemotherapy, azacitidine, etc) and allogenic hematopoietic cell transplant (HCT). In addition to disease-specific data, we will also collect data related to comorbidities, quality of life and healthcare resource utilization. Data from the pre-specified interim analysis are presented.
Results: A total of 21 CMML patients (38% women) with a median age of 75 years (range 51-91 years) have been recruited between January and October 2013. The median follow-up time was 3.8 months in CMML patients. Morphological subtypes according WHO classification were CMML-1 (blasts count <10%) in 15 patients (71%) and CMML-2 (blasts count 10% to 19%) in 5 (24%). According to FAB criteria, 17 patients (81%) had CMML-MD depending on absolute leukocyte count at diagnosis (WBC ≤13x109/L) and 4 (19%) had CMML-MP (WBC >13x10^9/L). Karyotype was normal in 15 patients (83%). Three patients displayed cytogenetic abnormalities, 2 patients with trisomy 8 (isolated or with one additional abnormality) and 1 patient with complex karyotype (a finding of ≥3 chromosome abnormalities) and thus the CMML-GESMD cytogenetic risk classification was low/intermediate/high risk in 62%/19%/10% of patients, respectively. The CPSS was low/int-1/int-2/high in 29%/33%/24%/5% of patients, respectively. Seven out of 21 patients were transfusion dependent at diagnosis. After diagnosis, 16, 4 and 1 of CMML patients were considered candidate to SP, AT and HCT strategy, respectively and median time to AT initiation from diagnosis was 7.1 weeks. The reasons for observation just after diagnosis (SP group; n=16) were the risk-disease, symptomatology of pathology, patient's age, and comorbidities. Of those patients considered candidate for AT (n=4), 3 received other low-dose chemotherapy and 1 other therapy (erythropoietin and azacitidine), respectively. Only one patient was considered candidate for HCT and received azacitidine prior the transplant. In the last follow-up, 3 patients have died (14%) after a median of 1.7 months and 1 patient developed AML after 2.9 months.
Summary and Conclusions: CMML patients were treated on an individualized therapy strategy after diagnostic evaluation and prognosis assessment. More data on disease progression in routine clinical practice may be useful in characterizing the newly diagnosed CMML patients.

PB1582

EXPERIENCES WITH 10-COLOR FLOW CYTOMETRY IN MDS DIAGNOSTICS

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Background: Peripheral blood cytopenias of unknown etiology are a significant cause of referrals to hematology units. In some cases, where the diagnostic golden standard, cytomorphology (CM), reveals only minimal dysplasia not sufficient for a definitive diagnosis of MDS or no dysplasia at all, MDS can be verified by cytogenetics (CG) or by molecular abnormalities. In a significant proportion of cases, additional diagnostic approaches are needed to diagnose or rule out MDS. Multiparameter flow cytometry (MFC) has been shown to be able to increase the diagnostic yield, and its inclusion to the standard diagnostic work-up has been recommended.
Aims: A pilot study to explore the possibility of increasing MFC sensitivity with a wider array of colors to detect distinct aberrancies in antigen expression using simultaneously altogether 10 antibody conjugates.
Methods: 36 new patients with unclear cytopenia in one or more lineages (median age 69 yrs, range 32-87 yrs) were analyzed in parallel by CM and MFC. CG was performed in cases with clear clinical suspicion of hematologic malignancy. MFC was performed with 10-color flow in 4-5 tubes following ELN recommendations (Westers, Leukemia 2012;26:1730-) in myeloid progenitor cells (MPC), granulocytes, and monocytes. As a modification to the ELN protocol, the novel AML stem cell-associated antigen, C-Type Lectin Molecule-1 (CLL-1) together with CD38 was studied on MPCs. Our analysis also included the robust four parameter flow cytometric score (FCM score) originally reported by Della Porta and ELN (Haematologica 2012;97:1209-). In their study, a FCM score ≥2 was significantly associated with MDS. As MFC controls we had bone marrow samples from six healthy subjects.
Results: On the basis of CM and CG the patients were grouped to: 1) patients with confirmed MDS or CMML (12 pts, 33%), 2) patients with evidence of mild dysplasia not sufficient to diagnose MDS and with normal CG (6 pts, 17%), and 3) patients with no evidence of dysplasia in CM (18 pts, 50%).
MPCs defined as CD34+CD117+ cells or cells of monocytic lineage co-expressed lymphoid markers in 5/12 pts of group 1), in 3/6 pts of group 2), in 0/18 pts in group 3), and in none of the healthy controls. The most common aberrant lymphoid marker was the NK cell antigen CD56. In line with previously published results, the CLL-1 was expressed on 32-45% of CD38+ MPCs of the healthy controls. In one MDS case, it was not expressed at all and in another MDS case it was aberrantly expressed on 85% of CD38dim/neg MPCs.
The FCM score was ≥2 in 10/12 (83%) in group 1), 1/6 (17%) in group 2), 1/18 (6%) in group 3), and in none of the healthy controls.
Summary and Conclusions: We found 10-color flow cytometry feasible in MDS diagnostics. It allows better characterization of small blast populations and analysis of a wider selection of antigens in fewer tubes compared with conventional routine 4-5 color flow. As interesting findings in this pilot analysis still collecting new samples, we found CD56 as the most frequently aberrantly expressed lymphoid antigen, and the aberrant expression of CLL-1, previously described in AML, in two MDS patients.

PB1583

CLINICAL EVOLUTION OF PATIENTS WITH NEWLY DIAGNOSED MYELODYSPLASTIC SYNDROME DEPENDING ON INITIAL TREATMENT STRATEGY. A REPORT FROM ERASME STUDY

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Background: Myelodysplastic syndromes (MDS) are a group of stem cell neoplastic disorders characterized by a very heterogeneous prognosis. New treatments have been included, but data from the general population on how these treatments are being used and their impact on the evolution remain scarce.
Aims: To describe the "real world" management of MDS and Chronic Myelomonocytic Leukemia (CMML) patients and to analyze the impact of the time to therapy initiation from diagnosis and the therapeutic strategy chosen on the clinical outcomes. Here we present an intermediate analysis with the data of the group of MDS patients included in the ERASME study.
Methods: The ERASME (CEL-SMD-2012-01) study is an observational, post-authorization, prospective, multicentre study that will include a total of 600 patients with MDS and CMML and follow them during three years or until death. Primary endpoint is to describe the disease progression, based on the time to therapy initiation from diagnosis and the initial therapeutic strategy chosen. The patients are classified in 1) low/Int-1 IPSS, 2) int-2/high IPSS and 3) CMML. The initial management of the patients is classified in Observation & support (SP) (including growth factors), active therapy (AT) (chemotherapy, azacitidine, lenalidomide, etc) and allogenic hematopoietic cell transplant (HCT). In addition, we will also collect data related to comorbidities, quality of life and healthcare resource utilization. Data from the pre-specified interim analysis are presented.
Results: A total of 120 patients (48% women) with a median age of 76 years (range 37-88 years) have been recruited between January-October 2013. For

MDS patients the diagnosis were refractory cytopenia (RC) 6%, RC with ringed sideroblasts 6%, RC with multi-lineage dysplasia 39%, refractory anemia with excess of blast (RAEB-1) 15%, RAEB-2 27%, MDS with isolated del 5q 3% and unclassifiable MDS 4%. Group-1 included 68 patients (57%) and group-2 52 (43%). The median time to AT initiation from diagnosis was 24 and 11.6 weeks in group-1 (n=10) and group-2 (n=1), respectively. The IPSS-R was very low/low/intermediate/high/very high in 21/12/15/40/12% of MDS patients, respectively. The median follow-up time was 4 months. After diagnosis, 57/31/12% of patients were considered candidate to SP, AT and HCT strategy, respectively; the picture for group-1, group-2 was 87/9/4% and 17/60/23% for SP/AT/HCT strategies, respectively. The reasons for observation after diagnosis (SP; n=68) were the risk-disease in 90% of patients, symptomatology of pathology in 71%, patient's age in 57%, and comorbidities in 46%. Of those patients considered candidate for AT (n=37), 76% and 11% received azacitidine or AML-like chemotherapy/AZA, respectively. Fifteen out of 120 patients (13%) were considered candidate for HCT, moreover 1, 4, and 10 of these HCT patients were considered to receive transplant front line (without prior treatment), after AML-like chemotherapy or after azacitidine, respectively. Six of 15 HCT patients have already received transplant, 2 patients died before transplant and 7 are still waiting for the transplant. In the last follow-up, 19 patients have died (16%) after a median of 4.2 months and 6 patients developed AML after 4.2 months. For the MDS cohort, the leukemia free survival for the SP therapies was 9.03 months and the overall survival for AC/TPH therapies were 7.43/6.60 months, respectively.

Summary and Conclusions: MDS patients were treated on an individualized therapy strategy, risk-adapted basis after diagnostic evaluation and prognosis assessment. Most patients with higher risk MDS were considered candidate for AT while most patients in the lower risk group received supportive therapy.

PB1584

ICE INDUCTION PLUS AZA MAINTENANCE AS A FIRST LINE THERAPY IN HIGH RISK MDS PATIENTS: CETLAM MDS-09 RESULTS.

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Background: Myelodysplastyc syndrome is a group of clonal hematologic malignancies that evolves heterogeneously, being transformation to AML and comorbidities related to prolonged cytopeniathe most shocking features.Thus, high-risk MDS patients have an overall survival median of 12 months. What is more, the ALO-HSCT has become the only curative option. Under the CETLAM MDS-09 protocol, different strategies to improve high-risk MDS treatment and outcome were approached, considering the use of polychemotherapy followed by azacitidine for those patients without available donor or no HSCT candidates.

Aims: Assess treatment efficacy in terms of Overall Survival (OS) and Event Free Survival (EFS)in high-risk MDS patients.

Methods: Since May 2009 to date, patients diagnosed with MDS (accordingly to WHO classification, 2008) from 15 CETLAM hospitals have been studied: Patients without comorbidities, good performance status and no HSCT candidates have been stratified according to cytogenetics. Induction with ICE (Idarrubicin 10 mg/m^2 IV days 1, 3 and 5, Cytarabin 100mg/m^{2}IV days 1 to 7, Etoposide 100mg/m^2 IV days 1 to 3) followed by 5-AZA maintenance (5-AZA 75 mg/m^2 x 5 days every 28 days) were administered to those with good karyotype. Eastern Cooperative Oncology Group (ECOG) Performance Status and Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) were used to assess patient status and the International Prognosis System (IPSS) for MDS to calculate the risk. IBM SPSS© was used for descriptive analysis, performing survival analysis with Kaplan Meyer method.

Results: We have studied 28 patients, 23 men (82.1%) and 5 women (17.9%), with a median age of 66.7 years (40.6-75.9). According to WHO MDS classification, there were 3 RCDM (10.7%), 1 RCDM-RS (3.6%), 1 RAEB-I (3.6%), 10 RAEB-II (35.7%) and 3 MDS-U (3.6%). Also 10 secondary AML (35.7%) were studied (6 of them with a blast count >30%). Following the IPSS, we have 8 high-risk patients (28.6%), 10 intermediate-II (35.7%), 4 intermediate-I (14.3%) and 6 (21.4%) unclassifiable to have a blast count more than 30%. Regarding patient status, 27 (96.4%) had ECOG ≤2 and 23 (82.1%) an HCT-CI score ≤3. The overall survival (OS) median was 17.66 months (CI 95%:11.47-23.85) with a follow up median of 13.83 months (0.99-45.53). The event free survival (EFS) was 11.51 months (CI 95%:4.92-18.09). The median of AZA cycles was 4.5 (0-27). The Overall Response Rate (ORR) achieved after ICE induction was 71.4% and the ORR after 6 cycles of AZA maintenance was 55%. 5 patients (17.9%) had treatment failure after ICE and 2 (7.1%) died during ICE induction, both of them due to infectious events. One patient had Stable disease after ICE (3.6%).

Summary and Conclusions: Despite having a short follow up and a relatively small sample, it seems to be a benefit of receiving ICE induction in terms of prolonging OS in this set of patients.

PB1585

AZACITIDINE IMPROVES SURVIVAL IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES WITH 20-30% BONE MARROW BLASTS AND POOR-RISK CYTOGENETIC CATEGOR

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Background: Karyotype is a key prognostic factor in MDS and AML. Azacitidine (AZA) has shown survival advantage in high-risk MDS and AML (20%>30% blasts) in a randomized phase III trial. However, data on outcome of MDS and AML (20-30% blasts) with adverse karyotype treated with AZA and compared with conventional regimens are scarce.

Aims: To evaluate the role of AZA in MDS and AML with myelodysplasia-related changes (20-30% blasts) with poor-risk cytogenetic categories in an unselect cohort of patients (pts) treated with AZA or conventional care regimens.

Methods: Forty pts were retrospectively analyzed (MDS, n=32. AML, n=8). Karyotype was reported according to ISCN. Analysis included pts diagnosed from 2000 to 2013 and those with cytogenetic categories of poor (IPSS) or poor/very poor (IPSS-R). Patients were divided into two groups: Those who received AZA (AZA group, from 2009 to present; n=23, including: RCMD=5, RAEB-2=13, AML=5) and those who did not (non-AZA group, ECOG≤2,prior to AZA approval, from 2000 to 2009; n=17. RCMD=6, RAEB-1=4, RAEB-2=4, CMML=2, AML=3).Non-AZA group included intensive chemotherapy and non-intensive approaches (low-dose ARA-C). Response to therapy was defined by IWG MDS 2006 criteria.

Results: There were no differences between both groups in terms of WHO subtype, age, bone marrow (BM) blast% and distribution of pts with chromosome 3 abnormality (abn) or having ≥3 chromosomal abn and pts with monosomal karyotype (MK) *versus* any other adverse category, although a trend to higher BM blast% and chromosome 3 abn/≥3 abn was noticed in the AZA group as compared to non-AZA cohort (14% *vs* 6% and 73% *vs* 53%, respectively). At last follow-up, 10/40 (25%) patients are alive. Median OS was 14 months for AZA pts *vs* 6 months for non-AZA (*P*=0.07). Median OS for responding patients to AZA was 20 months (12.9-27). Overall response rate (CR/marrow CR/PR) was 56% (CR=47%, mCR=8%) in the AZA group. Interestingly, 41% of pts with chromosome 3 abn/≥3 chromosomal abn and 45% of pts with MK achieved CR with AZA, with median OS for responders of 16 and 14 months in each category, respectively. Four pts from each cohort underwent SCT. In the multivariable Cox proportional hazard model including SCT as a time-dependent covariate (Table 1), only treatment with AZA significantly influenced on survival (HR 0.33, 95% CI 0.146-0.973;*P*=0.04). A trend towards a better outcome was also observed in patients treated with IC (P=0.08). Receiving SCT (time-dependent covariate) did not have impact on survival (*P*=0.15). Severe thrombocytopenia (<50x10e^9/L) was associated with a poor outcome (*P*=0.055; HR=0.860, 95% IC: 0.007-1.059).

Table 1.

Parameter	HR (95% CI)	P
SCT	1.08 (0.239-4.883)	0.9
AZA	0.33 (0.146-0.973)	0.04
IC	0.33 (0.96-1.177)	0.08
Abn3/>3 abn	1.06 (0.432-2.616)	0.8
Plat<50x10^9/L	0.33 (0.146-0.973)	0.05

Summary and Conclusions: These data suggests a potential benefit on overall survival for azacitidine in pts with MDS and AML with myelodysplasia-related changes and adverse karyotype. Severity of karyotypic abnormalities did not preclude the possibility of achieving response to AZA. Tolerability and no need for hospital admission may favor the clinical decision whether to offer this approach to pts aged >65 years with MDS or AML (20-30% blasts) with adverse cytogenetics.

PB1586

RISK FACTORS IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic Syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by peripheral-blood cytopenias and risk of progression to acute myeloid leukemia. The clinical, morphological and prognostic heterogeneity is not sufficiently addressed even in current classification systems.
Aims: We investigated the role of WT1 gene expression and its association with the expression of the chemokine receptor CXCR4 on bone marrow CD34+ cells of MDS patients.
Methods: From January 2007 to January 2014 BM samples from 73 MDS patients (according to WHO classification: 22 RA, 12 RCMD, 10 RAEB I, 8 RAEB II, 9 RARS, 10 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and every 6 months. WT1 gene expression was evaluated by methods of real-time quantitative PCR (RQ-PCR). Surface CXCR4 expression were measured flow cytometrically.
Results: At diagnosis, 29 BM samples (10 RA, 7 RCMD, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. A significant correlation was found between WT1 expression levels, blast cell percentage and CXCR4 over-expression on blast cells (as defined by CXCR4 mean fluorescence intensity ratio thresholds of more than 5). After 6 months, 9 patients (2 RA, 5 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 and CXCR4 expression and a further elevation of WT1 expression level after 6 months.
Summary and Conclusions: WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. A strong association is present between the level of WT1 expression and the blast percentage and the CXCR4 over-expression. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. Our results justify further investigation into the role of CXCR4 in MDS and suggest that WT1 and CXCR4 should be incorporated into the risk assessment of MDS patients.

PB1587

EPIDEMIOLOGY SURVEY, RESULTS FROM THE ARGENTINE MYELODYSPLASTIC SYNDROME REGISTRY. MDS STUDY GROUP OF THE ARGENTINE SOCIETY OF HEMATOLOGY

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Background: Little is known about the incidence and epidemiological patterns of Myelodysplastic Syndromes (MDS) in Latin American region. The requirement of high quality exchangeable data has become a priority all over the world.
Aims: To register MDS cases reported in our region, to review and analyze the data.
Methods: From January 2007 through February 2014, fourteen Argentine Hematology Centers reported 479 adult patients (pts). FAB and WHO classification were taken into account, as well as IPSS, WPSS, and IPSS-R. Informed Consent was required.
Results: Median age was 71 (range: 17-94), women 206 (43%), M/F ratio: 1.3, primary MDS (p-MDS) 425 (89%). FAB (422 evaluable pts), subtypes: RA 56%, RARS 7%, RAEB 17%, RAEB-T 6% and CMML 15% (Proliferative CMML 34%). WHO (355 pts): RCUD/RS 9%, 5q- 4%, RCMD 61%, RAEB-1 10%, and RAEB-2 16%. Median follow-up: 17 months, median OS: 43 months; deaths: 182 (40%), and Acute Myeloid Leukemia progression: 93 (19%).

Prognostic Scoring Systems	Results
IPSS (n=352)	L (43%), Int-1 (40%), Int-2 (12%), H (5%)
WPSS (n=308)	VL (29%), L (41%), I (22%), H + VH (8%)
IPSS-R (n=345)	VL (29%), L (40%), I (11%), H (11%), VH (8%)

Cytogenetic in p-MDS according to IPSS-R (377 pts) showed: VL 3%, L 76%, IM 13%, H 4% and VH 4%, and the percentage of altered cytogenetic studies in secondary MDS (s-MDS) was 46%. Myelofibrosis (MF) was evaluated in 374 pts: MF0+MF1 (88%) and MF2+MF3 (12%), with a median survival of 47 and 16 months, respectively (*p=0,004*). Treatment reported: EPO (43%), iron chelation (4%), hypometilanting agents (27%), and Bone Marrow Transplant (3%).
Summary and Conclusions: Although Argentine Registry is young with a relatively short follow-up, we were able to observe a high incidence of RA, CMML and a predominance of lower risk groups. There was a good correlation among different Scoring Systems, MDS subtypes and time to AML progression. In s-MDS, the number of altered cytogenetic studies was relatively low. The presence of Myelofibrosis was useful to predict survival. Erythropoietin and Hypomethylating agents were frequently used.

PB1588

COMPARATIVE ANALYSIS OF CLINICOPATHOLOGIC FEATURES IN HYPOPLASTIC MYELODYSPLASTIC SYNDROME AND APLASTIC ANEMIA: COULD THE TWO REPRESENT THE SAME DISEASE ENTITY?

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Background: Hypoplastic myelodysplastic syndrome (hMDS) has clinical and morphologic manifestations overlapped with those of aplastic anemia (AA). In the majority of cases, diagnostic distinction between hMDS and AA is difficult, and often impossible. Overt MDS or acute myeloid leukemia (AML) has been seen in patients treated for AA. The pathogenesis is currently unclear.
Aims: To investigate the clinicopathologic distinction between hMDS and AA.
Methods: Eighteen cases of hMDS and 9 cases of MDS/AML secondary to AA (sMDS/AML) were identified in our databases from 2002-2012, and were retrospectively analyzed for clinical presentations and pathologic features, in comparison with 59 cases of AA.

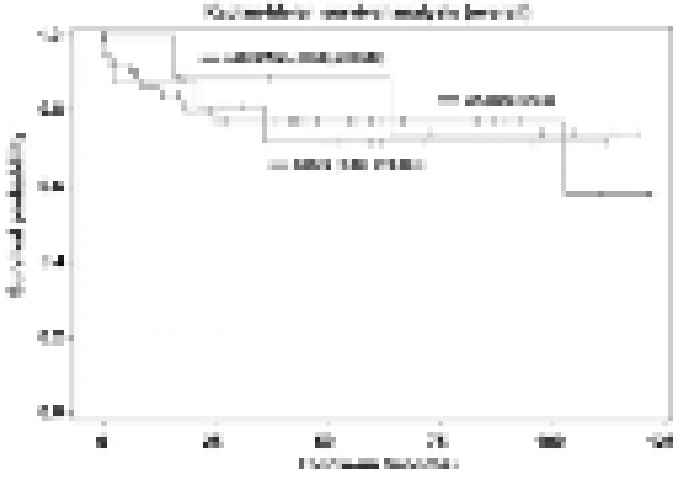

Figure 1.

Results: At diagnosis, the median age was 49.5 years (range, 5-87) for hMDS, 47 years (16-72) for sMDS/AML and 41 years (2-99) for AA. The male/female ratios were 1:1, 2:1 and 3:2, respectively, for the 3 groups. Among the 3 groups, the clinical presentations were similar with the majority manifesting anemia-related symptoms and laboratory evidence of moderate-severe pancytopenia. The morphologic features of initial marrow biopsies were indistinguishable among the three, all showing markedly decreased cellularity with hematopoietic hypoplasia. Cytogenetic study was performed in 17/18 hMDSs, 8/9 sMDS/AMLs and 48/59 AAs. Clonal cytogenetic abnormalities were detected in 16/17 (94.1%) hMDSs, while all cases of AA showed no abnormal changes. All the 8 cases of sMDS/AML demonstrated no cytogenetic abnormalities, and were initially diagnosed as AA. Due to similar clinical presentations, all the patients received conservative therapy at the beginning, primarily with immunosuppressive therapy (IST), until confirmed refractoriness and worsening

pancytopenia, when nonmyeloablative stem cell transplant (SCT) was considered. Eventually, SCT was given to 17/55 (30.9%) patients in AA, 7/18 (38.9%) in hMDS and 3/9 (33.3%) in sMDS/AML. For 9 patients who later developed sMDS/AML, the median interval between the diagnoses of AA and sMDS was 27.5 months (8-77), and the one between AA and sAML was 12 months (12-16). Interestingly, -7/-7q constituted 55.6% (5/9) of cytogenetic abnormalities in sMDS/AML, the proportion similar to that in hMDS (8/17 [47.1%]). After further treatment, 2/9 (22.2%) patients died in sMDS/AML group, with median follow-up of 37 months (6-86), compared to 4/14 (28.6%) in hMDS (median follow-up of 36 months [0.5-112]) and 12/55 (21.8%) of AA (median follow-up of 26 months [0.23-122]). Survival analysis demonstrated no significant difference between AA and hMDS as well as between hMDS and sMDS/AML (Figure 1).

Summary and Conclusions: Because of no distinguishable clinical and morphologic features between hMDS from AA, the diagnosis of hMDS is largely relied on cytogenetic analysis. About 50% of diagnostic hMDS harbor -7/-7q, the feature distinct from other de novo MDS. AA (13.4% in our series) may transform to overt MDS/AML 1-6 years after the diagnosis. While the role of treatment in the transformation is currently unclear, it may be a natural course of AA given a similar composition of cytogenetic abnormalities in sMDS/AML and hMDS. Due to the significantly overlapped pathologic features and similar clinical outcome, AA and hMDS may represent the same disease entity with the majority manifesting indolently with IST and a minority evolved to an aggressive course. While close monitoring of "AA" with cytogenetic study may be justifiable at this time, an early distinction between the two in many cases need identifying additional clonal markers because of the limitation of cytogenetic analysis, particularly in this disease group.

PB1589

BIOSIMILAR EPOETIN IN ELDERLY PATIENTS WITH LOW-RISK/INTERMEDIATE 1 MYELODYSPLASTIC SYNDROMES IMPROVES ANEMIA, QUALITY OF LIFE AND BRAIN FUNCTION

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Background: Myelodysplastic syndromes (MDS) are a group of clonal myeloid disorders characterized by ineffective hematopoiesis with progressive cytopenia and a variable risk of transformation to acute myeloid leukemia (AML). MDS primarily affects elderly patients and its annual incidence is 5 cases per 100,000. Cytopenia and its complications (fatigue, infections, bleeding), the risk of disease progression, and patients' age and co-morbidities can all affect patients' physical and psychological status. Quality of life in MDS is compromised by functional and disease-specific components and has been found to be associated with hemoglobin (Hb) levels and with transfusion dependency. Transfusion dependence is associated with reduced survival in patients with lower-risk MDS due to iron overload, cardiac failure, and progression to myeloid leukemia. Erythroid-stimulating agents (ESAs) have two potentially useful actions in the treatment of anaemia in the setting of MDS syndromes: correcting anaemia to improve QOL and avoiding/reducing the need for transfusions. However, some MDS patients are refractory to this treatment and little is known about the exact molecular mechanisms underlying the effect of ESAs in these subjects. A biosimilar epoetin α was approved by the European Medicines Agency (EMA) in 2007, and is marketed as Binocrit®. Binocrit was developed using Eprex/Erypo (the Janssen Cilag recombinant erythropoietin α authorized in the EU in 1994) as the reference product. Comparable efficacy was confirmed in treatment of anemia by administration of intravenous Binocrit in 478 chronic renal failure patients receiving hemodialysis . Emerging data suggest that Binocrit is effective and safe for the treatment of patients with cancer and chemotherapy-induced anaemia.

Aims: To evaluate the effectiveness of a biosimilar epoetin α (Binocrit) for the treatment of anemia in low/intermediate 1 risk MDS patients and to evaluate the impact of ESAs on QOL.

Methods: we studied a group of 24 consecutive MDS patients aged over 65 years and treated with Bio similar epoetin α (40,000 IU once a week). 14 patients were male and 10 patients were female. The average age was 72 years (range 65- 84 years). Criteria for eligibility consisted of adult age, newly diagnosed MDS, International Prognostic Scoring System (IPSS) score<2 and at least one cytopenia. 15 patients had refractory anemia (RA), 5 refractory cytopenia with multilineage dysplasia with or without ringed sideroblasts (RCMD±RS), 1 patient had refractory anemia with excess of blasts type I (RAEB I), and 3 patients had refractory anemia with ringed sideroblasts (RARS). All patients received biosimilar epoetin α at a fixed dose of 40,000 IU once a week subcutaneously for a minimum of 12 weeks. Responsive patients continued with 40,000 IU/week for further 12 weeks. Changes in QOL were assessed by the Functional Assessment of Cancer Therapy-Anemia (FACT-An). Cognitive function was assessed using a psychometric test, the mini-mental state examination (MMSE).

Results: All patients completed 12 weeks of therapy. Sixteen patients (66.67%) achieved an erythroid response (ER), 14 patients (58.33%) achieved a major response, while two patients had a minor response (8.33%). Briefly, in order to classify groups of patients according to international response criteria, patients'response was defined as "major" when Hb increased more than 2 g/dL for patients with pretreatment Hb values smaller than 11 g/dL or, in red blood transfusion-dependent patients, when they reached transfusion independence. By contrast, response was defined as "minor", when Hb increased 1 to 2 g/dL in patients with pretreatment Hb values smaller than 11 g/dL and, for red blood cell transfusion-dependent patients, when transfusion requirements decreased by a rate of 50% [21]. Non-responders were considered patients who achieved neither major or minor response Eight patients were non-responders (33%), of whom 5 patients were low risk and 3 intermediate I risk. Six responders were low risk, and 10 were intermediate-1 risk. Mean hemoglobin (Hb) values were significantly higher after treatment in both responders ($p<.001$) and non-responders ($p_s<.03$). ER was maintained after 24 weeks. Statistically significant positive correlations between improvement in Hb and variations in patients' mini-mental (Spearman's Rho = .54 $p<.01$) and FACT-An scores (Spearman's Rho = .59, $p<.003$) were demonstrated

Summary and Conclusions: our study gives preliminary encouraging data showing that Biosimilar epoetin α has comparable efficacy to that of other ESAs in the treatment of anaemia in MDS patients. These data should be confirmed with studies enrolling a larger number of patients, and with a direct comparison with originator ESA. Use of biosimilar ESAs can provide savings in hospital budgets, expand access to ESA treatment, and provide physicians with the latitude to treat anaemia earlier (in accordance with current labels and guidelines) Patient responding to Biosimilar epoetin α have similar clinic characteristics to MDS patients who respond to originator products, and similarly they could maintain sustained responses to treatment

PB1590

ACUTE MYELOID LEUKEMIA PROGRESSION IN MYELODYSPLASTIC SYNDROMES, RESULTS FROM THE ARGENTINE REGISTRY

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Background: At least, one third of patients with Myelodysplastic Syndromes (MDS) develops Acute Myeloid Leukemia (AML) at any time of the follow-up. This subgroup of patients depict clinical and laboratory features, specific MDS subtype, and especially poor prognosis.

Aims: To describe and analyze clinical features of adult MDS patients that had progressed to AML

Methods: This is a multicenter retrospective study of 197 (23%) adult MDS patients that progressed to secondary AML (s-AML), from a database of 840 patients diagnosed from 1981 to 2013. Data belong to the MDS Registry sponsored by the Argentinean Society of Hematology and to a previous multicenter study. Informed Consent was required. Patients were classified following FAB and WHO criteria and 13 patients presented with secondary MDS. The median age was 64 (17-89) years with 74% above 60 years, a male/female (123/74) ratio of 1.7. During the follow-up, with a median overall survival of 14.3 months, 173 (88%) died. BM transplanted patients were censored till the moment of the procedure. Using a log-rank test and *Kaplan-Meier*, we performed a univariate analysis to examine the effects of age at MDS diagnosis (limit of 70 years), gender, haematological parameters and, IPSS, WPSS and IPSS-R scores on time to Leukemia transformation (TTL) and on the overall survival (OS).

Results: FAB classification was evaluable in 184 patients distributed into: RA 37 (20%), RARS 7 (4%), RAEB 71 (39%), RAEB-T 39 (21%), CMML 30 (16%).

WHO classification was evaluable in 134 patients: RCUD/5q- 10 (7%), RCMD 34 (25%), RAEB-1 33 (25%), and RAEB-2 57 (43%). Age (limit of 70 years), percentage of bone marrow blast (0-<5, 5-10, 11-20, >20), hemoglobin level (limit of 10 g/dL), platelets (limit of 100000 /µL) and neutrophil count (limit of 800 /µL), LDH level, cytogenetic group of risk (according to the IPSS-R), and red blood cell transfusion requirements were significant predictive variables for prognosis (Kaplan-Meier and Long-Rank test, p<0.05). According to our database, most patients received supportive care with vitaminotherapy; erytroid and neutrophil stimulating factors, and 158 required red blood cells transfusion support. Chemotherapy was administered once the leukemic phase of the disease was diagnosed (68 patients) or for stem cell transplant (14 patients), hypomethylating agents were administered in 66 patients, and 2 lenalidomide.
Summary and Conclusions: Our multicenter Argentinean cohort showed a rate of 23.4% of patients who progress to s-AML, similar to previous reports. Clinical parameters, age, classifications and the applied scoring systems were useful tools to evaluate prognosis in our series. As expected, we observed a higher gender ratio, a lower median age and an increased incidence of higher risk groups, but the shorter time to AML in lower risk group will be matter of future studies.

Table 1.

PB1591

EFFICACY OF PSYCHOLOGICAL NURSING ON MYELODYSPLASTIC SYNDROME

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Background: Patients with myelodysplastic syndrome always have much anxiety.Anxiety can affect the therapeutic efficacy.Psychological nursing is needed on the baisis of traditional nursing.Psychological nursing supplies patients with psychological intervention to alleviate anxiety.Patients with less anxiety can have a good therapeutic alliance.
Aims: To analyze the efficacy of psychological nursing on myelodysplastic syndrome.
Methods: 192 myelodysplastic syndrome patients were divided randomly into 2 groups:psychological nursing group(A group) 85 cases and control group(B group) 97 cases.A group accepted routine nursing combinating with psychological nursing.B group accepted routine nursing. Analyze HAMA scores of each group respectively between prior treatment and aftrer 10 days of treatment and compare HAMS scores of two groups prior treatment and aftrer 10 days of treatment.
Results: Prior treatment,HAMA score of A group 19.37±6.73 points and HAMA score of B group 18.33±5.64 points,had no significant differences.After 10 days of treatment,HAMA score of A group 11.19±3.63 points and HAMA score of B group 14.52±4.10 points,had signicant differences.A group and B group both had significant differences between prior treatment and posttreatment.
Summary and Conclusions: Psychological nursing can ameliorate anxiety of myelodysplastic syndrome patients and should be advocated.

PB1592

HEAVY SMOKERS HAVE HIGHER FREQUENCY OF LEUKEMIC TRANSFORMATION THAN LIGHT SMOKERS IN PATIENTS DIAGNOSED WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal hematological disorder that is classified by the World Health Organization (WHO) as one of the myelodysplastic (MDS)/ myeloproliferative (MPN) overlap diseases. Smoking has been found to cause damage to somatic and germ cells, and in some studies smoking was defined as a risk factor to develop MDS. Even more recently, smoking appeared to have negative impact on overall survival (OS) in patients (pts) with low-risk MDS, but its impact in CMML is still unknown.
Aims: To study the effect of smoking on clinical outcome for patients diagnosed with CMML.
Methods: A single institution, retrospective study through chart review of all cases diagnosed with CMML at Mayo Clinic Rochester between 1994 to 2011 was performed. Smoking was classified into never/ ever smoker patients, all demographic information were obtained from the patients' medical records. Appropriate IRB approval was obtained in accordance with the Helsinki declaration. Comparison between groups' medians was done using Wilcoxon test, while survival estimates were calculated using Kaplan-Meier curves using JMP V9.
Results: Two-hundred and thirty five pts (out of 270) were found to be eligible, of which 53% (124 pts) were identified as ever-smoker (103 (44%) former smoker, 21(9%) current). Median age was 72 years, 68% of which were males. Median hemoglobin was 11 gm/dL, white blood cells (WBC) 12 x 10^9/L, platelets were 88 x 10^9/L, peripheral blood (PB) blasts 0, and bone marrow (BM) blasts 3%. Sixty five percent (148 patients) had diploid cytogenetics (CG). CMML2 was seen in 10% of group. Leukemic transformation (LT) to acute myeloid leukemia was documented in 32 pts (14%). Median OS was 534 days with a median follow-up was 311 days. No statistical difference was found upon comparison of never-smoker pts (group 1) to ever-smoker pts (group 2): Median age was 72 *vs* 71 years, hemoglobin 11 *vs* 10 gm/dL, WBC 12 x 10^9/L (both groups), platelets 95 *vs* 84 x10^9/L, PB blasts 0 (both groups), and BM blasts were 4% *vs* 3%, respectively. CG was diploid in 65% *vs* 35%, respectively. Males were more frequent in group 2 *vs* 1 (81% *vs* 53%, p <0.0001). Median overall survival (OS) was found to be similar in both groups (515 day *vs* 565 days, respectively (p=0.8)). There was no statistically significant difference for LT between group 1 *vs* 2 (16% *vs* 11%). On multivariate analysis, smoking (in addition to PB and BM blast) did not affect mOS, while (age, hemoglobin, and platelets) did impact mOS. Among the 124 ever-smoker pts, heavy smokers (HS) (≥ 1 pack/day) were found in 65% (33 pts), while light smokers (LS) (< 1 pack/day) were found in 35% (18 pts). LT was significantly higher in HS *vs* LS (21% *vs* 0, p=0.01), but mOS was not different (480 *vs* 534 days, p=0.8).
Summary and Conclusions: Smoking was found in about half pts diagnosed with CMML (53%), with more males being smokers than females. However, smoking did not affect pts characteristics and did not impact overall survival compared to never-smoker. Upon comparison with light smokers, heavy smokers had higher frequency of LT but did not affect mOS. More studies are needed to study effect of smoking on leukemic pts.

PB1593

PHARMACOKINETICS AND PRECLINICAL ACTIVITIES OF PRACINOSTAT AND ACTIVE METABOLITE SB991

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Background: Pracinostat (SB939) is an orally available histone deacetylase (HDAC) inhibitor being developed for hematologic malignancies including myelodysplatic syndrome (MDS) and acute myeloid leukemia (AML). SB991, an N-de-ethylation of Pracinostat, was shown to form as the primary metabolite following Pracinostat incubation with human, dog, rat and mouse microsomes.
Aims: To compare the exposure between Pracinostat and SB991 following oral Pracinostat administration in humans and dogs as well as the *in vitro* bioactivity of each compound.
Methods: Dogs received Pracinostat (10 mg/kg) via oral gavage, and PK samples were obtained 5 min, 15min, 30min, 1h, 2h, 4h, 6h, 8h, and 24h. Plasma was analyzed for Pracinostat and SB991 using an HPLC bioanalytical method. The HDAC inhibitory activities of Pracinostat and SB991 were measured *in vitro* using purified recombinant HDAC 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 enzymes. Functional HDAC inhibition was assessed by measuring DNMT1 protein (a measure of HDAC1 activity) and acetylated alpha-tubulin (a measure of HDAC6 activity) in MDA-MB-231 (human breast adenocarcinoma) cells by western blot. Humans (healthy volunteers) received Pracinostat 60 mg orally following an overnight fast, and PK samples were collected pre-dose and at 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours postdose. Plasma samples were analyzed for Pracinostat and SB991 using a validated bioanalytical method.
Results: SB991 displayed greater inhibitory activity than Pracinostat against all HDACs tested (Table 1). In addition, SB991 displayed greater bioactivity in MDA-MB-231 cells than Pracinostat as measured by a decrease in DNMT1 protein levels and increase in acetylated alpha-tubulin by western blot. Analysis of dog plasma samples showed SB991 to be formed by metabolism of Pracinostat after oral administration (Figure1). Non-compartmental pharmacokinetic analyses indicated an $AUC_{0\text{-}inf}$ of 6,900 ng*h/ml for Pracinostat and 13,700 ng*h/ml for SB991. C_{max} was 2,200 ng/ml for Pracinostat and 3,000 ng/ml for SB991. The human healthy volunteer study is currently underway and results will be reported.

Table 1. IC50 of Pracinostat and SB991 against a panel of HDACs.

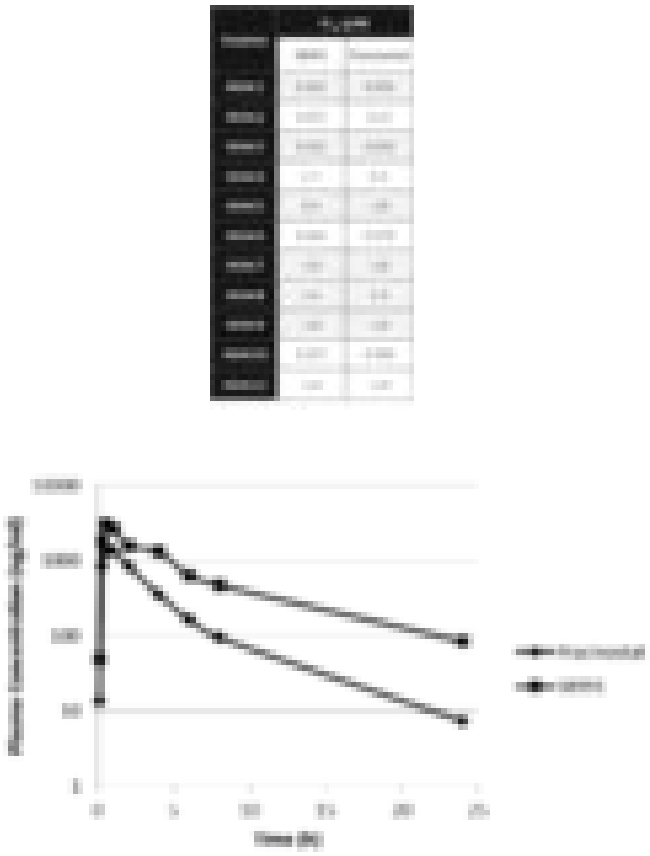

Figure 1. Plot of plasma concentration *vs* time for Pracinostat and SB991 after oral administration of 10 mg/kg Pracinostat to beagle dogs.

Summary and Conclusions: SB991 is a more potent HDACi than Pracinostat. In addition, the combined *in vivo* levels of Pracinostat and SB991 in dogs indicated a much greater exposure to HDACi than would be expected based on levels of Pracinostat alone. This increased exposure may distinguish Pracinostat from other HDACi under development. Thus, when considering the potential clinical efficacy of Pracinostat as a function of potency and exposure, it is valuable to consider the combined exposures to Pracinostat and SB991, rather than exposure to Pracinostat alone.

PB1594

DETECTION OF NUCLEOPHOSMIN 1 (NPM1) MUTATIONS IN MYELODYSPLASTIC SYNDROMES AND MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS: STILL A DIAGNOSTIC DILEMMA

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Background: De novo acute myeloid leukemia (AML) carrying mutations of the nucleophosmin 1 (NPM1) gene account for about 30% of all cases, 50-60% of AML cases with normal karyotype (NK), whereas NPM1 mutations are less frequently observed in secondary AML, arising from either myelodysplastic syndromes (MDS) or myelodysplastic/myeloproliferative neoplasms (MDS/MPN), ranging from 8% to 17% of the cases. Furthermore, NPM1 mutations are rarely found in patients diagnosed with either MDS or MDS/MPN (2%>4% of the cases), but scanty information is reported about immunohistochemical (IHC) examination for NPM1 in this clinico-pathologic setting.
Aims: We describe two cases observed at our Institution, with peripheral blood and bone marrow (BM) myeloid blast count <20%. Patient 1, a 57-year old woman, was initially diagnosed with atypical chronic myeloid leukemia, BCR-ABL negative, with NK. Patient 2, a 47-year old woman, was initially diagnosed with MDS/MPN-unclassifiable, with NK. Surprisingly, in both patients NPM1 mutation was documented by PCR analysis, in the absence of any other molecular abnormality. The IHC examination of BM trephine biopsy documented cytoplasmic NPM1 (NPMc+) staining in more than 20% of cells, so that, in both patients, AML with mutated NPM1 was finally diagnosed and treated with intensive chemotherapy, obtaining complete remission and subsequent favorable outcome. Based upon these observations, we have retrospectively analyzed 175 further adults with either MDS or MDS/MPN, consecutively observed over a period of 6 years (2008-2013). Overall, BM aspirate samples were available for molecular analysis to investigate the presence of NPM1 mutations in 135 cases (76.3%). For patients with NPM1 mutations, subcellular localization of NPM1 was investigated by IHC analysis, performed on BM trephine biopsy.
Results: A total of 177 patients, namely 140 MDS and 37 MDS/MPN (29 CMML) cases, 109 males and 68 females, of median age 77 years (range 47-93 years) were analyzed. BM aspirate and trephine biopsy morphologic examinations were available for 167 (94.4%) and 136 (76.8%) cases, respectively. Cytogenetic analyses were performed in 151 patients and showed NK in 79 cases (52.3%), while chromosomal abnormalities were documented in 71 cases (47.7%). Including patients 1 and 2, NPM1 mutations were observed in 4 out the 135 (3%) BM aspirates available for the molecular analysis. The IHC examinations, retrospectively performed on BM trephine biopsies from the two further elderly patients with RAEB-2 (patients 3 and 4, observed in 2010 and 2012, respectively), in whom NPM1 mutation was observed, documented NPMc+ staining in more than 20% of cells, suggesting AML with mutated NPM1. Of note, patient 3, a 79-year old man, developed AML after having received 6 cycle of 5-azacytidine, whereas patient 4, a 85-year old woman with pancytopenia, received best supportive care only.
Summary and Conclusions: According to Falini *et al.* (Blood 2007;109:874-85), caution is needed when defining a case as NPM1-mutated MDS, since NPMc+ AML frequently shows multilineage involvement and dysplastic features. Moreover, MDS and MDS/MPN cases with NPM1 mutation frequently progress to AML, within 0.5 to 14 months since diagnosis. The results of our study suggest that in the rare cases of either MDS or MDS/MPN with a blast count invariably <20%, but with NPM1 mutation on molecular analysis, AML may be under-diagnosed. IHC examination may be the method of choice to precisely evaluate the percentage of BM cells with NPMc+ staining, thus reaching a definite NPMc+ AML diagnosis.

PB1595

ASSESSMENT OF MORTALITY EVENTS IN MYELODYSPLASTIC SYNDROME (MDS): EXPERIENCE IN A SPANISH FACILITY.

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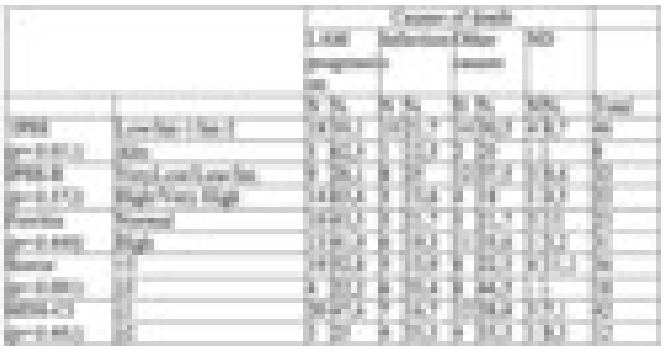
Background: MDS is a haematological malignant disorder characterized by a low life expectancy and high morbidity and mortality rates. Disease evolution and intensive chemotherapy strategies can lead to prolonged immunosuppression status that increase death related to infectious events. What is more, high-risk MDS patients have a high leukemia transformation rate.
Aims: Our goal is to assess different mortality events in MDS patients at our department.
Methods: From January 2002 to October 2013, data of all patients diagnosed with MDS in Son Llàtzer Hospital were collected. We used SPSS v.19 © statistics for descriptive statistical analysis (c^2 test to determine statistical relationships between main variables) and Kaplan -Meier for survival analysis. Different overall mortality causes were analysed according to prognostic groups IPSS and IPSS-R, comorbidity indexes (Sorror, MDS-CI) and ferritin levels.
Results: Of 112 patients, 96 were eligible for analysis: 57 men (59.4%) and 39 women (40.6%) with a median age of 77 years (33-94). Median follow-up was 28.3 months (1.3 to 120.9). The median overall survival was 41.8 months (95% CI: 34.39-49.22). The cumulative incidence of death at 3 years was 40% and 67% at 5 years. At the time of analysis 54 deaths (56.3%) were reported. The most common cause of death was LAM progression in 42.6% of cases (23 patients), with time to transformation median of 28.8 months. Secondly, infections in 20.4% (11 patients) followed by bleeding in 7.5% (3 patients had CNS haemorrhage and 1 patient had gastrointestinal bleeding secondary to angiodysplasia). The other causes were heart disease 9.3% (5 patients), other causes 9.3% (5 patients: 2 had liver disease with HCV positive serology, one had lower limb gangrene, 1 severe chronic obstructive pulmonary disease (COPD) and 1 bladder cancer) and toxicity procedure-related in 3.7% (2 patients who underwent allogeneic transplant). Regarding infectious events, 2 cases were bacterial sepsis (*Escherichia coli* and polymicrobial), one was fungal infection by *Aspergillus sp.* and no microbiologically documented infections in 8 patients. A statistically significant relationship between death causes in relation to prognosis IPSS groups (p=0.011) was observed and nearly significant regarding SORROR index (p=0.091). Moreover, no statistically

significant differences between the IPSS-R (p=0.372), MDS-CI (p=0.461) prognostic groups and ferritin levels (p=0.446) were observed (Table 1).

Table 1.



Summary and Conclusions: The most common death cause in our series was AML progression, followed by infections. Although the number of patients is small, it appears to be a tendency to die due AML transformation in adverse prognostic patients. In better prognosis subgroups it was observed an increased mortality related to infections, mainly secondary to immunosuppression or other comorbidities.

PB1596

SAFETY PROFILE OF AZACITIDINE IN THE TREATMENT OF THERAPY-RELATED MYELODYSPLASTIC SYNDROMES/ACUTE MYELOID LEUKAEMIA

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Background: The improvement of chemotherapeutic treatments for solid and haematological malignancies is leading to the observation of an ever-increasing number of therapy-related Myelodysplastic syndromes/Acute myeloid leukaemia (t-MDS/AML). A few recent studies have documented the efficacy of 5-azacytidine (AZA) in patients with t-MDS/AML. A comparable overall response rate has been reported between t-MDS/AML and *de novo* MDS/AML, but the former leads to inferior overall survival.
Aims: The aim of the present study was to supply data on the safety profile of AZA in t-MDS/AML.
Methods: We retrospectively collected clinical data from consecutive patients (aged ≥ 18 years old) with t-MDS/AML treated with AZA at 3 Hematology Centers. Patients received at least 2 cycles of AZA.
Results: Seventeen patients with t-MDS (n.13)/AML (n.4) (median age 68, range 44-79, M/F 7/10) were retrospectively selected from August 2006. Previous diagnosis was of solid malignancy for 10 patients, hematologic malignancy for 6 (non-Hodgkin lymphoma n.5, Chronic myelomonocytic leukaemia n.1) and amyloidosis for 1 patient. The median number of chemotherapy courses was 1 (range 1-5); four patients received high dose chemotherapy and autologous stem cell transplant; five patients also received radiotherapy. Underlying malignancy was still active in 5 patients when AZA was started. Eight patients (47%) were transfusion-dependent. The cytogenetic risk, known for 14 patients, was: good for 6 (42.8%), intermediate for 1 (7.1%), poor for 7 (50%). AZA was administered subcutaneously at the dosing of 75 mg/m^2/day for 7 days (schedule 5-2-2) every 28 days. The median number of administered cycles was 5 (range 2-22). None of the patients underwent allogeneic stem cell transplant. AZA was discontinued due to progression of the solid tumor (n.2, 11.7%), treatment failure (n.3, 17.6%), AE (infection) (n.5, 29.4%), death due to ischemic events (n.2, 11.7%) or respiratory failure (n.1, 5.8%). AZA is ongoing in 4 patients (23.5%). Hemoglobin reduction ≥ 1g/dL from baseline and not imputable to disease activity was reported in 58.8% of patients and was observed mainly in the first cycles (1-4). ANC reduction was present in 70.5% (G4 41.1%). 5/17 patients (29.4%) experienced a severe infection, which for 4/5 was fatal: 3/5 presented progressive MDS (1 also with active CLL); 1 with refractory amyloidosis previously treated with autologous SCT; 2/5 died in complete remission, 1 of them had received high dose chemotherapy and autologous SCT. Platelet reduction was observed in 52.9%. A grade 2 bleeding occurred in 2 patients (11.7%). Gastrointestinal toxicity (nausea, constipation, diarrhea) grade 1-2 occurred in 4 patients (23.5%). At the intermediate evaluation (4-6 months), response assessment was possible in 13 patients (76.4%) and we observed: 2 CR (15.3%), 3 PR (23%), 3 SD (23%), and 5 failure (38.4%). The median overall survival was 6 months (range 3-24).
Summary and Conclusions: Hematological and extra-hematological toxicity was comparable to that described in phase III studies for *de novo* MDS. The life-threatening infections mostly occurred in patients who failed to respond to AZA or were heavily pre-treated for a primary malignancy. Our OS resulted inferior in comparison to other reports, possibly due to the inclusion of patients with active primary malignancy. AZA could be a reasonable choice for the treatment of these patients, but larger studies are desirable.

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REAL LIFE EXPERIENCE OF AZACITIDINE USE FOR A VARIOUS MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA IN A COMMUNITY HOSPITAL. A UK SINGLE CENTRE RETROSPECTIVE STUDY

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Background: The real life practicality of using Azacitidine has been poorly reported.
Aims: The aim of this study was to analyse efficacy and practicality of Azacitadine in 41 patients with MDS Intermediate 1 and 2 /high risk, AML with 20-30% blasts, AML in complete remission post intensive chemotherapy (n=2), myeloid sarcoma (n=2), MDS/MPD (n=3) and CMML 2 (n=3).
Methods: Patient's baseline characteristics and outcomes were retrospectively analysed.
Results: Of the 41 patients, 26 were male and 15 were female; median age was 74.69 (range 36-89). Median performance status was 1. 13 patients had poor risk cytogenetics and 20 had >15% blasts. A median of 6 cycles of Azacitidine was administered (range 1-20). Patients were supported throughout their treatment with GCSF, antifungal and antiviral prophylaxis. Treatment continued until progression, transplant or death. Overall 25 patients had a haematological response, and 13 achieved complete remission (CR). Median overall survival was 399 days. There was a significant survival difference between patients with poor risk cytogenetics and those with normal or intermediate genetic abnormalities (median survival 267 *vs* 628 days, p=0.02). There was no significant difference in survival between late *vs* early responders although it should be noted that most responders (21/25) showed signs of haematological recovery within 1 cycle. In terms of toxicity 14 patients required hospitalisation at least once due to infections but mostly at the start of treatment. Three patients had mild self limiting GI toxicity. No patients refused treatment due to toxicity or quality of life issues. 36 patients received Azacitidine with a schedule of 5 + 2 + 2. Two patients received the 5-day regime as maintenance treatment post intensive chemotherapy. One of them received it for 7 cycles but failed to achieve haematological response, remained transfusion dependent and relapsed 2 years later. The other patient underwent allogeneic transplantation and died due to post transplant lymphoproliferative disorder with no evidence of relapsed AML. One patient was changed from the 5+2+2 schedule to a 6 weekly regimen due to prolonged cytopenias and is still in CR. In another patient with AML and 30% blasts who developed cytopenias during treatment with azacytidine, treatment cycle was reduced to 5 days. The patient is still in CR with regards to the bone marrow blast count. One patient went into complete remission from CMML 2 without any other prior therapy and was transplanted. He remains in CR. One patient had a remarkable complete response to Azacitidine from myeloid sarcoma of the bowel on histology.
Summary and Conclusions: Due to the above toxicities and cytopenias experienced by a significant number of patients, the cycle length of Azacitidine can be altered from the 5+2+2. Although Azacitidine treatment is regarded as well tolerated and "non-intensive" it is intensive in terms of timing, cycle length and supportive care especially within the first cycles. Our data suggest that Azacytidine is a safe option in elderly patients with co-morbidities but supportive care is of paramount importance for safe clinical practice maximizing the chance of the best possible response. Not recovering counts does not always signify lack of response as patients might have variable disease kinetics. More data is needed to support and justify altering the administration accordance to response.

PB1598

POLISH EXPERIENCE OF LENALIDOMIDE IN THE TREATMENT OF LOWER RISK MYELODYSPLASTIC SYNDROME WITH ISOLATED DEL(5Q) - A RETROSPECTIVE ANALYSIS

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Background: Lenalidomide has been approved for the treatment of lower-risk myelodysplastic syndrome (MDS) with 5q deletion (del(5q)). We present retrospective analysis of low-risk MDS with isolated del5q treated with lenalidomide, outside the clinical trials.
Aims: To analyse efficacy and toxicity of lenalidomide treatment in transfusion-dependent low-risk myelodysplastic syndrome with isolated del(5q) patients.
Methods: 36 red blood cell (RBC) transfusion-dependent patients have been included into the study. Patients received lenalidomide 10 mg/day on days 1-21 of 28-day cycles.
Results: 91.7% of patients responded to lenalidomide treatment: 72.2% has achieved erythroid response, 19.4% achieved minor erythroid response, 8.4% of patients did not respond to treatment. Response depended on number of previous treatment lines (p=0.0101), International Prognostic System Score (IPSS; p=0.0067) and RBC transfusion frequency (p=0.0139). Median duration of response was 16 months (range 6-60 months). Treatment was well tolerated. We observed hematological toxicity (grade 3 and 4): neutropenia - in 16 (44.4%) patients, and thrombocytopenia in 9 (25%) patients. Two patients (5.5%) progressed to high-risk MDS, and two subsequent progressed to acute myeloid leukemia. The overall survival of patients (60 months) in the study group was 79.0±8.8% (Figure 1).

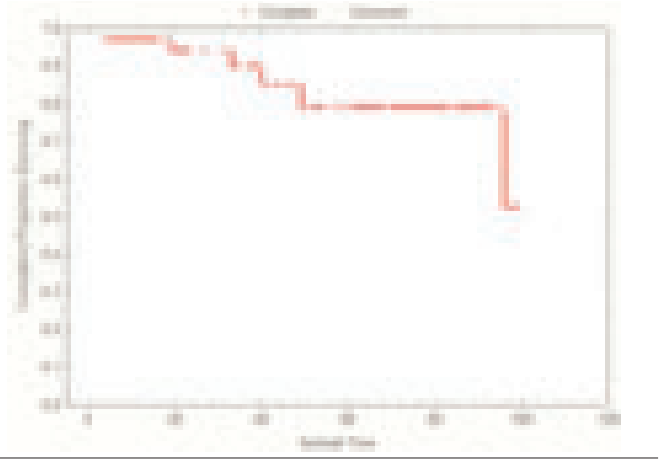

Figure 1.

Summary and Conclusions: Lenalidomide in this group of patients was beneficial for the treatment of RBC transfusion-dependency with well known safety profile.

PB1599

ADHERENCE TO GUIDELINES IN PRESCRIBING IRON CHELATION THERAPY IN MDS PATIENTS

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Background: Iron chelation therapy, according to the current guidelines, should be applied to all cases of transfusion dependent patients (pts) with a long life expectancy, after exposure to more than 20 RBC units or with ferritin levels above 1000 ng/dL.
Aims: To verify the appropriateness of iron chelation prescription in Myelodisplastic Syndomes (MDS) patients in Lombardia region.
Methods: The REL ("Rete Ematologica Lombarda") MDS Disease Registry was consulted after 2 years of activity (1 July 2011 - 30 June 2013) and the recorded data were analyzed.
Results: The Registry, at time of extraction, contained data of 218 pts from 10 centers in Lombardia region: 165 primary MDS, 29 t-related MDS and 24 MDS/MPN cases. Concerning primary MDS pts, IPSS was distributed as follow: low in 41 cases, int-1 in 75, int-2 in 27, high in 5, not valuable in 17. At the time of diagnosis the average hemoglobin level was 10.2 g/dl. In 20 patients it was below 8 g/dL. The average value of serum ferritin was 290 ng/mL. In 14 pts it was over 1000 ng/mL. Fourty-one pts were transfusion-dependent. In 45 patients was found an heart disease, with arrhythmia in 6 of them. During the follow-up 73 pts developed anemia with hemoglobin degree under 10 g/dL; of them 39 under 9 g/dL and 25 under 8 g/dL. Transfusional support was started in 63 cases. In 18 cases ferritin levels arise over 1000 ng/dL: of them, it raised over 2000 in 2 cases and over 3000 in one case. Heart disease was developed in one patient more. Iron chelation was started in 12 cases, and in 8 of them was on going at the end of the observation period. In the 4 cases in which the therapy was withdrawn, median time of treatment was of 228 days.
Summary and Conclusions: About half of pts was transfusion dependent after the period of observation. Ferritin levels above 1000 ng/mL were found in about 15% of pts. Assuming that one third of them had an int-2 or high IPSS risk (*i.e.* a bad life-expectancy), we can deduce that about the 10% of this of this population would had benefit by iron chelation. Actually, in only 6% of pts iron chelation therapy was started. We may conclude that in Lombardia region iron chelation therapy is under-prescribed respect to the indication of current guidelines.

PB1600

VERY POOR OUTCOME OF ACUTE MYELOID LEUKEMIA SECONDARY TO MYELODYSPLASTIC SYNDROME AFTER AZACITIDINE FAILURE IN A DAILY-LIFE SETTING

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Background: In the last decade, azacitidine has represented a major breakthrough in the setting of myelodysplastic syndromes (MDS), allowing to favorably alter the course of disease and to achieve survival benefits and improvement of quality of life. However, no curative effect is provided and acute myeloid leukemia (AML) transformation remains the most probable trend in this setting. Several studies have reported a poor outcome of AML arising from MDS after azacitidine failure, since no suitable therapeutic option is available. Moreover, the clinical presentation of AML in this setting may be rapidly aggressive and particularly devastating, reflecting a probable selection of a more aggressive malignant clone, despite the clinical responses previously achieved by azacitidine.
Aims: From our MDS database, we retrieved 26 patients (7 female) with a median age of 72 (32 – 82) years. At the primary diagnosis, 19 (73%) patients had refractory anemia with excess blasts, 6 (23%) myelodysplastic type-2 chronic myelomonocytic leukemia (CMML-2) and 1 (4%) refractory cytopenia with multilineage dysplasia. The IPSS at the primary MDS diagnosis was low, Int-1, Int-2 and high in 3 (12%), 8 (30%), 14 (54%) and 1(4%) patients, respectively. According to the approved indications in Europe, low and Int-1 patients were treated only after progression, at a median of 2.5 (0.5- 94.2) months from the primary MDS diagnosis; so that, at the start of azacitidine, IPSS was Int-2 and high in 22 (85%) and 4 (15%) patients respectively. Among them, 7 (27%) patients presented an abnormal karyotype. In 5 (19%), 5 (19%) and 4 (16%) of the 26 patients treated with azacitidine, partial response, complete response (CR) and hematological improvement (HI) were achieved (overall response: 54%).
Results: AML transformation occurred 9 (2-40) cycles and 12 (2-49) months after the starting of azacitidine; at the AML onset, a WBC count higher than 50.000/uL was observed in 50% of patients; moreover, 4 (15%) cases progressed to an oligoblastic (20 to 30% bone marrow blasts) AML. One patient presented a myelomonoblastic sarcoma of the anterior chest wall at the time of AML transformation. Cytogenetic analysis was available for 22 (84%) patients; 9 (41%) presented an abnormal karyotype. Given the advanced age, the poor performance status and the particularly devastating disease onset of AML in most patients, only 8 (31%) were suitable for high-dose cytarabine (HDAC)-based regimen and 2 (8%) underwent allogeneic hematopoietic stem cell transplantation (allo-SCT). No patients treated with HDAC-based regimen achieved a CR; moreover, among the two allo-SCT patients, one died because of early AML relapse and the other of transplant-related complications. Of the four patients who progressed to oligoblastic AML and continued to receive azacitidine, all achieved HI. With a median follow-up of 2 months (0-32) from transformation to AML, 6 (23%) patients are still alive, all of them with active disease. The median overall survival was 2 months (56 days) and the 6-months survival was 23% (Figure 1).

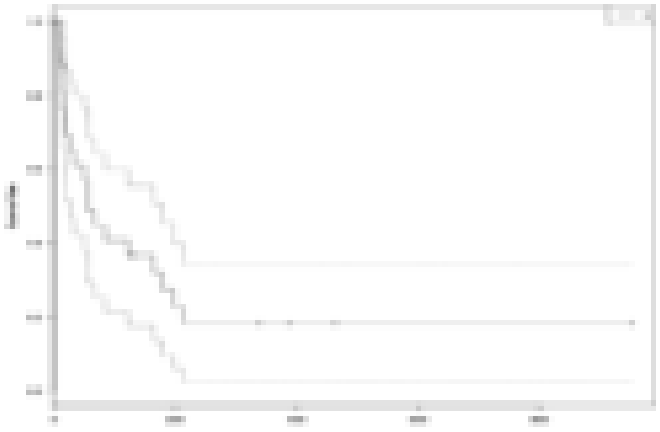

Figure 1. Overall survival: Kaplan-Meier survival curve with 95% confidence limits from AML progression for the 26 patients (time in days).

Summary and Conclusions: Our findings confirm the very poor outcome of these high risk leukemic patients with the current standard of care, including HDAC-based regimens and allo-SCT. Efforts aimed at the development of novel agents and effective AML treatments, including the association of azacidine with innovative agents, are urgently needed.

PB1601

MDS CLEAR PATH: A WEB-BASED EDUCATIONAL ALGORITHM FOR THE DIAGNOSIS AND MANAGEMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: MDS has significant implications for patients, including transfusion dependence, an increased risk of acute myeloid leukemia (AML) transformation and decreased survival. Therapeutic options are available that may reduce transfusion dependence, delay AML transformation, improve survival and improve quality of life. In 2011 the Canadian Consortium on MDS set out to develop Canadian recommendations for the management of MDS, termed MDS Clear Path.
Aims: Objectives were to develop a comprehensive tool for the management of MDS. Key decision points are identified, evidence provided and areas where data are lacking are identified.
Methods: A draft algorithm was developed by the steering committee and presented to a group of hematologists from across Canada in October 2011. Input was obtained from over 60 hematologists through nine regional meetings held between November 2011 and May 2012. Feedback was incorporated at a review in March 2013. The final steering committee review was completed in September 2013.
Results: The algorithm provides a step by step opportunity to diagnose, manage and treat MDS patients from any risk group and at any point in their course. It details investigations required, prognostic scores and management recommendations and features a prognostic calculator for all scoring systems. Information on therapeutic options (erythropoiesis stimulating agents, lenalidomide, azacytidine, immunosuppressive therapy, supportive care, iron chelation therapy, investigational agents and clinical trials with links to trial websites) is provided, including expected response, side effects and their management, and provincial reimbursement. Many points incorporated into the algorithm were by consensus built via input from the regional meetings. Where data from the literature are lacking but practical advice needed, the steering committee incorporated recommendations into the algorithm. The algorithm treatment wizard mode guides the user through a series of questions resulting in a treatment recommendation while the self-directed mode presents the therapeutic algorithm. All reference and supportive data are contained within the algorithm with information panels provided as appropriate. There is an option to search by words of interest. The algorithm is available in English or French at www.MDSClearPath.org and as an app that can be downloaded onto an ipad from the website or the Apple store.
Summary and Conclusions: Through a collaborative effort of Canadian hematologists, an internet/app based algorithm is currently available to support qualified health care providers in the diagnosis and management of MDS patients. Recommendations from the algorithm should assist in standardization of MDS care across the country. The algorithm includes indications and regimens which are not currently approved in Canada; product monographs contain complete indication and safety information. Content will be regularly updated to reflect advances made in the care of MDS patients. Ipads will be available during the poster session to enable conference participants to navigate the MDS Clear Path algorithm. This program was supported by an unrestricted educational grant from Celgene Inc.

PB1602

AZACYTIDINE THERAPY FOR MDS PATIENTS: RETROSPECTIVE MULTICENTRE REGIONAL EXPERIENCE IN PATIENTS REPORTED IN THE LIGURIAN MDS REGISTRY

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Background: In high-risk patients with myelodysplastic syndromes (MDS), the DNA methyltransferase inhibitors produce responses in up to 40% patients. Moreover Azacytidine (AZA) has shown a survival advantage when compared with conventional therapies. In clinical trials AZA is also used in low-risk patients failing to respond to recombinant human erythropoietin.
Aims: We retrospectively reviewed the clinical outcome of MDS patients treated with AZA according to common clinical practice in an Italian region (Liguria). MDS patients are registered in the Ligurian MDS registry which is affiliated to the FISM (Italian Foundation for Myelodysplastic Syndromes).
Methods: Herein we report the results of 56 patients treated with AZA in the last five years of whom complete haematological, cytogenetic and clinical informations are available. Median age was 71 years (range 41-85); male/female ratio was 30/26. MDS patients had RA or RARS (n.7, 12%), AREB-1 (n. 18, 32%), AREB 2 (n. 21, 37%), other subtypes (n. 10, 17%). Karyotype was normal in 37 patients (65%), complex in 7 (13%); 2 patients had trisomy 8 (4%) and 10 other alterations (18%). IPSS was intermediate-1 in 28 patients (50%), intermediate-2 in 23 (41%) and high in 5 (9%). The median follow up was 36 months. Forty-six patients had a transfusion-dependent anaemia. All low and int-1 risk MDS patients had a transfusion-dependent anaemia that was unresponsive to r-EPO.
Results: AZA was given at 75 mg/sqm with the "5+2" schedule every 28 days. Patients were delivered a median of 8 courses of treatment (range 3-44). Thirty-nine patients (69%) received concomitant recombinant alpha erythropoietin therapy (40 000 U once weekly). Major infections during therapy were recorded in 13 patients (23%), with no infection-related deaths. Forty-two patients (75%) achieved a hematological response, that was complete in 12 patients (21%) and partial in 25 (44%). Five patients had a hematologic improvement and 14 patients (25%) did not respond. A statistically significant reduction of transfusional support was observed in responding patients ($p<0.05$). Response was achieved after a median of 3 (range 2-12) and 5 (range 1-12) AZA courses in low/int-1 risk and int-2 / high risk MDS patients, respectively. Response lasted a median of 16 months (range 4-40). Leukaemia free survival (LFS) was affected by IPSS risk (36 months projected LFS was 56.9% and 20.4% in patients with intermediate-1 and intermediate-2/ high risk, respectively, p: <0.01) and WHO diagnosis ($p<0.01$). Projected overall survival (OS) was 89.9% at 12 months, 62,6% at 36 months and 45,6% at 60 months (median survival 51 months). OS was mainly affected by response to AZA (60 months projected OS was 60% and 26% in patients achieving at least a PR and in unresponsive patients, respectively; $p<0.01$), IPSS ($p<0.05$), age and WHO diagnosis ($p<0.05$).
Summary and Conclusions: These preliminary data confirm that AZA therapy is well tolerated and effective in the common clinical practice in all age and all risk MDS patients and can significantly reduce transfusional support. LFS and OS are mainly affected by IPSS risk, age and response to AZA.

PB1603

AZACITIDINE IN 'REAL LIFE' PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML): A SINGLE CENTRE EXPERIENCE

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Background: CMML is a clinically heterogeneous disease for which, according to recently reported experiences, hypomethylating therapies have provided significant clinical benefits.
Aims: Herein, we present the outcome of 11 patients (pts) who fulfilled the WHO 2008 criteria for CMML (CMML-2 in 7, CMML-1 in 2) or CMML-related acute myeloid leukemia (AML) with <30% bone marrow blast (2 pts) and have been treated with azacitidine (aza) at our institution between 2010 and 2014 after informed consent has been obtained.
Results: Median age at diagnosis was 76 years (range 62–86). Five pts had proliferative CMML. Four pts were transfusion dependent at some time point of disease course. Two out of 11 pts had abnormal kariotype (46,XY,Inv12 and 45,Y,-X, respectively). Two pts had secondary CMML; one to a 7-years lasting myelodysplastic syndrome (refractory anemia), whereas the other, who has undergone radio-chemotherapy for a solid tumor 3 years before, presented a likely therapy-related CMML. Prior therapies included cytoreductive therapy and erythroid stimulating agents. The MDAPS was low, Int-1, Int-2 and high in 1,2,4 and 2 CMML pts, respectively. Pts were treated with azacitidine, 75 mg/m^2 x 7 days, 5+2+2 schedule, every four weeks, subcoutaneously). Supportive care was given as required. Bone marrow (BM) response was assessed in 10 pts (following the sixth cycle in 9 pts and the fourth in 1); response was not assessed in 1 pt only, due to death (multiorgan failure) occurrence after second cycle. Responses were classified according to the modified IWG criteria; 5/9 evaluable pts achieved complete remission (CR) and 3 partial remissions (PR) with an overall response rate (CR+PR) of 73%; 2 pts maintained stable disease. No progressing pts continued the treatment. Two pts progressed to AML following the sixth and the fourteenth cycle respectively, after having obtained CR. With a median follow-up of 13 (2 – 31) months, 4 pts are alive and all of them continue to receive the treatment; six pts have died, 3 of AML, 2 of sudden cardiac death (with stable CMML), 1 of multiorgan failure (before response assessment); 1 patients was lost at follow up; median survival from therapy start was 15 months. Treatment was well-tolerated and no remarkable side effects were recorded.
Summary and Conclusions: In conclusion, despine the limited number of cases, our experience was encouraging; indeed, the use of aza in our hands achieved good responses in more than 70% of the treated pts, despite their high risk of disease and unfavorable prognostic profile.

PB1604

CYTOGENETIC ANALYSIS IN MYELODYSPLASTIC SYNDROME: AUDIT OF THE CURRENT PRACTICE IN A HAEMATOLOGICAL MALIGNANCY DIAGNOSTIC CENTRE. WHAT IS THERE TO BE LEARNT?

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Background: Identification of a clonal cytogenetic abnormality is essential for prognostic assessment of patients with Myelodysplastic Syndrome (MDS) (Revised International Prognostic Scoring System (IPPS-R)) and necessary for informing treatment decisions. According to the British Committee for Standards in Haematology (BCSH) guidelines (2013), cytogenetic analysis should be performed on all patients with suspected MDS having a bone marrow examination. Cytogenetic analysis is costly however, so it is crucial to ensure that the test is performed on appropriate samples. In many centres samples are cultured initially and subsequently analysed where needed. As significant numbers of these specimens are not analysed, we have implemented a procedure of immediate morphological screening of bone marrow aspirate samples prior to sending for cytogenetic culture.
Aims: We audited our current laboratory practice of immediate morphological screening of bone marrow aspirate samples before requesting cytogenetic analysis when investigating patients with possible MDS.
Methods: Using the Haematological Malignancy Diagnostic Links (HMDL) database, we reviewed 603 bone marrow samples received from the University Hospitals of Leicester NHS Trust, Kettering General Hospital NHS Foundation Trust and Northampton General Hospital NHS Trust over a 6 month period (March-August 2013). The key words 'myelodysplasia', 'uni-, bi-, pan-cytopenia', were used to select cases where MDS was suspected by the clinician. 116 bone marrows that met the criteria were assessed. Cytogenetic findings were reported in accordance with the International System for Human Cytogenetic Nomenclature Recommendations.
Results: Of the 116 samples, 52 were consistent with MDS according to the World Health Organisation (WHO) Revised Classification 2008 but only 29 of these were a new diagnosis and so appropriate for prognostic cytogenetic analysis. 14 samples were screened as possible MDS but this was not confirmed on detailed morphological reporting and in 7 of these cases cytogenetics were not subsequently analysed. As a result of bone marrow screening, 33 samples requested by clinicians were not sent for cytogenetic processing, as there was no evidence of dysplasia.
Of the 29 suitable cases cytogenetics could not be performed and as a result, the IPPS-R score could not be calculated for 5 new patients. For 3 of these no sample was received and in one case cytogenetic analysis failed. However, in 1 case the sample was rejected at the screening process resulting in the need for a repeat aspirate sample. Out of the 25 available reports, 10 showed an abnormal karyotype (40%). 10 cases were deemed intermediate or high risk by IPSS-R criteria and were considered for treatment with the nucleoside analogue, Azacitidine as per the TA218 National Institute for Health and Care Excellence (NICE) recommendations and the BCSH guidelines.
Summary and Conclusions: Immediate morphological screening of bone marrows referred as possible MDS has prevented the inappropriate processing of 43% of samples. This represents a significant cost saving. The audit has led to the introduction of a check procedure to ensure samples are not inappropriately rejected at screening.

PB1605

ASSISTED ADMINISTRATION OF SUBCUTANEOUS 5-AZACYTIDINE TO THE PATIENT'S HOME IS SAFE AND COST-EFFECTIVE

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Background: Administration of azacitidine is planned in-patient daily, there is no experience of its administration to the patient's home.
Aims: The aim of this work was to evalute the feasibility of a program of home administration of azacitidine capable of reducing the cost-of-illness, to increase adherence to treatment while maintaining the same safety of the therapy given in the hospital.
Methods: Between Jan 2005 and Dec 2012, 22 consecutive patents, (MDSs, n= 15; CMML, n= 4; AML, n= 3), were enrolled in the study.The pharmaco-economic analysis included assessment of direct costs (hospital inpatient, physician inpatient,physician outpatient, emergency department , nursing home care, specialists' and other health professionals' care,diagnostic tests, prescription drugs and drug sundries, and medical supplies),indirect costs incurred by care recipients and unpaid caregivers, induding time, productivity and travel cost.
Results: Azacitidine 75 mg/m2 day was administered as a subcutaneous injection for 7 consecutive days every 4 weeks.Median age of the patients was 71 years (range, 65-83. Median number of courses delivered to each patient was 9 (range, 3-31) Hematologic responses (CR/PR/mCR) were induced in 6 patients {27%} Median number of treatment courses to achieve any response was 2 (range 1 -6) Adverse events were evaluated for the first 6 courses for all patients, for a total of 124 courses. Major adverse events were cytopenia and cytopenia-related infection. Grade 3 or higher neutropenia was 64.4%) but incidence of febrile episode requiring intravenous antibiotics was 8,4% slightly lower than reported from the pivotal clinical study. Grade 3 or higher non-hematologic toxicities were infrequent. Injection site reaction 0.4 and site pain 0% Median follow-up duration of surviving patients was 46.9 months (range, 11.8-55-5). Of the 14 patients who where RBC transfusion dependent at baseline, 48.0% became RBC transfusion independent during the treatment period. Adherence to treatment was 100%>
Summary and Conclusions: In our experience, despite the high percentage of elderly patients of whom 36% living in rural area, it was possible to give treatment to all patients with total adherence. There has been a reduction in direct medical costs due to less use of hospitalization, a reduction of indirect costs by 63% due to the lower number of working days lost and a drastic reduction of travel costs with the same efficacy and safety of administration.

PB1606

MYELODYSPLASTIC SYNDROMES MANAGEMENT AND TREATMENT: A CONCEPTUAL FRAMEWORK

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Background: First-line treatment of myelodysplastic syndrome (MDS) patients with hypomethylating agents (HMAs) has been demonstrated in clinical trials to improve survival and/or delay progression to acute myelogenous leukemia (AML). However, most patients eventually relapse and, unless eligible for allogeneic hematopoietic cell transplant (HCT), face limited treatment options

and survival of only months. Despite the anticipated growth in the MDS population, little is known about the diagnostic and treatment decision-making undertaken by clinicians who treat non-transplantable MDS patients.
Aims: We sought to better understand MDS patient management and unmet needs by identifying clinicians' approaches for determining MDS: diagnosis; risk; progression; and treatment, particularly among higher-risk patients and after initial treatment failure.
Methods: We conducted in-depth, semi-structured interviews with nine hematology and/or oncology specialists (8 physicians; 1 nurse practitioner) in the US experienced in diagnosing and managing MDS patients.
Results: Diagnostic processes were consistent across providers and included blood work, physical examination, and often tests to rule out potential alternative diagnoses. Bone marrow biopsies (BMBs) were always performed to confirm the diagnosis. Providers calculated International Prognostic Scoring System (IPSS) and/or revised IPSS (IPSS-R) scores for almost all treatment-naïve patients. Some providers used just one system, while others used both due to specific trial protocol requirements and the limited real-world evidence regarding IPSS-R use in guiding treatment decisions. Initial treatment decision-making was driven by risk score, symptoms, transfusion dependency and, in some cases, performance status. Providers' management approaches were generally consistent for very low-risk (*e.g.*, active monitoring) and very high-risk (*e.g.*, HMA; HCT, if eligible) patients. There was substantial variation in response criteria (*e.g.*, change in BMB results, worsening cytopenias) used to determine progression from lower- to higher-risk MDS; several providers cited the need for a validated metric to address this inconsistency. Two-thirds of providers treated indefinitely with HMAs regardless of treatment response and given an absence of treatment-related adverse effects, citing long term or cyclical HMA benefits and lack of second-line options. Differences existed in methods used to determine HMA response, with the most common being periodic blood counts and/or BMBs. Providers noted that patients receiving HMAs either fail to respond or respond for some period of time and eventually experience treatment failure and MDS progression. Providers agreed that there is a significant unmet medical need for treating these second-line, non-HCT eligible MDS patients. Described as "limited," second-line treatments included clinical trials, cytotoxic induction chemotherapy, and supportive care. All providers stated that prognosis is poor regardless of approach.
Summary and Conclusions: The lack of treatment options for MDS patients, especially high-risk patients who do not respond to HMAs or have initial treatment failure, is an issue of profound importance. Providers use different approaches for these patients but agree that none offer significantly improved prognoses, leaving a considerable unmet need for second-line options. More research is required in determining optimal strategies for treating higher-risk and second-line MDS; assessing disease response in treated patients; and defining treatment failure.

PB1607

PROGNOSTIC SIGNIFICANCE OF CYTOGENETIC CATEGORIES IN MYELODYSPLASTIC SYNDROMES: SINGLE CENTRE STUDY FROM OMAN
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Background: Myelodysplastic syndromes (MDS) are clonal disorders of hematopoietic stem cells characterized by ineffective hematopoiesis, peripheral cytopenias and a variable risk of leukemic transformation. Chromosomal abnormalities play an important role in the underlying disease biology, predicting prognosis.
Aims: To investigate and compare IPSS-R prognostic stratification in MDS patients with the outcomes.
Methods: This retrospective study assessed 50 MDS patients (median age-60; range 1-86 years) with IPSS-R poor and very poor (n=5), intermediate (n=7) and good and very good (n=38) prognostic stratification. The median follow up was for 39.5months (range 1-70).They were analyzed for overall survival, leukemic progression, and according to the MDS associated cytogenetic abnormalities.
Results: 31 patients (62%) had normal cytogenetics. Amongst the MDS related abnormalities described, 4 (8%) had monosomy 7, 4 (8%) had trisomy 8 and one each had isochromosome 17q, del (11q) and del (5q). 14 had single abnormality, 3 showed double abnormalities, whereas one each had a complex abnormality and balanced translocation (t(5:12)). In the IPSS-R cytogenetic groups, the median survival was 34months for the good risk, 32 months for the intermediate group but 15.5 months for the poor risk subgroups($p<0.05$, chi square test). Although the overall mortality was 26%, it was 22%, 29% and 36% in the IPSS-R good, intermediate and poor subgroups respectively. Leukemic transformation was seen in 22% patients with an overall mortality of 55%. However, leukemic transformation occurred in 3%, 43% and 55% in the IPSS-R good, intermediate and poor subgroups respectively (Figure 1).

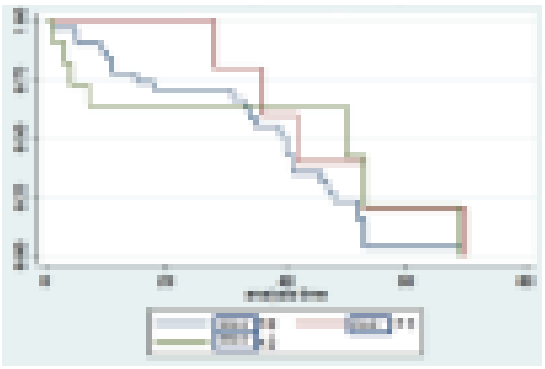

Figure 1. Kaplan-Meier survival estimates.

Summary and Conclusions: The IPSS-R can effectively stratify the prognosis of MDS based on cytogenetics even in a small single centre study of 50 patients with the three Kaplan Meier curves can be seen to diverge initially. However, with effective therapy like BMT, it was seen that progressively the lower curve superimposes with the first indicating the better long term outcomes.

PB1608

PEDIATRIC MYELODYSPLASTIC SYNDROMES: HOW DO THEY DIFFER FROM ADULTS?
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Background: Although uncommon, pediatric myelodysplastic syndromes [MDS] are not rare but its incidence is underestimated. Furthermore, unlike adults, it frequently presents as bone marrow failure syndrome in contrast to refractory cytopenias, and *per se* there is considerable difficulty in assigning the overlapping morphological features seen with bone marrow failure syndromes.
Aims: To study the clinical presentation, overall survival and correlate WHO 2008 Classification of MDS cytogenetic changes in childhood MDS *versus* Adults.
Methods: This retrospective study assessed 14 pediatric MDS patients (Median Age-11.5; range 1-16 years) and compared them with 39 adult MDS patients (Median age-65, range 23-86 years) from a single tertiary institution in Oman. Pediatric MDS patients were analyzed for initial presentation, type of progression, leukemic transformation and overall survival as well as according to the MDS related cytogenetic abnormalities. The median follow up was for 43 months (range 1-69).
Results: The commonest presentation was refractory cytopenia of childhood [RCC] in 43%, followed by RAEB-T [29%], RAEB [21%] and JMML [7%]. Seven patients (54%) had normal cytogenetics. Amongst the MDS related abnormalities described, four (31%) had monosomy 7 whereas, one each (8%) had trisomy 8 and trisomy21 with del (10q). Overall mortality in the pediatric MDS patients was 29%. Six patients [43%] demonstrated leukemic transformation with 50% mortality. Seven patients [50%] underwent Bone marrow transplantation (BMT) with six long term survivors [85%] (Figure 1).

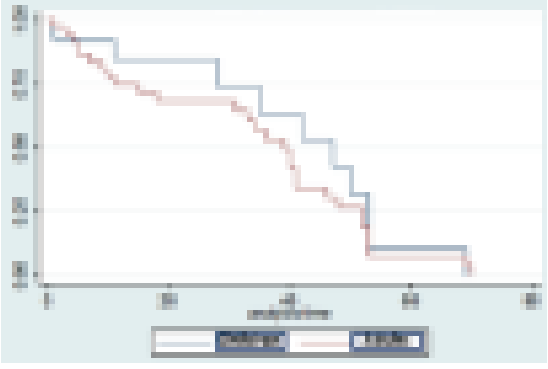

Figure 1. Kaplan-Meier survival estimates.

Summary and Conclusions: Although there are several fundamental differences between childhood and adult MDS patients, the overall survival in these MDS cohorts did not show any statistically significant differences in the

Kaplan Meier survival analysis. Early treatment with BMT is likely to result in good long term prognosis in spite of the poor cytogenetic features.

PB1609

HYPOALBUMINEMIA IS AN INDEPENDENT ADVERSE PROGNOSTIC FACTOR FOR OVERALL SURVIVAL IN MYELODYSPLASTIC SYNDROMES

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Background: Low serum albumin level is known to be an adverse prognostic factor in patients with malignances such as multiple myeloma. The negative prognostic value of low serum albumin has been reported by the Moffitt Cancer Center group in a large series of 767 patients (Komrokji *et al.* Am. J. Hematol. 2012;87:1006-1009).
Aims: The aim of this study is to confirm the prognostic value of serum albumin in different MDS risks groups and cytogenetic groups (Schanz *et al.* J. Clin Oncol. 2012;30:820-829).
Methods: Patient's data were obtained from our MDS database. The primary objective was to check the prognostic value of serum albumin for overall survival (OS). Patients were divided into 2 groups according to the levels of serum albumin at diagnosis (< 4 gr/dl y > 4 gr/dl). We used Kaplan-Meier analysis to estimate OS and the log-rank test to compare survival estimates between the two groups. Cox-proportional hazards regression was used for multivariate analysis.
Results: We analyzed data from 53 patients diagnosed of MDS between January 2012 and January 2014. The median age was 69 years. The median of serum albumin was 4,1 gr/dl. MDS were classified in low risk (IPSS low and intermediate-1 or WPSS very low, low and intermediate) 68%, and high risk 32%. Baseline characteristics of the patients in the two groups were:

Table 1.

The median OS for all patients was 25 months (95% CI: 13,2-38). Age (< 60 *vs.* ≥ 60 years) and the MDS cytogenetic subgroups (Very good/Good *vs* Intermediate/Poor/Very poor) did not reach prognostic value. However, the IPSS-WPSS MDS subgroup risk [mean OS 13,6 (95% CI: 7,7-19,5) *vs* 31,8 months (95% CI: 13,3-50,3), p=0,019)] and the albumin levels(< 4 *vs* ≥ 4 gr/dl) [mean OS 9,2 (95% CI: 2,9-15,4) vs 31 months (95% CI: 14,9-47,1), p<=0,0001), see figure] showed statistically significant prognostic value in the univariate analysis. After adjustment for MDS subgroup risk, OS was statistically significantly different among MDS patients with serum albumin<4 gr/dl [Hazard Ratio = 0,112 (95% CI: 0,03-0,4); p=0,001] (Figure 1).

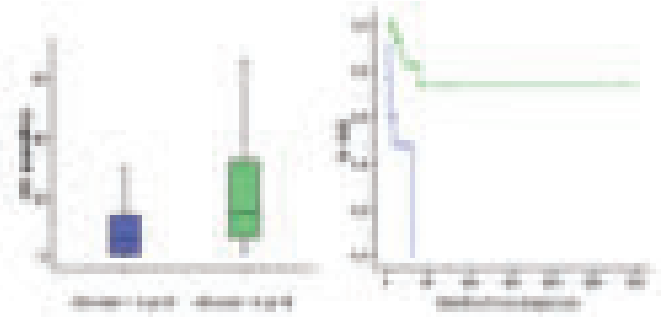

Figure 1.

Summary and Conclusions: In this retrospective analysis patients with serum albumin less than 4 gr/dl at diagnosis have significantly reduced OS, and showed its value as an independent prognostic factor for OS after adjustment for MDS IPSS-WPSS subgroups risk and cytogenetics. Serum albumin should be taken into account as a new prognostic factor in MDS and might be studied as a surrogate factor of performance status and co-morbidities.

PB1610

ORIGINATOR ERYTHROPOIETIN ALPHA *VS* BIOSIMILAR ERYTHROPOIETIN ALPHA *VS* ERYTHROPOIETIN ZED PLUS LIPOFER AND B12 AND FOLATES IN PATIENTS WITH REFRACTORY ANEMIA. FIVE CENTER RETROSPECTIVE STUDY

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Background: Biosimilar drugs, including erythropoirtin zed, have a similar, but usually not inferior although not hidentical effects of originator drugs. Safety is hidentical to originator drugs.
Aims: Aim of this study is to verify if in MDS patient with refractory anemia biosimilar erythropoietin alpha and erythropoietin zed, are not inferior to erythropoietin alpha in terms of safety, efficacy and costs.
Methods: This study is a retrospective study.Between july 2008 and december 2013, 101 patients affected by refractory anemia were studied.Median follow-up was 16 months (R10-28).Patients received in group A erythropoietin alpha 40000 IU sc/weekly.In group B patient received biosimilar erythropoietin alpha 40000 IU sc/weekly.In group C patient received biosimilar erythropoietin alpha 40000 IU sc/weekly.In all three arms patients received lipofer 14 mg 2 tablets orally/day calcium levofolinate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally.In group A median age was 70 years (R63-73), M/F: 15/28.In group B median age was 64 years (R60-70), M/F: 24/19.In group C median age was 68 years (R62-73), M/F: 10/5.IPSS was low in 30 patients and int-1 in 12 patients, karyotype showed –Y in two patient, del 20q in one patient, trisomy 8 in two patients in group A.IPSS was low in 32 patients and int-1 in 10 patients, karyotype showed –Y in one patients, del 20q in one patient in group B.IPSS was low in 11 patients and int-1 in 4 patients, karyotype was normal in 9 patients and not evaluable in 6 patients.Patients with 5q- were excluded from this study.Median level of haemoglobin was 9 g/dl in group A (R8-11), 8.7 g/dl (R8.5-10.5) in group B and 8.5 in group C. Cost of every month of erythropoietinic therapy was calculed dividing for each patient the sum of complete erythropoietic therapy for each month of follow-up, then in each group of patients median cost of erythropoietic therapy was calculed.
Results: Group A patients increased Hb level of 1 g/dl after a median time of 5 weeks (R4-9), after a median time of 3.5 weeks (R3-8) in group B and after a memedian time of 4 weeks in group C. No relevant side effects were observed in all three groups groups . Erythropoietin alpha was reduced in group A because Hb achieved a level > 12g/dl after a median of 12 weeks (R 4-18).Biosimilar erythropoietin alpha was reduced in group B because Hb achieved a level > 12g/dl after a median of 10 weeks (R 3-16).Erythropoietin zed was reduced in group C because Hb achieved a level > 12g/dl after a median of 9.5 weeks (R 3-15).In group A maintenance dose was administered with a median of every two weeks (2-4), in group B maintenance dose was administered with a median of every three weeks (2-5), in group C maintenance dose was administered with a median of every three weeks (2-4).Median cost for every month of erythropoietic therapy was 1536 euros/month (R1240-1850) in group A , 1354 euros/month (R954-1550) in group B, 1300 euros/month (R1300-1380) in group C. Five patients need transfusion support in group A,6 patients need transfusion support in group B and 5 in group C.
Summary and Conclusions: Biosimilar erythropoietin alpha and erythropoietin zed plus lipofer and B12 and folates support seems to be safe, feasible, probably equally cost-effective and substantially not inferior to originator erythropoietin alpha support in patients affected by refractory anemia. This study needs confifrmation on a larger cohort of patients.

PB1611

PEDIATRIC MYELODYSPLASTIC SYNDROMES: PROGNOSIS, MANAGEMENT AND EVALUATION

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Background: Myelodysplastic syndromes (MDS) are a rare disease in children, accounting for less than 5% of all hematological malignancies. The definition and classification of childhood MDS child can be confusing, due to frequent overlapping between forms of MDS and myeloproliferative disorders (MPD). MDS may occur as a previous phase to the development of acute myeloid leukemia, whose progression is usually rapid and fatal if not treated early.
Aims: To analyze clinical characteristics, prognostic factors and outcome of pediatric patients diagnosed with MDS at our center
Methods: We studied 10 patients diagnosed at our center with childhood MDS

(7 juvenile myelomonocytic leukemia (JMML), 1 amegakaryocytic thrombocytopenia, 1 Refractory cytopenia with multilineage dysplasia (RCMD), 1 Refractory anemia with excess blasts-1 (RAEB-1)), with a minimum follow-up of 120 days. Qualitative variables were analyzed using chi -square and nonparametric tests were used for quantitative variables.
Results: The mean age was 4 years (r, 1-13) . Median leukocyte count at diagnosis was 42.61 x10^9/L (r, 3.67-233) with a median fetal hemoglobin of 16%(r, 1-55). One patient presented with unfavorable cytogenetics (monosomy 7) and the remaining nine with an intermediate prognosis. Seven patients (70%) underwent allogeneic HSCT. All donors were HLA- identical, 5 of which were unrelated donors. Bone marrow was used in 4 cases and umbilical cord in 3. The conditioning regimen was myeloablative in 100% of patients. Thymoglobulin+cyclosporine+methotrexate was used in all cases as GVHD prophylaxis . Four patients (57%) developed acute GVHD, grade II – IV in two cases. No chronic GVHD was observed in the analyzed series. Six (60%) presented hepatomegaly at diagnosis, observing 83% (5) of exitus in these patients. Nine patients (90%) developed splenomegaly, splenectomy prior to HSCT was performed in only one (13%), with no affect on mortality. We observed 6 (60%) deaths, 4 of these in patients with JMML. Of the 7 patients that underwent HSCT, 57% (4) are still alive, while 100% (3) of those that didn't receive HSCT have died.
Summary and Conclusions: Pediatric patients with MDS have a fatal evolution as described in the literature. In our series leukocyte count at diagnosis and the presence of hepatomegaly were identified as prognostic factors. The longest survival rates were achieved after HSCT, which should be considered as the first therapeutic option, as it is the only treatment with proven healing capacity in these patients.

PB1612

VALIDATION OF DIFFERENT PROGNOSTIC CLASSIFICATIONS IN PATIENTS DIAGNOSED WITH MYELODYSPLASTIC SYNDROME (MDS) IN THE NEW MILLENIUM

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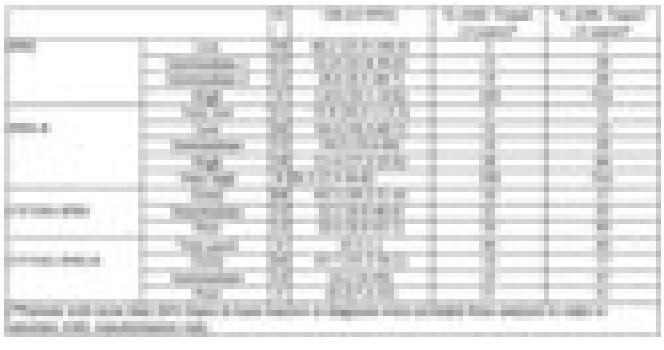
Background: To determine a proper therapeutic approach is essential for MDS, due to the wide heterogeneity of disease presentation at diagnosis. For that purpose, we have studied different prognostic classifications: IPSS, IPSS-R and WPSS.
Aims: The aim of our study was to assess the adaptation of these classifications to the results obtained in our patient series.
Methods: Data of all patients diagnosed with MDS at Son Llàtzer Hospital were prospectively collected from January 2002 to February 2014. We used SPSS statistics v.19 © for descriptive and univariate statistical analysis, using Kaplan-Meier for survival analysis and log-rank test to compare the curves.
Results: Of 112 patients, 96 were eligible for analysis: 57 men (59.4%) and 39 women (40.6%) with a median age of 77 years (33-94). According to FAB: RA was 26 (27.1%), 20 RARS (20.8%), 30 RAEB (31.3%), 6 RAEB-t (6%) and 14 CMML (14.6), while according to WHO, 7 were RA (7.3%), 6 RARS (6.3%), 16 RCMD (16.7%), 9 RCMD-RS (4%), 12 RAEB-1 (12.5%), 13 RAEB-2 (13.5%), 5 RCUD (5.2%), 5-5q MDS (5.2%), 15 MDS / MPD (15.6%), 1 SMDI (1%) and 1 was not available. Six patients (6.3%) were diagnosed with secondary AML. The median overall survival (OS) was 41.81 months (95% CI 34.4-49.21) with a median of follow up of 31.38 months. The overall rate of transformation to leukemia after 5 years was 29%. Statistically significant differences were observed in OS according to IPSS (p=*0.000*) and IPSS-R (p=*0.000*). Moreover, no significant differences were found based on WPSS (p=*0.312*), Sorror comorbidities index (p=*0.532*) or according to ferritin levels groups (p=*0.167*) in terms of OS (Table 1).

Table 1.



Summary and Conclusions: In our series, IPSS and IPSS-R rating have been useful for predicting the prognosis in terms of OS as well as the risk to AML transformation. However, some groups of cytogenetic risk at IPSS-R required a larger sample since we have not observed concordance respect to risk of transformation to AML as it appears in the rest of the literature

PB1613

THE ASSESSMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROME ACCORDING TO REVISED INTERNATIONAL PROGNOSTIC SCORING SYSTEM

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous myeloid clonal disease. Prognosis can be determined by international prognostic scoring system (IPSS) which is based on both chromosomal abnormalities and blast ratio in bone marrow. Prognosis may be also specified by World Health Organization prognostic scoring system (W-IPSS) which consists of chromosomal abnormalities, blast ratio in bone marrow and the requirement for blood transfusion. Revised international prognostic scoring system (R-IPSS) has been defined recently. This system refers to cytopenia for each blood cell and the blast ratios of<2% or 2-5% as separate categories. Risk groups have been described as very low, low, intermediate, high and very high.
Aims: In this study, we aimed to compare the IPSS with R-IPSS.
Methods: This is a retrospective evaluation of 130 patients (65 men and 65 women) who were diagnosed with MDS at the Department of Hematology in Adnan Menderes University Medical Faculty Hospital between 2008 and 2012. The IPSS and R-IPSS criteria were used for this evaluation. Fifty patients had MDS RA whereas RAEB-2 existed in 17 patients. The mean patient age was 69±12 years while the median overall survival was 41±13 months (Figure 1).
Results: Both IPSS and R-IPSS were found to be statistically similar when they were compared by one way analysis of variance and two-paired t-test.

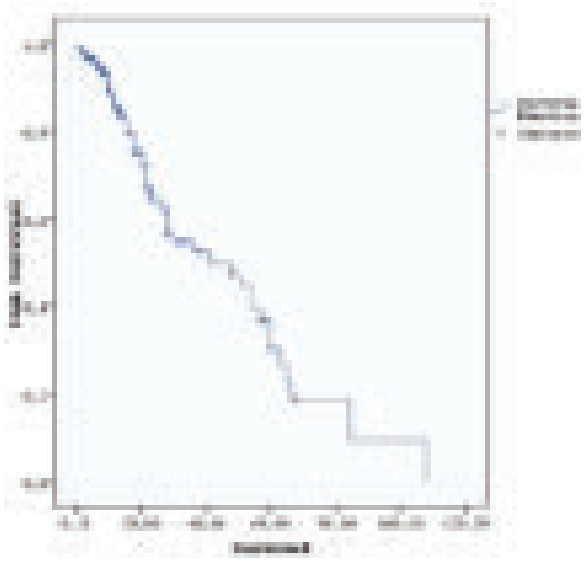

Figure 1.

Summary and Conclusions: Literature indicates superiority of R-IPSS in prognostic evaluation of patient with MSD. However, the present study has been unable to point out an advantage of R-IPSS over IPSS. Such discrepancy may be attributed to the relatively small cohort size and the differences in treatment regimens. Large-scale studies are needed to confirm the validity of these findings.

PB1614

AZACYTIDINE THERAPY FOR MDS, AML AND CMML: TOLERABILITY, EFFICACY AND SURVIVAL. A SINGLE CENTRE EXPERIENCE

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Background: Azacytine (AZA) is a first-in-class hypomethylating drug with documented activity mainly in MDS, but also in AML and CMML.

Aims: We report the results of the treatment with AZA in our Centre in patients with high and low risk MDS, AML and CMML. We evaluate 1) tolerability, 2) efficacy and 3) survival data.
Methods: We reviewed 54 patients (29 males/25 females) with high-risk MDS (n=23), low-risk MDS (n=8), AML (n=19) and CMML (n=4) treated with AZA with at least 2 courses of therapy. Median age of the group was 69.5 years (29-84). Thirty-two patients (59.2%) had a transfusion-dependent anemia, eleven (20.3%) a transfusion-dependent thrombocytopenia, twenty-two (40.7%) a neutrophil count below 500/mm3 and seven (12.9%) a leucocyte count above 10.000/mm3.
Five days AZA dosing schedule was used in 26 (48,1%) patients and seven days in 23 (42.6%). Five patients (9.3%) alternated both schedules. AML and high-risk MDS patients received 7.9 (2-25) and 10.1 (3-47) median number of AZA cycles respectively.
According to the IPSS score at the time of AZA treatment onset, MDS patients (n=31) were classified as low (4), intermediate-1 (12), intermediate-2 (11) and high (4) According to IPSS-R they were low (7), intermediate (3), high (10) and very high (11).
Among high-risk MDS, AZA was the frontline treatment in 22 and second line in 1 patient. In AML patients, AZA was the frontline treatment in 7, second or third line in 10 and maintenance treatment in 2.
Results: 1) Regarding tolerability: overall, AZA was very well tolerated. Dose reduction was needed only in 4 patients: 3 due to hematologic toxicity and 1 due to non-hematologic toxicity.
2) In terms of efficacy: 25 patients (46.3%) achieved haematological improvement: 5 in AML (CR 1, PR 4), 14 in high-risk MDS, 3 in low-risk MDS and 3 in CMML. Transfusion independence was achieved in 13 patients (24.1%): 3 in AML, 8 in high-risk MDS, 1 in low-risk MDS and 1 in CMML. Bone marrow blast reduction was observed in 5 AML patients (26.3%) and in 4 (17.4%) with high-risk MDS.
3) Median overall survival was 11.8 months (1-49): 9 months in AML pts (1-24), 12.2 months in high-risk MDS (4-49), 18.1 months in low-risk MDS (4-43) and 9.7 months in CMML (5-17). In high-risk MDS as well as AML, we could not find any statistically significant association between survival and the following parameters: Sorror comorbidity index, transfusion-dependent anemia, transfusion-dependent thrombocytopenia, neutrophil count below 500/mm3 and prior chemotherapy treatment.
Summary and Conclusions: Data from the use of AZA in our Institution shows: 1) An excellent tolerance to the treatment with a high grade of compliance. 2) Good efficacy rates, with nearly half of patients achieving haematological improvement and nearly a quarter achieving transfusion independence. This benefit was seen in all subgroups of patients. 3) Sorror comorbidity index, transfusion-dependent anemia, transfusion-dependent thrombocytopenia, neutrophil count below 500/mm3 and prior chemotherapy treatment were not related to survival.

Bone marrow failure syndromes incl. PNH - Clinical

PB1615

DIAMOND-BLACKFAN ANEMIA IN A KOREAN INSTITUTE: GENOTYPING AND LONG-TERM OUTCOME

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Background: Diamond-Blackfan anemia (DBA) is characterized by a rare congenital red cell aplasia, often in association with physical anomalies, short stature, and a predisposition to cancer. DBA is a disorder of aberrant ribosome biogenesis and function. Mutations in more than 10 ribosomal proteins have been identified.
Aims: This study was aimed to characterize Korean patients with DBA. Clinical and hematological manifestations, genotyping and phenotypic correlation, treatment response to steroids, long-term outcome and the effect of L-leucine supplementation were evaluated.
Methods: Medical records of 10 patients who were diagnosed of DBA at Chonnam National University Hospital between 1992 and 2013 were retrospectively reviewed.
Results: Four patients were males. The median age at diagnosis was 3 months (range, 1 to 18 months). Median initial hemoglobin level was 5.9 g/dL (range, 1.9 – 9.4 g/dL). Heart defects were seen in 4, and polydactyly in 3. Among 9 patients tested, 7 had mutations (77%): *RPS19*, 4; *RPL5*, 2; *RPS26*, 1. All patients initially responded to steroids, having hemoglobin level ≥ 9 g/dL with the median of 10 days (range, 7-32 days). Three patients are currently off steroid therapy. Minimum dose of oral prednisolone (≤ 0.2 mg/kg/day) was required to maintain hemoglobin ≥ 9 g/dL in 6 cases. L-leucine at 2,000mg/m^2/day was supplemented in 2 patients who were short and had required transfusions despite steroid treatment. At 6 months both patients showed improvement of appetite and growth spurt as well as the increase of hemoglobin. With the median follow-up of 10 years, no malignancy has been identified. Three patients had short stature.
Summary and Conclusions: The majority of Korean DBA patients had mutations, but genotype-phenotype association was not apparent in our study. Thirty percent of patients achieved a remission, but still many patients are on steroid treatment. L-leucine as a translation enhancer seems to be beneficial without significant side effects. Further studies are warranted to closely monitor steroid side effects and the development of malignancy. The potential role of L-leucine should be validated in trials incorporating more DBA patients.

PB1616

CLINICAL AND IMMUNOLOGICAL ASPECTS OF MYCOPHENOLIC ACID TREATMENT OF PATIENTS WITH APLASTIC ANEMIA

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Background: Aplastic anaemia (AA) can be successfully treated with immunosuppressive therapies (IST) with response rates 70% and good long-term survival. Future progress may be achieved by using of new regimens and new agents for the treatment of patients with AA. One of the additional immunosuppressive agents to standard anti-thymocyte globulin/cyclosporin (ATG/CsA) schemes is mycophenolic acid (MFA) which may improve the response rate and survival of patients with AA. Scientific data show, that despite a strong theoretical rationale for its use, MFA-therapy did not result in the improvement of response when compared with historical standard ATG/CsA treatment.
Aims: To demonstrate the results of treatment of AA patients with MFA drug products.
Methods: 14 patients with AA (9-severe AA; 5-non-severe AA) were treated with MFA between 2008 and 2013 at the Russian Institute of Hematology and Transfusiology. Immune cell parameters were evaluated using multi-color flow cytometry.
Results: Positive clinical and hematological effect was observed in 10 patients. MFA therapy was effective in patients who had shown good results after the initial standard IST with inability to continue it due to adverse effects of CsA. Analysis of peripheral blood lymphoid subsets of patients with AA receiving MFA-regimens have revealed the same tendencies as for “traditional” immunosuppresive therapy: decrease of CD3+ T-cells from 87,7% to 72,6%, increase of NK-cells (CD16/56+) from 7,7% to 13,1%, decrease of activated NKT (CD16+/3+) cells from 13,1% to 9,1%, and normalization of CD19+ B-cells. Positive dynamics of the main immune parameters correlated with clinical

efficacy evidencing the influence of MFA products on the key mechanisms of AA pathogenesis, mediated via T-cell immunity. Good tolerability has been shown for two types of MFA products: Cellsept and Myfortic without any significant adverse effects, even with long duration of treatment (up to 40 months).
Summary and Conclusions: Our findings suggest the possibility of using mycophenolic acid products in the second or third line treatment of patients with AA, in which there is no possibility of standard IST with full doses.

PB1617

WOLMAN DISEASE PRESENTING AS FAMILIAL HEMOPHAGOCYTIC LYMOPHOHISTOCYTOSIS

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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is a rare, autosomal recessive disease that is usually evident in the first few months or years of life. Major signs and symptoms include hepatomegaly, splenomegaly , anaemia, leucopenia or thrombocytopenias. These manifestations resemble many inborn errors of metabolism and lysosomal storage diseases in which hemophagocytic lymphohistiocytosis have been reported as a secondary association.
Aims: Molecular characteristics of the Egyptian patients with Wolman disease presenting as FHL.
Methods: The study included patients presented to the Medical Genetics Center for genetic counseling with clinical and laboratory criteria consistent with FHL and no mutation found in known FHLH genes. Molecular diagnosis was done by sequencing of LIPA gene.
Results: Two out of 20 patients (10%) were found to have mutations in LIPA gene consistent with Wolman disease. The first is a 2 month old boy with homozygous mutation G929A (W130X) and the second is a 3 month girl with c.398delC (S133X) mutation. The absence of prominent fever, the huge hepatomegaly and the severe failure to thrive were characteristics of these patients.
Summary and Conclusions: LIPA gene should be excluded in patients with clinical and laboratory characteristics of FHL and negative molecular testing especially if not associated with prominent fever and have huge hepatomegaly or severe failure to thrive .

PB1618

THE PRESENCE OF PNH CLONE IN APLASTIC ANEMIA PATIENTS IS A PROGNOSTIC FACTOR OF GOOD ANSWER ON IMMUNOSUPPRESSIVE THERAPY

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Background: The immunosuppressive therapy (IST) increases treatment efficacy of patients with aplastic anemia (AA) significantly but 20-30% of them are still refractory. According to international research data the detected PNH clone in AA patients can be a determining factor of good response to IST.
Aims: The main purpose was both detection and monitoring of PNH clone in AA patients and evaluation of its importance for IST effectiveness.
Methods: From May 2011 till November 2013 38 de novo AA patients were studied, the follow-up median (Me) was 12 months, and the median age was 26 years. The patients were divided into two groups: the 1st one consisted from patients with PNH clone (>0,01%), and the 2d one from patients without PNH clone. All patients underwent IST (ATG and cyclosporine). The treatment response was evaluated as hematological improvement (HI) in case if hemoglobin>80 g/l, granulocyte>1,0x10^9/l (for SAA>0,5 x10^9/l), Tr>30x10^9/l). PNH clone was detected by flow cytometry following ICCS guideline with sensitivity 0,01% on granulocytes.
Results: Before IST the PNH clone was detected in 55,2% of patients: Me RBC – 0,1%, Gr – 0,76%, Mon – 5%. HI had been reached by 3 months at 47,4% of patients, 77,8% of them were from 1st group. Among the patients who had not achieved HI (52,6%) by 3 month after therapy started many of them were from 2d group (65%). Other 35% of not HI patients were from 1st group and their difference was only 5,8% PNH clone on monocytes *versus* 0,7% in HI positive patients from 1st group. When the final analysis was made 73,7% of patients had reached HI: 47,4% from 1st group and 26,3% from 2d group in which six patients showed PNH clone appearance and persistence during IST. In patients from 1st group with HI some changes in PNH clone size were observed: in one patient PNH clone disappeared or became less 0,01% and 3 of them showed an increase of the clone size with clinical intravascular hemolysis.
Summary and Conclusions: Positive response was more achieved in the group of patients with initially detected PNH clone. We had observed the appearance and persistence of PNH clone in patients with positive IST response initially negative by PNH clone.

PB1619

EVALUATION OF SPECIMEN STABILITY FOR DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) USING FLOW CYTOMETRIC MEASUREMENT OF MONOCYTIC CD55/CD59

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Background: Flow cytometric analysis of glycophosphatidyl-inositol (GPI)-linked proteins, CD55/CD59 expressed on the surface of neutrophils and monocytes is a fast, inexpensive method for diagnosis of Paroxysmal nocturnal hemoglobinuria (PNH). Although CD55/CD59 analysis of neutrophils is the most sensitive method, it has limitations that flow cytometric analysis should be performed within 4 hours after the collection of specimens. In cases of referred specimens from other laboratories, it is practically impossible to proceed the test within 4 hours.
Aims: In this study, we analyzed the CD55/CD59 expression on the surface of monocytes which are relatively stable than red blood cells and granulocytes, and we compared the effect of time after specimens were prepared.
Methods: Specimens from 53 patients who were suspected as PNH were included in the flow cytometric analysis of CD55, CD59 expression on the surface of red blood cells, neutrophils and monocytes. In order to examine the effects of time, three PNH- negative specimens were analyzed for the measurement of CD55, CD59 in three types of cells at time of 0, 4, 8, 24, 48, 72 hour after the specimens were prepared.
Results: 24 specimens among 50 samples showed positive results in PNH study with less than 95% expression of CD55 or CD59. The remaining 29 specimens were negative in PNH study with more than 95% expression of both CD55 and CD59. CD55/CD59 positivity rate of the 24 specimens was 76.2%/80.9%, 90.5%/90.5%, 81.9%/90.5% for red blood cells, neutrophils and monocytes, respectively. There was no effect in results measured on three types of cells at different time periods.
Summary and Conclusions: : In patients with PNH-positive results, the positivity rate of CD55/CD59 in monocytes was higher than that in red blood cells, wheras that of CD59 was similar in neutrophils. The measurement of CD55/CD59 in monocytes is useful for the diagnosis of PNH when CD55/CD59 is negative in red blood cells but positive in neutrophils. Previous studies have recommended that CD55/CD59 analysis in neutrophils should be done within 4 hours of specimen collection. However, we have demonstrated that three types of cells including red blood cells and monocytes were all stable 72 hours after the specimen collection.

Myeloma and other monoclonal gammopathies - Biology

PB1620

CD137 LIGAND SIGNALING INDUCES OF PROLIFERATION AND SURVIVAL OF MULTIPLE MYELOMA CELL

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Background: CD137 ligand (CD137L) is mainly expressed by antigen presenting cells, including mature dendritic cells, monocytes /macrophages and B cells. While, the new studies have testified that the CD137L is expressed by various carcinoma cells too. However, the expression of CD137L and the function on multiple myeloma cells is unknown.
Aims: To explore the expression of CD137L and the function on multiple myeloma cells.
Methods: We identified the constitutive expression of CD137L by flow cytometry on U266, RPMI 8226, LP1, MY5 and KMS-11 of Multiple myeloma (MM) cell lines as high as 96%, 97.5%, 89%, 93% and 94%.But, CD137 expressed on the cell surface was low as 4%, 5%, 1%, 2%, 5% respectively. Now that, CD137L was expressed very strongly on MM cell lines, next, we investigated CD137L expression on primary MM cells from 126 BM samples of patients diagnosed of active multiple MM. We found CD137L protein was expressed by a select group of CD45-CD38++CD138+cells (n=126), but not as higher as MM cell lines. Separating those cases in different grouping according to osteolytic bone disease and plasmacytoma, we found highest expression of CD137L in cases with no osteolytic bone disease or no plasmacytoma and the lowest levels of CD137L in cases with more osteolytic bone disease(*P*=0.012). However, CD137L expression was not or hardly detectable on normal plasma cells confirmed by CD45+CD38++CD138+ CD56- CD19+.
Results: *in vitro* study, U266 cells were cultured in 24-well plates or in 96-well plate pre-coated with mAb 1F1 or irrelevant mouse IgG. The proliferation and survival of U266 was enhanced by stimulating- CD137L mAb (1F1) than those induced by control mouse IgG by cell counting and CFSE assay at incubation for 48h. In addition, the cell cycle analysis showed that CD137L induces proliferation and increases the number of cells in the S phase from 36.1% to 42.5% after 72h incubation. Apoptosis was assessed by the externalization of phosphatidylserine, assayed by the Annexin V and PI apoptosis detection. The U266 cells were stained at incubation for 48h and analyzed by flow cytometry. The percentage of apoptosis cells was 19.6% *VS* 21.2% with no statistical significance. We surveyed the cytokine profiles during the incubation of U266 cells cultured for 2 days with different stimuli with mAb 1F1 compared with the control group. Intracellular cytokine staining showed that treatment of cells with 1F1 increased the production of IL-6 from 3.8% to 63.9% by Flow cytometry. When neutralizing anti-IL-6 mAb was added to the culture medium, the cells were cultured for 48 h in pure medium or plus Fc or CD137–Fc protein and the cell proliferation measured by WST-8 was 0.79 *VS* 0.80 *VS* 0.72, the average of 6-well plates absorbance.1F1-induced cell proliferation was effectively inhibited. An increase of these cytokines might explain why CD137L expression could stimulate the proliferation of U266. Finally, the U266 cells were treated with bortezomib and the growth of cells was analyzed by WST-8 assay. It demonstrated that bortezomib could inhibit the function of 1F1 and the inhibition ratio of bortezomib was 22%, 51% and 58% at 24h, 48h and 72h.
Summary and Conclusions: In our study, CD137L is a promoting proliferation factor. This suggests the possibility that its expression on MM cells may be directly target for immunomodulatory therapy for MM.

PB1621

EXPRESSION OF ANGIOGENIC FACTORS AND MATRIX-METALLO PROTEINASES IN BONE MARROW AND PERIPHERAL BLOOD PLASMA CELLS OF MULTIPLE

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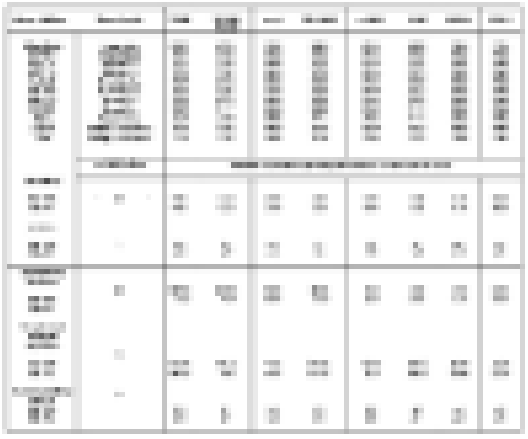
Background: Bone Marrow (BM) angiogenesis is involved in the pathogenesis and progression of multiple myeloma (MM). Moreover, studies on BM biopsies indicate that changes in BM composition from benign conditions such as M-GUS to early stage multiple myeloma (smoldering myeloma, SMM) and to advanced multiple myeloma (MM) follow a precise pattern, including an increase in the degree of angiogenesis and secretion of matrix-metalloproteinase-degrading enzymes (MMP) (1).
Aims: We compared the angiogenic potential and MMPs gene expression in BM and peripheral blood plasma cells from M-GUS, SM and active MM patients.
Methods: Among the pro-angiogenic factors and the MMP enzymes, we analysed by PCR the expression of VEGF, Ang-2, MUC18/MCAM, bFGF, and MMP-2 and MMP-9. e-CADH (epithelial cadherin) and the VE–CADH (vascular endothelial cadherin) genes. Epithelial and MM cell lines were used as controls. PC were separated from BM and PB specimen with antibodies against CD138 as described by manufacturer (Voden-Stem Cell Technology Inc., Milan, Italy) from patients with the following diagnoses: 7 M-GUS, 20 SMM, 20 MM, (4 of the were extramedullary MM). After total cellular RNA extraction, we performed oligo- $d(T)_{16}$-RT and primer-specific-PCR. Primer sequences were designed in house. The house-keeping b-2-microglobulin gene was used as internal control.
Results: Molecular expression of angiogenic factors and metallo-proteinases is summarized in the Table 1 below. BM-PC expression pattern of angiogenic factors and MMP was more restricted compared to PB-PC. Expression was constantly absent in M-GUS PC with exception for a weak MMPs positivity for PB-PC. No angiogenic markers were expressed alone, but always in association with others including VEGF+MUC18/MCAM+Ang2 (particularly in SMM) or bFGF and the MMPs (particularly, in 5 SMM and the symptomatic MM). Similarly to M-GUS, 7 out of 20 SMM did not co-express angiogenic factors, while the remaining 13 co-expressed several markers. We detected e-CADH expression in 100% of BM-PC (16/16) and in 87.5% (14/16) of symptomatic MM and in 5 SSM which shared a similar expression pattern of angiogenic factors. No e-CADH expression was detected in extra medullary BM-PC and in the PB-PC of MM.

Table 1.



Summary and Conclusions: Our preliminary data indicate that increasing angiogenic factors expression follows the disease *spectrum* from M-GUS to MM. In particular, early markers of angiogenesis such as VEGF, Ang2 and MUC 18/MCAM seem to be expressed on SMM while the co-expression of all the above markers together with bFGF and the MMPs is associated with the active disease. Our data on e-CADH, an epithelial cell adhesion molecule, found to be expressed by MM-PC represent a novel unexpected finding deserving further investigation.

Reference

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PB1622

LIGHT INVOLVEMENT IN MULTIPLE MYELOMA-OSTEOLYTIC BONE DISEASE

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Background: Multiple myeloma (MM)-osteolytic bone disease occurs in about 70% of newly diagnosed patients, and up to 90% at relapse. It is characterized by an unbalanced bone remodelling, due to increased osteoclast (OC) activity and impaired osteoblast repair. Osteoclastogenesis is regulated by members

of TNF superfamily. Among these, there is LIGHT/TNFSF14 recently implicated in the pathogenesis of joint diseases with increased bone resorption, as the rheumatoid arthritis. LIGHT is expressed by activated T-cells, monocytes, granulocytes, spleen cells, and immature dendritic cells, and it is described as a potent T-cell co-stimulatory molecule.
Aims: Here, we purposed to investigate a possible role of LIGHT in the mechanisms of the enhanced osteoclastogenesis occurring in MM-osteolytic bone disease.
Methods: Peripheral blood (PB) and bone marrow (BM) aspirates were obtained from 40 patients (23M/17F, median age: 64 years), newly diagnosed as having symptomatic MM with or without osteolytic bone disease, smoldering MM (sMM) or Monoclonal Gammopathy of Undetermined Significance (M.G.U.S.). Osteolytic lesions were detected by skeleton standard radiography, and in some cases also by spine and pelvis nuclear magnetic resonance. The control group included PB and BM aspirates from 15 patients with non-neoplastic disease without any skeletal involvement as well as PB from 25 healthy donors matching for age and sex with the patients' group. Patients and controls gave their written informed consent to the study, approved by the local Ethical Committee and performed according to the Declaration of Helsinki. By means of flow cytometry, Western Blotting and real-time PCR, LIGHT expression was evaluated in freshly purified CD14+ monocytes, CD2+ T-cells and neutrophils from PB and BM aspirates of patients and controls. OCs were obtained from unfractionated PB mononuclear cells (PBMCs) cultured in the presence or in the absence of an anti-LIGHT neutralizing monoclonal antibody (mAb). Mature OCs were identified as multinucleated tartrate-resistant acid phosphatase (TRAP) positive cells.
Results: In the CD14+ monocytes, $CD2^+$ T-cells and neutrophils isolated from PB and BM of patients with MM-osteolytic bone disease, at both protein and mRNA levels LIGHT was found more expressed than in the cells isolated from the patients with symptomatic MM without bone disease, sMM, M.G.U.S, non-neoplastic disease without any skeletal involvement, and healthy donors. The *in vitro* effect of the anti-LIGHT mAb on osteoclastogenesis resulted in a significant reduction of the OC formation ($p<0.001$).
Summary and Conclusions: Our findings support a possible involvement of LIGHT in the mechanisms of the osteoclastogenesis occurring in MM-osteolytic bone disease.

PB1623

BORTEZOMIB INDUCED EXPRESSION OF HEME OXYGENASE 1 VIA ENDOPLASMIC RETICULUM STRESS

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Background: The proteasome inhibitor Bortezomib (BO) is one of the most effective drugs in the treatment of Multiple Myeloma (MM) but eventually patients became refractory to this drug. Heme Oxygenase 1 (HO-1) is a cytoprotective microsomal enzyme that catalyzes the degradation of heme into to carbon monoxide, biliverdin and ferrous iron. It plays a pivotal role in inflammation, oxidation and apoptosis and it is increased by endoplasmic reticulum (ER) stress. It has been also recently described that Bortezomib is able to increase HO-1 expression (Barrera, Cell Cycle 2012).
Aims: We have investigated the effect of BO on 2 MM cell lines (U266, SKM-M1). As expected, we observed that BO induced apoptosis after 24h of incubation. We have studied the ability of BO to induce endoplasmic reticulum (ER) stress and we have used as positive control the effect of Thapsigargin, a known inducer of ER stress.
Methods: Apoptosis was evaluated by cytometric analysis (annexin V). Using western blot, we assessed HO-1 and protein markers of ER stress. The compartimentation of HO1 was observed in myeloma cell lines by confocal microscope
Results: Similarly to Thapsigargin, BO was able to induce the expressionof Bip, IRE1alfa , Ero1, PERK and CHOP (some proteins involved in the activation of ER stress) in multiple myeloma cells after 6 h with a peak after 24 h of treatment. Noteworthy, the increased expression of CHOP has to be considered as an index of ER-stress-induced apoptosis, suggesting that induction of apoptosis in multiple myeloma cells by BO can be attributed also by triggering ER stress. Both BO and thapsigargin were also able to induce a significant increase in mRNA levels of HO-1 after 3 hours (h) of treatment with a maximum peak after 6h.All these effects were reverted by adding the chemical chaperone 4-Phenylbutyric acid (4 – PBA). Moreover, flow cytometry analysis revealed that the levels of reactive species of Oxygen (ROS) were increased after 1h of BO treatment with a peak after 3h. By confocal microscope we observed that HO-1 is localized both in the cytoplasm and also in the nucleus of MM cell line and that blockage of HO 1 nuclear translocation, with a selective inhibitor of the protein cleavage, E64, induces this MM cell line to become more sensitive to the cytotoxic effect of BO.
Summary and Conclusions: Our data suggest that BO- induction of HO1 is probably due to the ability of bortezomib to induce ER stress. We hypothesize that HO-1 induction may be involved in the mechanisms of MM drug resistance and that the cytotoxic effect of bortezomib can be potentiated when HO-1 is inhibited to translocate into the nucleus of MM cells.

PB1624

SPHINGOSINE 1-PHOSPHATE AS A THERAPEUTIC TARGET IN MULTIPLE MYELOMA CELLS

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Background: Multiple myeloma (MM) is one of the common hematological malignancies and is a uniformly fatal disorder of B cells characterized by accumulation of abnormal plasma cells in the bone marrow. Clinical outcomes for patients of MM have improved with the treatment, including the proteasome inhibitor. However many patients have a relapse of disease and develop drug resistance. Since the prognosis remains poor for patients with refractory disease, the new therapeutic strategies are required to treat against refractory MM patients. Sphingosine-1-phosphate (S1P) is a potent bioactive sphingolipid. Two isoforms of sphingosine kinases (SphKs), SK1 and SK2, catalyze the formation of the S1P in mammalian cells. SphK has also been shown to be up-regulated in the variety of cancer types.
Aims: S1P is involved with cell proliferation, angiogenesis, cell transformation, oncogenesis and cell survival in malignancies such as multiple myeloma. One of the S1P analogue, fingolimod (FTY720), which is an orally active immunomodulatory drug and developed for the treatment of multiple sclerosis. SKI-I, which is a non-lipid pan-SphK inhibitor and ABC294640, which is selective inhibitor of SK2, is currently being investigated in a pivotal phase 1 clinical trial against solid tumors. Therefore S1P and SphKs may present attractive targets for MM treatment.
Methods: In this study we investigated the efficacy of fingolimod, SKI-I and ABC294640 by the use of MM cell lines, which is RPMI8226, MM1.S, MM1.R, and primary MM patients samples.
Results: 72 h treatment of fingolimod exhibits cell growth inhibition of MM cell lines in a dose dependent manner. Treatment of SKI-I and ABC294640 also exhibits cell growth inhibition in a dose dependent manner. Because S1P is the ligand for a family of five G-protein-coupled receptors with distinct signaling pathways that regulate angiogenesis and chemotaxis, we next evaluated the chemotactic response of human umbilical vein endothelial cells (HUVEC). We determined the S1P receptors expression profiles of HUVECs by RT-PCR. Our results indicated that at least S1P1 and S1P3 were expressed in HUVECs. We found that 4 h treatment of S1P significantly induced the migration of HUVECs. Treatment of HUVECs with fingolimod inhibited S1P-stimulated chemotaxis. We also found that S1P-induced chemotaxis of HUVECs was abolished by the SKI-I and ABC294640. These facts suggested that intracellular SK1 and SK2 may play the important role in S1P induced chemotaxis of HUVEC. We next investigated the S1P concentrations in MM patients by enzyme-linked immune sorbent assay (ELISA) because S1P may be a potent tumorigenic growth factor that releases from tumor cells. We found that plasma concentrations of S1P were significantly higher in patients with MM compared with normal samples. We also found that conditioned medium from MM cell line had chemotactic activity for HUVECs.
Summary and Conclusions: These results implicate that S1P may be a novel biomarker for early stage of MM and S1P might be an important bioactive sphingolipid involved in angiogenesis. In this study, we also demonstrate that fingolimod, SKI-I and ABC294640 have potent preclinical anti-tumor activity in MM and also inhibit the angiogenesis. These facts offer unique opportunities for novel therapeutic strategies for the treatment of multiple myeloma.

PB1625

MAST CELL DENSITY IN BONE MARROW CORRELATES WITH BOTH ANGIOGENIC AND PROLIFERATIVE ACTIVITY IN MULTIPLE MYELOMA

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Background: There is increased mast cell density (MCD) in multiple myeloma (MM) bone marrow (BM), being correlated with the disease stage. Mast cells produce various mediators, promoting MM growth in multiple manners.
Aims: The purpose of this study was to estimate whether BM MCD correlates with markers of disease activity, such as levels of LDH, BM Ki-67 proliferation index, and with the angiogenic potential, estimated by BM microvascular density (MVD) in MM.
Methods: We studied in 42 newly diagnosed active MM patients (19 male,

mean age 66.5±10.1 years, 26 IgG, 12 IgA, 4 light chain, ISS: 12 stage I, 14 II and 16 III), and in 22 healthy controls the immunohistochemical expression of CD31, mast cell thryptase, Ki-67 and CD38, in order to estimate MVD, MCD, Ki-67 proliferation index (Ki-67 PI) and infiltration by plasma cells respectively, as well serum levels of LDH.
Results: All values were higher in active MM patients compared to healthy population ($p<0.001$ for all cases). They also were in parallel with ISS stage ($p<0.003$ for infiltration, $p<0.004$ for LDH and $p<0.001$ for the other cases). Moreover, BM MCD correlated positively with Ki-67 PI ($r=0.537$ $p<0.0001$), BM infiltration ($r=0.431$ $p<0.004$) and MVD ($r=0.360$ $p<0.02$).
Summary and Conclusions: Mast cells are inflammatory cells, increased in MM BM, participating in many aspects of the disease. They may release various mediators, increasing directly and indirectly myeloma growth. They may also contribute to increased angiogenesis, through various mechanisms. Overall, mast cells seem to be important cells in MM biology, rendering them alluring targets for therapy.

PB1626

MYELOID AND LYMPHOID IMPAIRMENT IN MONOCLONAL GAMMOPATHY: AN IMMUNOPHENOTYPE STUDY
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Background: In Multiple Myeloma (MM), but not in the monoclonal gammopathy of unknown significance (MGUS), the immune function is impaired as consequence of an immunologically hostile microenvironment and cellular defects, including reduction of immuno-surveillance and T-cell immunoparesis. Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of myeloid cells with peculiar immunosuppressive properties against T-cells, well described in solid tumors and recently evaluated in MM.
Aims: We hypothesized immune suppression, detectable at the same time in both myeloid and lymphoid arms can play a role in favoring MM progression and its clinical presentation.
Methods: We evaluated by flow cytometry main suppressive subpopulations investigable in peripheral blood within lymphoid (T-reg, CD62L T cells) and myeloid arm (three phenotypes of MDSC: mo-MDSC, G-MDSC, CD34+-MDSC) in 30 newly diagnosed MM patients treated upfront with lenalidomide compared with 30 MGUS and 30 healthy subjects matched for sex and age.
Results: We found that MM patients exhibit an increased levels of percentage of circulating G-MDSC ($p<0.001$) and mo-MDSC ($p=0.029$) and absolute number of CD34+MDSC ($p=0.009$), with the concomitant down-regulation of CD62L expression on CD8+T-lymphocytes ($p<0.0001$) and an increased percentage of T-reg ($p=0.01$). All patients except 3 obtained at least a partial remission (reduction of more than 50% of monoclonal component present at baseline). After induction therapy, all MDSC subtypes evaluated were significantly reduced ($p=0.003$). None of the immunological parameters at baseline was predictive of the response quality achievable or of progression free survival (PFS), except percentage of G-MDSC. Patients with G-MDSC>60% had a longer PFS than those with less than 60% (13.9 *versus* 42.6 months, $p=0.028$). G-MDSC percentage was independent from any clinical variable at diagnosis except the neutrophils to lymphocyte ratio (r square 0.60, $p<0.0001$). T-reg cells progressively reduced from the first to the last administered cycle (respectively, $p<0.001$).
Summary and Conclusions: Taken together our data suggest that MM expansion needs immunoparesis involving T-reg and MDSC cells in order to maintain poor tumor-specific immune response, phenomenon that can be overcome by new drugs, such as lenalidomide.

PB1627

Abstract withdrawn

PB1628

IMMUNOHISTOCHEMICAL EXPRESSION OF ENDOGLIN ESTIMATES BONE MARROW NEOANGIOGENESIS IN MULTIPLE MYELOMA
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Background: Bone marrow (BM) microvascular density (MVD), represents the angiogenic process and is prognostic in multiple myeloma (MM). Endoglin (CD105) is highly expressed on endothelial cells in sites of active neoangiogenesis but not when they are in rest. The estimation of CD105-MVD seems to be prognostic in several solid tumors.
Aims: The purpose of the study was to estimate if endoglin is a reliable marker of BM neoangiogenesis in MM.
Methods: We measured in 47 patients with active MM (24 male, mean age 67.4±10.4 years, 29 IgG, 14 IgA, 4 light chain, ISS: 10 stage I, 17 II and 20 III), in 19 of them who responded to bortezomib-based therapy (8 in CR, 11 in VGPR), and in 20 controls: serum levels of soluble CD105 and basic-fibroblast growth factor. We also estimated immunohistochemically BM MVD using both CD31 (being an established marker) and CD105.
Results: All values were higher in active MM, in parallel with ISS stage and decreased after effective treatment. CD105-MVD estimation was lower than CD31-MVD both in the entire group of active MM patients and in each stage separately ($p<0.001$ for all cases), but not in remission patients. CD105-MVD correlated with all angiogenic markers.
Summary and Conclusions: CD105-MVD is following the known behavior of CD31-MVD in MM, suggesting that it is a reliable marker of BM neoangiogenesis in MM. Its prognostic impact remains to be proven.

PB1629

CIRCULATING LEVELS OF SOLUBLE FAS LIGAND IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) plasma cells seem to be apoptosis-resistant. Therefore, the system of Fas/FasL may possess other properties.
Aims: The purpose of the study was to correlate serum levels of soluble Fas ligand (Fas-L) in MM patients with markers of disease activity.
Methods: We studied 57 newly diagnosed patients with active MM (28 male, median age 67 years, range 54-83 years, with paraprotein: IgG in 33, IgA in 18 and light chain disease in 6 patients, concerning staging: 13 in ISS I, 22 in II and 22 in III) and 22 age and sex-matched, healthy controls. Values of beta-2 microglobulin (B2M) and C-reactive protein (CRP) were measured on routine examination. Serum levels of soluble Fas-L and interleukin-6 (IL-6) were measured by ELISA, whereas bone marrow infiltration by malignant plasma cells was estimated immunohistochemically using CD38 as marker (routine examination).
Results: Serum levels of measured parameters were higher in active MM patients compared to controls ($p<0.001$ for all cases). All values were also increasing in parallel with the disease ISS stage ($p<0.001$ for all cases). Significant positive correlations were found between serum levels of soluble Fas-L with IL-6 ($r=0.460$), percentage of BM infiltration ($r=0.490$ $p<0.001$ for both cases) and LDH ($r=0.271$ $p<0.04$), but not with values of CRP and B2M.
Summary and Conclusions: It seems that serum levels of soluble Fas-L are in parallel with disease activity. This observation suggests that the system of Fas/Fas-L may not induce apoptosis in MM cells but, on the contrary, may promote tumor growth, using other, non-apoptotic pathways. The non steady correlation with all markers of disease activity suggests an indirect manner on promotion of MM growth. By these means, activation of Fas/Fas-L system in MM bone marrow microenvironment may up-regulate invasion-related genes, may promote recruitment of pro-inflammatory cells and moreover may possess immunosuppressive role against T cell mediate cytotoxicity.

PB1630

ANTINEOPLASTIC EFFECTS OF VARIOUS TARGETED DRUGS ON PRIMARY MYELOMA CELLS AND FIVE DEFINED MYELOMA CELL LINES
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Background: Multiple Myeloma (MM) is a plasma cell malignancy characterized by monoclonal paraproteinemia, bone marrow plasmocytosis, and osteopathy. During the past few years, novel effective treatment strategies have been developed, but MM is still a clinical challenge and many patients relapse. Therefore, several attempts have been made to identify new effective agents in MM.
Aims: We examined the effects of 15 targeted drugs on growth and survival of five defined MM cell lines (MM1.S, NCI-H929, OPM-2, RPMI8226, U266).
Methods: Apoptosis was measured by morphology evaluation and microscopy as well as by Annexin/PI-staining and Caspase-3-staining by flow cytometry and proliferation was determined by ^{3}H-thymidine uptake. In consecutive experiments, we examined the effects of the most potent drugs on apoptosis

and proliferation of primary neoplastic cells obtained from patients with MM
Results: Of all drugs tested, the PI3-kinase blocker NVP-BEZ235, the pan-Bcl-2 inhibitor obatoclax, the Hsp90-inhibitor 17AAG, and the PLK-1 blocker BI2536 showed major growth-inhibitory and apoptosis-inducing effects (IC50<1μM) in all cell lines tested. All four compounds were found to inhibit the *in vitro* proliferation of MM cells at pharmacologically meaningful concentrations (IC50 <1 μM). Growth-inhibitory drug effects were accompanied by signs of apoptosis. Apoptosis-inducing effects of 17AAG, BI2536 and NVP-BEZ235 were seen in primary bone marrow-derived myeloma cells as well as in the CD20+/CD27+/CD138- subfraction of putative myeloma stem cells. Next, we examined cooperative antineoplastic effects of these drugs. Major cooperative effects on proliferation of MM cell lines were obtained with the following combinations: 17AAG+NVP-BEZ235 (MM1.S, OPM2, RPMI8226, U266), 17AAG+BI2536 (MM1.S, OPM2, RPMI8226, U266), 17AAG+obatoclax (MM1.S, NCI-H929, OPM2, RPMI8226), BI2536+NVP-BEZ235 (MM1.S, NCI-H929, OPM2, RPMI8226), BI2536+obatoclax (MM1.S, OPM2, RPMI8226), and NVP-BEZ235+obatoclax (MM1.S, RPMI8226).
Summary and Conclusions: In conclusion, several different targeted drugs induce anti-proliferative and apoptosis-inducing effects on human MM cells. Whether these effects also occur *in vivo* in MM patients remains so far unknown.

PB1631

SERUM LEVELS OF INTERLEUKIN-22 CORRELATE WITH MYELOMA ACTIVITY

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Background: Interleukin-22 (IL-22) is a pro-inflammatory cytokine, participating in several aspects of diseases.
Aims: The purpose of the study is to estimate the participation of IL-22 in the inflammatory process of multiple myeloma (MM).
Methods: We studied 51 newly diagnosed active MM patients (25 male, mean age 69.4±13.2 y, 25 IgG, 14 IgA, 2 light chain, ISS: 14 stage I, 16 II and 21 III), 22 of them after effective treatment (CR and VGPR) and 18 controls. We measured serum levels of IL-22, IL-1beta, B2microglobulin (B2M) and paraprotein, as well the degree of bone marrow infiltration.
Results: Serum levels of IL-22, IL-1beta and B2M were significantly higher in active MM patients (p<0.001 for all cases). All values were increasing in parallel with ISS stage (p=0.025 for paraprotein, p<0.001 for the other cases). Serum levels of IL-22 correlated positively with IL-1beta (r=0.614), B2M (r=0.631) and infiltration (r=0.566, p<0.0001 for all cases), but not with paraprotein level. All values decreased significantly in the responders (p<0.001 for all cases). It is remarkable that pre-treatment values of IL-22 were, not significantly, lower in responders compared to entire group. Furthermore, post-treatment values of IL-22 were significantly higher in responders compared to controls (p<0.001).
Summary and Conclusions: IL-22 follows MM activity, suggesting an important participation in its biology. This increased occurrence of IL-22 in bone marrow microenvironment may enhance myeloma proliferation and growth, and moreover, may participate in the mechanisms of immune deregulation of the disease. It seems to be part of the inflammatory process, which remains active even after remission.

PB1632

SERUM LEVELS OF SELECTED PARAMETERS OF BONE MICROENVIRONMENT PREDICT OVERALL SURVIVAL IN MULTIPLE MYELOMA PATIENTS REGARDLESS OF TREATMENT REGIMEN USED

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Background: Several cytokines have been identified as potent inductors of myeloma bone disease (MBD) or growth factors in multiple myeloma (MM). Their serum levels often correlate with the extent or aggressiveness of the disease, and, even with prognosis of MM.
Aims: Our aim was to assess serum levels of selected parameters of bone marrow microenvironment with respect to survival functions.
Methods: We have assessed serum levels of 6 selected parameters of bone marrow microenvironment – hepacyte growth factor (HGF), syndecan-1 (SYN), osteopontin (OPN), macrophage inhibitory protein 1α and 1β (MIP-1α, MIP-1β), osteoprotegerin (OPG) and endostatin (END) in a cohort of 52 patients with MM with regard to overall survival (OS), progression free survival (PFS), time to progression (TTP) and duration of response (DOR) after induction treatment line. The patients were treated using biological based regimens (*i.e.* with thalidomide, bortezomib and lenalidomide) or with high-dosed chemotherapy with support of autologous stem cell transplantation. For statistical estimation we used Mann-Whitney U test, ROC analysis, Kaplan-Meier analysis, Cox regression and log rank test at p<0,05.
Results: Out of the 6 evaluated parameters, we found significant correlation with OS in HGF (p=0,042), OPN (p=0,006) and OPG (p=0,029), the other parameters did not have significant differences. Based on these results we constructed ROC curves to find optimal cut-off value with the best discriminatory potential. For HGF the optimal cut-off value was 1919 ng/l with 70% sensitivity and 68% specificity. Patients with HGF<1919ng/l had significantly longer OS than patients with HGF ≥ 1919ng/l (M 6,6 *vs* 3,1 years, p=0,001). In the case of OPN, the optimal cut-off level was 97 ug/l with 68% sensitivity and 84% specificity, dividing the cohort into a group of patients with good prognosis with OPN<97 ug/l and poor prognosis with OPN ≥ 97 ug/l (M 8,0 *vs* 2,9 years, p<0,0001). The optimal cut-off level for OPG was 5,2 pmol/l with 69% sensitivity and 64% specificity. Patients with OPG<5,2 pmol/l had a benefit in survival with respect to patients with OPG ≥ 5,2 pmol/l (M 6,1 *vs* 3,6 years, p=0,015). There were no significant differences between any of the selected parameters with respect to induction treatment line, *i.e.* to PFS, TTP and DOR. Using the logistic regression and Cox regression analysis we constructed a model for short survival. Significant predictors of short survival were OPN ≥ 101 ug/l and OPG ≥ 5,2 pmol/l. OPN ≥ 101 ug/l significantly increased the risk of poor prognosis 5,3 times (95%CI: 1,4 - 19,9; p=0,013). OPG ≥ 5,2 pmol/l significantly increased the risk of poor prognosis 4,3 times (95%CI: 1,2 - 16,2; p=0,030).
Summary and Conclusions: Analysis of serum levels of the parameters of bone microenvironment contributes to closer understanding of biological mechanisms occuring in MM. Our analysis revealed three parameters with prognostic potential in multiple myeloma. Patients with high serum levels of hepatocyte growth factor, osteopontin and osteoprotegerin had significantly shorter overall survival regardless of treatment modality used. There were, however, no differences in PFS, TTP and DOR after the first line treatment suggesting long term outcomes of bone microenvironment processes rather than short term influence of the tumor burden.
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PB1633

ROLE OF MONOCLONAL AND POLYCLONAL COMPONENTS IN THE EVALUATION OF K/L RATIO IN PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS)

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Background: In a previous study we observed a significant correlation between immunoparesis and abnormal K/L ratio in patients with MGUS. We confirmed these results after an updated analysis of 123 cases. In patients with immunoparesis (n-64) K/L ratio is abnormal in 43 (67,1%), in those without immunoparesis (n-59) it is abnormal in 23 (38,9%) (P<0,001). K/L ratio can be altered due to the monoclonal chain levels increase or to polyclonal chain levels decrease. The first phenomenon is related to the burden disease, while the second is caused by a physiological loss of immune response.
Aims: Aim of this study was to investigate if both the phenomena are present in MGUS patients and to verify which one is a stronger predictor of progressive disease.
Methods: Patients were stratified in five groups (according to the polyclonal light chain value): 0 when the value was below the lower limit, 5 when it was above the upper limit and 1, 2, 3 and 4 according to the quartile in the normal range. We then correlated polyclonal light chain levels decrease (groups 0,1,2) with K/L ratio and with the presence of immunoparesis. Monoclonal light chain levels increase could be another cause of abnormal K/ L ratio so we correlated monoclonal light chain increase beyond the upper limit of normal range with K/L ratio.
Results: In patients with normal K/L ratio (n-57), polyclonal chain levels are decreased in 28 (49,1%). In those with abnormal K/L ratio (n-66), they are decreased in 54 (81,8%). P<0,0001. In patients without immunoparesis (n-59) polyclonal chain levels are decreased in 34 (57,6%), while in those with immunoparesis (n-64), they are decreased in 49 (76,5%). P=0,034. In patients with normal K/L ratio (n-57) the monoclonal chain levels are upper normal range in 22 (38,5%). In those with abnormal K/L ratio (n-66%) it is upper normal range in 60 (90,9%). P<0,0001. We could not stratify patients according to the monoclonal component (IgG *vs* IgA) because of the limited number of cases included in this study.

Summary and Conclusions: These data suggest that an abnormal K/L ratio could be related either to a reduced activity of immune system with a lower production of polyclonal light chains either to a higher production of monoclonal chains due to the burden disease. We need a longer follow up and a larger number of patients to clarify the role of these two factors in predicting the risk of progression.

PB1634

THE ROLE OF ADIPOCYTOKINES IN MONOCLONAL GAMMOPATHIES – A PRELIMINARY STUDY

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Background: Multiple myeloma (MM) is a neoplastic disease characterized by the growth of malignant plasma cells in the bone marrow (BM) with a subsequent overproduction of a monoclonal protein and development of bone lesions and soft-tissue masses. This malignancy starts with a monoclonal gammopathy of undetermined significance (MGUS). In the transition from MGUS to MM, the BM microenvironment is believed to have a central role by producing cytokines as Interleukin-6, Interleukin-1b and tumor necrosis factor, that are correlated with the induction of angiogenesis and suppression of cell-mediated immunity, which are important factors for the progression to MM. Adipocytokines are a group of hormones produced by the adipose tissue that play an important role in energy homeostasis, hematopoiesis, immunity, inflammation and angiogenesis. Moreover, adipocytokines can exhibit pro-inflammatory (*e.g.* leptin) or anti-inflammatory (*e.g.* adiponectin) properties. There are studies that have shown that adipocytokines may have a role in the pathogenesis of some cancers such as colon and breast cancer and even hematological malignancies such as acute myeloid leukemia. Recent evidence showing that increased body mass index has a positive association with both mortality and incidence of MM, suggests that adipocytokines may have a role in the pathogenesis of MM.
Aims: This work aim to explore the role of adipocytokines, namely adiponectin, leptin, resistin, MCP-1 and TNF-α in the pathogenesis of Monoclonal Gammopathies and correlate these adipocytokines levels with patient's clinical characteristics.
Methods: A total of 34 monoclonal gammopathies patients (15 MGUS and 19 MM patients, those 3 with smoldering MM and 16 with symptomatic MM) and 37 healthy individuals were included in this study, after informed consent. MGUS patients had an average age of 70,9 years (ranged between 41 and 90 years), 60% female and 40% male. MM patients age ranged between 56 and 86 years (average of 73,5years), being 52,6% female and 47,4% male. Adiponectin, leptin, resistin, MCP-1 and TNF-α levels were quantified on the peripheral blood (PB) and BM using ELISA commercial kits.
Results: Our preliminary results have shown higher levels of resistin in PB of patients with monoclonal gammophaty (15,65±13,96 ng/mL) and MM (18,90±16,78 ng/mL) compared to controls (9,22±5,33 ng/mL). Besides that, resistin levels in PB are higher in symptomatic MM patients (20,91±17,61 ng/mL) when compared with controls (9,22±5,33 ng/mL) and with smoldering MM patients (8,17±1,79 ng/mL). The adiponectin levels were lower in symptomatic MM patients (6,93±5,70 µg/mL) compared with patients presenting smoldering MM (14,84±3,23 µg/mL). However, adiponectin was higher in smoldering MM patients (14,84±3,23 µg/mL) than those with MGUS (8,60±4,86 µg/mL). Furthermore, symptomatic MM patients that exhibit an IgG monoclonal component, had higher levels of MCP-1 in both PB (194,05±93,36 ng/mL) and BM (484,35±323,48 ng/mL). On the other hand, those who overproduce κ light chains show higher levels of MCP-1 in PB (242,25±155,39 ng/mL). According to ISS classification, stage 3 patients, also show higher levels of MCP-1 in PB than those on stage 1 (stage 3: 200,97±88,18 ng/mL; stage 1: 109,59±3,97 ng/mL).
Summary and Conclusions: Our study suggests that adipocytokines may be envolved in the pathogenesis of MM by creating a pro-inflammatory state in the BM that contributes to the carcinogenesis process. These findings might contribute to a better understanding of this malignancy, may allow the development of new treatment approaches and to improve the prognosis classification.

PB1635

18F-FDG PET/CT AND 99MTC-MIBI SCINTIGRAPHY LOCALIZE ACTIVE MYELOMA LESIONS AND CORRELATE WITH INCREASED SERUM LEVELS OF THYMIDINE KINASE DETECTED BY NOVEL METHOD DIVITUM™

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Background: Cell proliferation activity is a central independent prognostic factor and one of the targets for personalized and risk-adapted treatment in multiple myeloma (MM). Increased activity of thymidine kinase (TK) in serum is associated with higher proliferation rates in hematological malignancies. Imaging methods such as 99mTc-MIBI scintigraphy or 18F-FDG PET/CT can visualize active myeloma lesions and their positivity is a negative prognostic factor.
Aims: The study aimed at comparing two methods for evaluating thymidinekinase TK in serum – an older RIA method and novel DiviTum™ – in patients with MM and MGUS, and also comparing them with biochemical markers and degree of activity evaluated by imaging methods 99mTc-MIBI scintigraphy and 18F-FDG PET/CT.
Methods: Serum thymidinekinase TK levels were evaluated by DiviTum™ and an RIA method (TK REA kit by Immunotech);The study analyzed correlation of TK activity in serum with biochemical markers reflecting activity of MM: β2-m, LDH, the ratio of kappa to lambda (κ/λ) free light chains and percentage of bone marrow plasma cells (BMPC). 99mTc-MIBI scintigraphy and 18F-FDG PET/CT were performed at the time of diagnosis. The degree of activity was expressed semiquantitatively. Scans were classified as 0 (normal activity), 1 (diffuse positivity) or 2 (focal positivity).
Results: We found a strong positive correlation between TK in serum evaluated by DiviTum™ and by TK REA.. The DiviTum™ analytic method extended the detection range and was able to detect higher levels of TK than the RIA method. DiviTum™ technique found positive correlation with β2-m (r = 0.497) and LDH (r = 0.502) and moderate positive correlation with BMPC (r = 0.368). Significantly higher TK values measured by TK REA and DiviTum™ in the group of patients with MM (stages I, II or III) than in those with MGUS. Increased TK levels were observed in MIBI- or PET/CT-positive patients. Analysis of repeated measurements of TK in serum during treatment of MM patients found a correlation between change in TK measured by DiviTum™ and LDH during treatment.
Summary and Conclusions: Analysis revealed a significant correlation between TK in serum and LDH, β2-m and BMPC. Increased levels of TK in serum were observed in MIBI- or PET/CT-positive patients. Combination of positivity of imaging methods which can localize active tumor lesions and increased levels of TK in serum can have an impact on decision-making and optimization of the therapeutic approach.
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PB1636

PLASMA LEVELS OF MACROPHAGE CHEMOTACTIC PROTEIN-1 IN ASSOCIATION WITH PARAMETERS OF TUMOR BURDEN AND OTHER HUMORAL PROGNOSTIC FACTORS IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: The underlying role in pathogenesis of multiple myeloma (MM) and clinical relevance of the chemokine Macrophage chemotactic protein-1 (MCP-1) has not yet been fully elucidated. However, previous data suggest it's contribution in the homing of malignant plasma cells in bone marrow and angiogenesis.
Aims: The aim of this preliminary retrospective study was to determine potential association between pretreatment MCP-1 plasma levels, parameters of tumor burden (Durie-Salmon Stage- DSS and International Staging System- ISS) and some humoral prognostic factors, such as plasma levels of Vascular enothelial growth factor (VEGF), osteopontin (OPN), C-reactive protein (CRP) and lactate dehydrogenase (LDH). The association of plasma MCP-1 levels and patient survival was also evaluated.

Methods: diagnoses were established at the Department of Hematology, Clinical Center Rijeka, during the period from 2010 to 2012, according to the International Myeloma Working Group Criteria. Plasma levels of MCP-1, VEGF and OPN were measured by ELISA method in 45 untreated patients with MM (22 male and 23 female, median age 69 years, range 44-86) and 24 healthy controls (12 male i 12 female, median age 67 years, range 35-83). The values of LDH, CRP, as well as DSS and ISS are obtained from patient's medical records. The association of plasma MCP-1 concentration with patient survival was evaluated using Kaplan-Meier method, and differences between groups (high *vs.* low plasma levels) were tested by the log-rank test.
Results: certain amounts of MCP-1 were detected in plasma samples from all patients and controls. The median plasma level of MCP-1 in MM patients was 109.5 pg/ml (range 8.3 - 289.5 pg/ml) while median level for healthy controls was 104.9 pg/ml (range 69.5 – 234.5 pg/ml). There was no statistically significant difference in the values of MCP-1 between MM patients and control group ($p<0.949$). A significant positive correlation between plasma MCP-1 concentration and plasma OPN level ($r=0.418$; $p=0.006$) was determined, while there was a negative correlation between plasma MCP-1 and CRP levels ($r=-0.339$; $p=0.05$). However, no statistical differences were observed between plasma levels of MCP-1 and different stages of DSS ($p=0.276$) and ISS ($p=0.434$). Also there was no statistically significant association between MCP-1 plasma levels and VEGF ($p=0.745$), LDH ($p=0.268$) or patient surrvival ($p=0.638$).
Summary and Conclusions: in present pilot study the possitive associtaion between plasma levels of MCP-1 and OPN was established which can sugest the potential role of this chemokine in the pathogenesis of bone disease and angiogenesis. Furthermore, this association can implicate possible involvement of MCP-1 in the regulation of OPN secretion. Because of negative association between MCP-1 and CRP, which is surrogate measure of IL-6 and tumor aggressiveness, the pathogenetic and prognostic role of this chemokine should be clarified in the further prospective analysis of a large group of myeloma patients.

PB1637

CLINICAL CHARACTERISTICS AND PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA FROM NORTH-EAST BRAZIL: A SINGLE CENTER STUDY

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Background: Multiple myeloma (MM) is an incurable plasma cell neoplasm, characterized by genetic heterogeneity and particular immune deficits. The anti-inflammatory interleukin-10 (IL-10) production is under the influence of gene promoter functional single nucleotide polymorphisms (SNP) that can interfere with clinical outcome.
Aims: To describe clinical characteristics in a MM patient series diagnosed from 2001 to 2013 in a public single center from Natal, north-eastern Brazil, and to explore clinical impact of IL-10 proximal promoter SNPs (-1082A/G, -819C/T, -592C/A) genotypes and haplotypes.
Methods: *IL10* SNPs were genotyped with three experimental haplotyping-PCRs. Associations between gene polymorphisms and clinical/laboratory data were assessed by χ^2 and Fisher tests. Overall (OS) and progression free (PFS) survival analysis were performed by Kaplan Meier method and log rank tests.
Results: From the 38 patients enrolled in the study, 60.5% were female (sex ratio= 1.5). Median age at diagnosis was 62.5y (35-79). IgG/kappa was the most common type of MM (68.4%). Most of the patients were in Durie and Salmon stage IIIA (68.4%). Patients were equally distributed between stages I, II and III (36.8%, 21.1% and 31.6%, respectively) of the International Staging System. BM plasma cell ranged from 12 to 82% (median=20%). Plasmacytoma was observed in 44.7% of patients, being the thoracic curve of the vertebral column the most frequent site (N=6/17, 35.3%). Infections and paresthesia affected 39.5% and 28.9% of cases, respectively. Treatment was performed according to CTD protocol in 60.5%, MPT in 23.7%, Tal/Dexa in 10.5% and VAD in 5.3% of cases. According to the International Myeloma Working Group (IMWG), 15.8% of patients achieved complete response (CR), 23.7% very good partial response (VGPR), 37.1% partial response (PR), 7.9% stable disease (SD) and 10.5% progressive disease (PD). Eight patients were eligible for autologous stem cell transplantation (A-SCT). *IL10* (-1082A/G) genotype frequencies were 51.6% (AA), 32.3% (AG) and 16.1% (GG), and 45.2% (CC), 41.9% (CA) and 12.9% (AA) for *IL10* (-592C/A) SNP. Both SNPs were in Hardy Weinberg equilibrium At diagnosis, women exhibited lower ISS (ISS I= 54.5%; ISS II=22.5%; ISS III=22.5%) compared to men (ISS I= 16.7%; ISS II=25%; ISS III=58.3%, p=0.06). Most women achieved VGPR or PR (18/21, 85.7%, p=0.017), compared to men (4/14, 28.6%). OS was 86.5% and PFS was 69.4% in a follow up time of 125 months. *IL10* (-1082A/G) genotypes defined different OS: 93.8% (AA), 77.8% (AG), 80% (GG), p=0.001, Figure 1; and PFS: 80% (AA), 55.6% (AG), 75% (GG), p=0.042. The CTD-treated group had OS of 90.9% and PFS of 78.3%. In this group, patients underwent A-SCT had longer OS (0/6; 100% *vs* 2/16; 87.5%, p= 0.039) and carriers of the GCC *IL10* haplotype exhibited a worse OS compared to non carries (1/9, 88.9% *vs* 1/11, 90.9%; p= 0.046). Conversely, carriers of the -1082A allele had a better OS (1/16, 93.8% *vs* 1/4, 75%; p= 0.046). The MPT-treated group had OS of 77.8% and PFS of 62.5%.

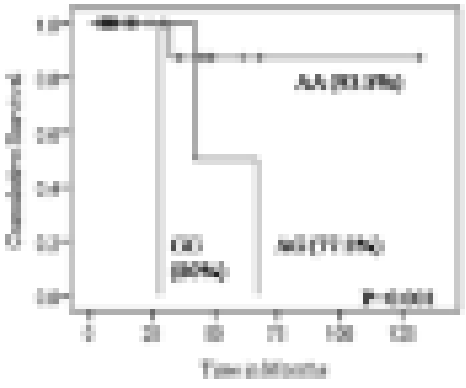

Figure 1.

Summary and Conclusions: Clinical presentation and response to treatment revealed gender-related differences not found worldwide, with women being diagnosed at early disease stages and achieving better responses. The causes of this (either related to bias in referral, or social and/or biological characteristics of the population) are not evident and warrant further research. IL10 SNPs were associated with differential survival; carriers of the -1082G allele exhibited a worse OS and PFS, while the -1082A allele was associated to a better survival.

PB1638

INTERACTION BETWEEN MONOCYTES AND BONE MARROW MICROENVIRONMENT IN PATHOGENESIS OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is an incurable haematological malignancy characterized by the clonal proliferation of malignant plasma cells within the bone marrow. The current data on MM disease progression indicate that bone marrow microenvironment plays crucial role in pathogenesis of MM. Myeloma cells contacts with bone marrow stromal cells (BMSCs), which secrete factors/cytokines, promoting tumor cell growth and survival. Paracrine secretion of cytokines, such as IL-6, insulin-like growth factor-1, inflammatory protein-1a in BMSCs promotes MM cell proliferation and protects against drug-induced cytotoxicity.MM lytic bone disease is caused by osteoclast activation and osteoblast inhibition. Disease-related bone complications result in significant morbidity due to pain, pathologic fractures and spinal cord compression. The bone microenvironment creates a supportive niche for MM progression. Osteoclasts and BMSCs, along with extracellular matrix and cytokines stimulate myeloma cell proliferation and confer chemoresistance. Therefore, the reciprocal interactions among tumor cells, osteoclasts, osteoblasts, and bone marrow stromal cells impact both the establishment and progression of MM.In current study, monocyte can directly promote osteogenic differentiation of mesenchymal stem cells through cell contact interactions and production of osteogenic factors. This mechanism is mediated by the activation of STAT3 signaling pathway in the mesechymal stem cells that leads to the upregulation of osteoblasts-associated genes such as Runx2 and alkaline phosphatase (ALP), and the downregulation of osteoblast inhibitors such as DKK1 to drive the differentiation of mesechymal stem cells into osteoblasts.
Aims: We revealed the role of monocyte, a component of bone marrow microenvironment, in the MM progression.
Methods: We investigated the proliferation of MM cell lines cultured alone or co-cultured with BM stromal cells, monocytes, or a combination of BM stromal cells and monocytes.The growth inhibitory effect of MM cell lines, monocytes and BMSCs was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetra-sodium bromide (MTT; Chemicon International, Temecula, CA) dye absorbance.To evaluate growth stimulation and signaling in MM cells, MM cells were cultured in BMSC coated 96-well plates in the presence or absence of monocyte. DNA synthesis was measured by [3H]-thymidine uptake, with [3H]-thymidine added during the last 8 h of 48 h cultures. All experiments were performed in quadruplicate.
Results: We observed increased proliferation of MM cell lines in the presence of either BM stromal cells or monocytes compared to cell line-only control. Furthermore,the co-culture of BM stromal cells plus monocytes induced the greatest degree of proliferation of myeloma cells. In addition to increased proliferation, BMSCs and monocytes decreased the rate of apoptosis of

myeloma cells. Our results therefore suggest that highlights the role of monocyte as an important component of the BM microenvironment.
Summary and Conclusions: Our results therefore suggest that highlights the role of monocyte as an important component of the BM microenvironment.

PB1639

THE IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS OF INTERLEUKINS ON PAIN RELIEF AND ANALGESIC CONSUMPTION IN THE TREATMENT OF MULTIPLE MYELOMA PATIENTS WITH PAINFUL BONE DESTRUCTIONS BY RADIOTHERAPY

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Background: Multiple myeloma (MM) cells interact with bone marrow stromal cells stimulating transcription and secretion of proinflammatory cytokines like IL-6 and IL-10, which are implicated in the progression and dissemination of MM. IL-6 is one of the most important cytokines that contributes to MM cells survival and proliferation, while IL-10 is not only the most potent factor for B-Cell differentiation, but it stimulates MM cell growth as well. Regulation of cytokines secretion is under genetic control through genetic polymorphisms in their coding and promoter sequences. It seems that single nucleotide polymorphism (SNP) in the promoter region of various genes may regulate the plasma concentrations of cytokines. Cytokines could be also hypothesized to function as pain modulators as peripheral nociceptors are sensitized by proinflammatory cytokines.
Aims: To determine if the SNP of IL-6 and IL-10 cytokines could influence the analgesic response of radiotherapy in the treatment of painful bone destructions in MM patients.
Methods: From 2012 until 2013, 30 patients (19 women and 11 men, median age: 67 years) with multiple myeloma and painful bone destructions were enrolled in the study. Patients with painful bone destructions were treated with palliative radiotherapy. Pain was evaluated according to the visual analogue scale with score endpoints ranging from 0 (no pain at all) to 10 (worst imaginable pain). A pain score ≤ 4 was classified as mild, 5 – 7 as moderate and ≥ 8 as severe. Pain score and analgesics usage were measured prior to radiotherapy as well as 4, 12 and 24 weeks afterward. Opioid analgesics were converted to the morphine-equivalent daily dose (mg/day). Genomic DNA was extracted from peripheral blood leukocytes and IL-6 and Il-10 gene promoter polymorphisms were analyzed with polymerase chain reaction-restriction.
Results: 60% (18/30) of the patients reported severe pain prior to radiotherapy, which decreased to 13% (4/30) at the first follow up visit (p <0.001). The Mean Morphine Equivalent Daily Dose (MEDD) on admission to the hospital was 75 mg/day (range 10 – 260 mg/day) which decreased to 46 mg/day (range 0 – 140 mg/day) at the first follow up visit after radiotherapy (p=0.033). A significant parameter in pain relief was: age<65 years (p=0.029). We analyzed 3 SNPs (-597G/A, -572G/C, -174G/C) in IL-6 gene promoter region and 3 SNPs (-592A/C, -819C/T, -1082A/G) in IL-10 gene promoter and their association with pain severity and analgesic consumption. Due to the small sample size we did not find significant relations, but there was a borderline association for patients who are IL6 -597A/A and G/G carriers assumed to be at higher risk for severe pain prior to radiotherapy (p=0.07) while for patients who are IL10 -1082A/A carries: the median pain score decreased faster (p=0.08). Patients with genotypes IL6 -597A/A and IL6 -174C/C required a smaller dose of opioids (p=0.06). Patients who are IL10 -1082A/G carriers are prone to respond better to radiotherapy than other patients (p<0.05).
Summary and Conclusions: SNP of IL-6 and IL-10 cytokines can influence the analgesic response of radiotherapy. Patients with genotype Il-10 -1082A/G respond better to radiotherapy.

PB1640

BLOOD CONCENTRATION OF LENALIDOMIDE WOULD CHANGE WITH THE EXISTENCE OF CLARITHROMYCIN

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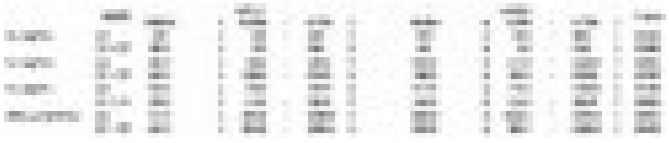
Background: Multiple myeloma is still lethal disease. However, the prognosis of this disease has been improving according to the administration of novel agents. Among of these novel agents, lenalidomide is confirmed the validity of consolidation-maintenance setting by a randomized controlled study. Recently, the combination of clarithromycin, lenalidomide, and dexamethasone (BiRd) was evaluated as therapy for treatment-naive symptomatic multiple myeloma (MM), with overall response at 2 years of 90%. However, mechanism of clarithromycin against myeloma cells is not still clear. It was reported that single-agent clarithromycin to have no efficacy in a phase 2 pilot study of 23 patients. However, some investigators argued that the addition of clarithromycin to weekly dexamethasone enhances the corticosteroid effect and may therefore increase both the overall response rate and toxicity. As known, lenalidomide is a weak substrate of P-glycoprotein, a membrane efflux transporter ubiquitously expressed in human tissues, such as the small intestine, whose activity could decrease the bioavailability of lenalidomide. Clarithromycin is known as inhibitor of P-glycoprotein in the intestinal wall. Therefore, we examined whether blood concentration of lenalidomide would change with the existence of clarithromycin.1
Aims: To investigate whether blood concentration of lenalidomide would change with the existence of clarithromycin.
Methods: Lenalidomide 15 mg (Revlimid; Celgene Corporation, Tokyo, Japan) was orally administered once daily at 08:00 hours according to the recommendations (day 1-21) of a 28-day cycle. Dexamethasone (20mg) was administrated on day 1,8,15, and 22. Orally, from day 8 to 21, Clarithromycin 400mg was administrated twice daily. On day 7and 14 of Bird therapy, whole-blood samples were collected just before oral lenalidomide administration, and at 1, 2, 4, and 6 hours thereafter. Pharmacokinetic analysis of lenalidomide was carried out using the standard non-compartmental method using WinNonlin (version 5.2; Pharsight Co, Mountain View, CA). The elimination half-life was calculated from the log-linear regression of the terminal phase of the concentration–time curve using at least 3 sampling points (elimination half-life = ln2/ke; ke = elimination rate constant). The total AUC was calculated using the linear trapezoidal rule.1
Results: Fifteen patients were enrolled in this study from April 2012 to February 2013. In some patients, blood concentration of lenalidomide increased administration of clarithromycin. These patients had wild type of ATP-binding Cassette Sub-family B Member 1 (ABCB1) C3435T genotype (C/C) (6 patients). The other patients who were not affect to clarithromycin administration were mutated types (C/T(6 patients) or T/T (3 patients)). The significant association between gingival overgrowth and the 3435TT genotype was confirmed by logistic regression analysis (p<0.046) (Table 1).

Table 1.



Summary and Conclusions: In patients who had wild type of ABCB1 genotype (C/C), blood concentration of lenalidomide significantly increased with the existence of clarithromycin. We need to do further examination whether blood concentration and an ORR are related.

PB1641

SERUM LEVELS OF FLT3 LIGAND ARE ASSOCIATED WITH MYELOMA ACTIVITY

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Background: FLT3 ligand (FLT3L) and its receptor FLT3 participate in the normal development of lymphocytes.
Aims: The aim of the study is to measure serum levels of FLT3L in active MM patients and to estimate if they are associated with known factor of disease activity.
Methods: We studied 58 patients with active MM (33 male; 30 with IgG, 19 with IgA, 9 with light chain paraprotein; 17 stage I, 19 stage II, 22 stage III, according to ISS) and 30 age and sex-matched, healthy controls. We measured serum levels of FLT3L and interleukin-6 (IL-6) by ELISA. We also recorded serum values of CRP, LDH and beta2 microglobulin (B2M), being measured routinely.
Results: Serum levels of FLT3L and the other parameters, were higher in active MM patients (p<0.001 in all cases). They all were, also increasing in parallel with ISS disease stage (p<0.001 in all cases). Moreover, serum levels of FLT3L correlated positively with values of IL-6 (r=0.400 p<0.002), CRP (r=0.489 p<0.0001) and LDH (r=0.434 p<0.001).
Summary and Conclusions: Serum levels of FLT3L reflect MM disease activity. Therefore, they could be useful markers, justifying both a more in-depth study of its participation in MM biology and the rational for potential use as a therapeutic target.

PB1642

DEK EXPRESSION IN NORMAL AND MALIGNANT PLASMA CELLS

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Background: Copy number variations are between the most frequent chromosomal abnormalities found in multiple myeloma (MM), a plasma cell neoplasm. *DEK*, which locates on chromosome 6p22.3, was initially identified in acute myeloid leukemia as a partner of abnormal *DEK-CAN* fusion. Increased expression of *DEK* provides proliferative advantage to epithelial cells whereas it enhances differentiation of myeloid cells. Increased copy number of chromosome 6p22.3 was correlated with upregulation of *DEK* expression in melanoma, urinary bladder cancer and retinoblastoma. Although amplification of 6p22.3 region where *DEK* locates was detected in about 20% of MM patients, whether this variation affects the expression of *DEK* in MM remains elusive.
Aims: We aimed to determine the expression level and copy number of *DEK* in multiple myeloma patients.
Methods: We isolated CD138^{positive} and CD138^{negative} cells from frozen or fresh bone marrow cells using the EasySep™ CD138 positive selection kit (Stem Cell Technologies, Vancouver, BC). Purity of the CD138^{positive} population was confirmed by flow cytometry. We analyzed the expression level of *DEK* mRNA in CD138^{positive} and CD138^{negative} cells from MM patients (n=41) and controls (pooled 3 bone marrow samples) using reverse transcription quantitative PCR (RT-qPCR). The copy number of *DEK* was determined in the same set of samples (CD138^{positive} MM cells and controls) using quantitative PCR (qPCR). In addition, DEK and CD138 expression was analyzed in formalin-fixed paraffin embedded (FFPE) bone marrow samples from 56 MM cases, 12 cases of monoclonal gammopathy of undetermined significance (MGUS), and 8 controls samples using immunohistochemistry (IHC).
Results: Immunohistochemical stains demonstrated moderate to high level of expression in the myeloid and erythroid cells, while DEK protein was not detectable in both normal and neoplastic CD138^{positive} plasma cells using FFPE bone marrow samples of MGUS, MM and controls. Immunohistochemical analysis of lymph nodes used as a positive control showed DEK expression in all lymphocytes, more extensively in the germinal center cells, but not in CD138 ^{positive} plasma cells. Accordingly, RT-qPCR results showed that *DEK* mRNA expression was significantly lower in CD138^{positive} MM cells compared to both CD138^{negative} cells (predominantly myeloid and erythroid cells) (P<0.0001) or control bone marrows (P<0.0009). There was a continuum of expression of *DEK* mRNA in the plasma cells without separation into a clear population of *DEK* overexpressors. By contrast, copy number of *DEK* was increased (between 1.5-2 fold) in 4 out of 41 MM samples by qPCR, but was not correlated with the expression level of *DEK* mRNA in these samples. In this limited number of cases, the percentage of samples showing *DEK* amplification (10%, 4/41) was slightly lower than the previously reported percentage of 20%.
Summary and Conclusions: Our results suggest that DEK expression level is downregulated in normal and malignant plasma cells which is independent of the copy number of *DEK*. Lack of detectable DEK protein might serve as an additional useful immunohistochemical marker for the diagnosis of MM.

Myeloma and other monoclonal gammopathies - Clinical

PB1643

RONEPARSTAT, A NOVEL HEPARANASE INHIBITOR AS A POTENTIAL TOOL FOR MULTIPLE MYELOMA THERAPY

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Background: Heparanase (HPSE), an enzyme that cleaves heparan sulfate chains, has been associated with aggressive growth of several tumor types. HPSE activity upregulates expression of genes that support tumor growth and also remodels the extracellular matrix within the tumor microenvironment thereby facilitating cell invasion associated with cancer growth, angiogenesis and inflammation. This makes HPSE a promising new and unexploited therapeutic target with multiple potential applications, including cancer and other diseases (*e.g.*, diabetes, inflammatory diseases). Roneparstat (SST0001), a 100% N-acetylated glycol split heparin(1), is a potent HPSE inhibitor devoid of any significant anticoagulant effect. In preclinical testing, Roneparstat significantly inhibited subcutaneous (sc) growth of tumor xenografts utilizing multiple myeloma (MM) cell lines RPMI-8226, MM1.S, CAG and KMS-11. In the myeloma tumors, Roneparstat inhibited angiogenesis and diminished levels of HGF, VEGF and MMP-9(2). Roneparstat also diminished HPSE induced shedding of syndecan-1, a heparan sulfate proteoglycan known to be a potent promoter of myeloma growth(3). In addition Roneparstat had an anti-metastatic effect in models of breast, pancreatic and melanoma cancers.
Aims: Although new therapies over the last decade have improved treatment of patients with MM, essentially all patients eventually develop resistance to drugs and succumb to the disease. Thus, the identification of innovative and effective treatments for MM is needed. In a model of MM using drug resistant MM.1R cells, the combination of Roneparstat with dexamethasone significantly inhibited tumor growth(2). This prompted evaluation of Roneparstat in MM patients, in combination with dexamethasone.
Methods: A First in Man, multicentre, international, phase I clinical study is currently ongoing in advanced heavily pre-treated refractory MM patients who have exhausted all available anti-MM therapies. Roneparstat is administered sc, with a starting flat dose of 25 mg defined according to ICH S9 guidelines. Dose escalation is combined with an escalation in the number of days of treatment (from DX5Q28D to DX5QW1,W2 Q28D); in absence of relevant toxicity, the dose levels are escalated in a doubling fashion, otherwise the dose escalation is based upon the type and severity of toxicity seen. Each cohort plans 3 + 3 patients. aPTT is used as a surrogate measurement of Roneparstat plasma concentration in pharmacokinetic studies. The pharmacodynamic effect of the drug on the coagulation cascade and any antitumor effect are also evaluated.
Results: The study is ongoing with 8 patients enrolled to date. Two patients received 1 cycle of therapy, three patients 2 cycles, one 4 cycles, one 5 cycles and one is not evaluable. No DLTs were observed. Roneparstat so far has been well tolerated both systemically and locally.
Summary and Conclusions: Nonclinical pharmacology studies suggested Roneparstat activity against hematological malignancies, in particular multiple myeloma, with an additive or synergistic effect with dexamethasone. The currently ongoing study will permit evaluation of the Maximum Tolerated Dose and will establish the Recommended Dose for further Roneparstat development as combination therapy in MM.

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PB1644

A PHASE TWO STUDY OF LENALIDOMIDE, METRONOMIC DOSES OF CYCLOPHOSPHAMIDE AND PREDNISONE IN THE TREATMENT OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS OLDER THAN 75 YO

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Background: Multiple Mieloma (MM) remains incurable. There is an increasing interest in the treatment of the oldest MM patients (75yo or older) due to increasing of life expectancy. The current challenge is to incorporate the new drugs in tolerable schedules that may improve treatment-results without compromising the quality of life of such population of patients.
Aims: Our aim was to assess the response rate according to the International Myeloma Working Group (IMWG) criteria with the combination of lenalidomide, prednisone, and metronomic doses of cyclophosphamide in newly diagnosed MM patients older than 75 years-old.
Methods: This is an open-label, multicenter, single-arm, phase II study in patients older than 75 years with newly diagnosed MM. After signing the informed consents, patients were treated with lenalidomide, cyclophosphamide and prednisone combined during 21 days in 28-day cycles (lenalidomide10, 15 and 25 mg/day in the first three cycles respectively; cyclophosphamide 50 mg/every other day; prednisone 50 mg/every other day). Treatment was administered until disease progression or unacceptable toxicity. The primary endpoint of this study was to assess the response rate according to the IMWG criteria. Response rates were assessed at the end of each 3 cycles. Secondary endpoints were: time to progression (TTP), overall survival (OS), progression free survival (PFS), early mortality, treatment regimen tolerability and treatment regimen toxicity.
Results: A total of 21 patients (57,1% women) with a median age of 80,6 years (range 76,1 – 90,4 years) were recruited between June 2009 and June 2011.The ISS at diagnosis was I for 2 patient (9,5%), II for 5 patients (23,8%), III for 13 patients (61,9%) and non available for 1 patient (4,8%). The overall median time to treatment initiation from diagnosis was 17 days. Overall response rate in the study was 71,5% (sCR 0%, CR 14,3%, VGPR 14,3%, PR 38,1%, SD 4,8%). Median time to response was 91 days (CI 95% 88,5 – 96,3). Median response duration was 365 days (CI 95% 343,8 – 386,2). Median TTP was 460 days (CI 95% 333,3– 586,3). The mean overall survival was 728 days (CI 95% 616 – 841). A total of 58 adverse events were related to the study medication. The most frequent related adverse events were diarrhea and neutropenia. Serious adverse events were reported in 11 patients but only 5 of them were considered as treatment-related.
Summary and Conclusions: The results of the study provide evidence that the combination of lenalidomide, cyclophosphamide and prednisone were effective and well-tolerated in patients older than 75 yo with newly diagnose of MM. A larger prospective trial is required in this special population for a better knowledge of this combination or others.

PB1645

IMPACT OF COMORBIDITY INDICES ON TREATMENT RESPONSE AND SURVIVAL IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a heterogeneous disease with 20% of patients having a survival period of less than 2 years, but more than 15% of patients have more than a 10 year survival period. Therefore it is important to identify disease features and prognostic factors that may allow better tailored therapeutic intervention. Because it is a disease involving a relatively older population with a median age of diagnosis at 69 years, besides advanced age and poor performance status, comorbidities are also empirically used as prognostic factors in treatment determination. As not all comorbidities may affect the outcome, weighted comorbidity measurements are frequently used in older patients but it is not well known which of them are really prominent at MM.
Aims: The purpose of this study was to assess whether and which comorbidity indices predict survival in a real life population of MM. We calculated the Charlson Comorbidity Index (CCI), CCI-age combined index, Hematopoietic cell transplantation-specific comorbidity index (HCT-CI) and Freiburger comorbidity index (FCI) retrospectively for 66 MM patients and compared their impact on treatment responses and overall survival.
Methods: The mean age of the 66 MM patients who varied between 34-90 years of age was 66.55 (±12.149) and nearly 60% (n=40) of the patients were men and 40% were women (n=26). As a result of the assessment (n=66) the mean and median survivals were determined to be 32.87 and 39.01 months respectively with no significant difference between genders. In order to compare the comobidity scores the patients were separated into the three risk groups of low, medium and high in accordance with literature.
Results: When the patients were separated into two groups taking the median age 67 as reference it was observed that the average survival period in the advanced age group (age≥67) is 27 months and 37 months in the younger group (age<67). However this was not found to be statistically significant (p=0.172). In the performed assessment the relation of calcium, albumin, beta-2 micro globulin, ISS and Durie-Salmon staging, ECOG and Karnofsky performance scales with survival were statistically significant (p<0.05). The ISS staging (p=0.006) and ECOG performance state were the factors that have a significant impact on treatment response. When the response to treatment is examined the response to treatment are found to be meaningfully worse (p=0.042, p=0,016, p=0,03 respectively) in CCI, CCI-age, HCT-CI high risk groups while FCI's response to treatment effect was not significant. When survival is examined while there is no meaningful relation with CCI, CCI-age and HCT-CI indices, it is observed that survival is 40.7 months, 38.5 months and 21.2 months respectively in the FCI-CI low, medium and high risk groups (p=0,006). When the factors comprising the FCI scoring were each examined individually it was observed that pulmonary and renal comorbidity scores do not have a significant impact on survival on their own but the Karnofsky performance state has a significant relation with survival on its own when coded as under and over 70 (p<0.001).
Summary and Conclusions: In light of these findings the conclusion can be made that during the initial assessment of patients the presentation of performance state and comorbidity in addition to staging is an important tool in predicting the prognosis of patients. In the assessment of comorbidities with different scores it has been presented that the FCI developed in MM patients is the only index with a significant effect on overall survival however, contrary to other indices, its relation with response to treatment has been found not to be signicant.

PB1646

A SINGLE CENTRE CLINICAL EXPERIENCE OF THE EFFICACY AN SAFETY PROFILE OF SUBCUTANEOUS VERSUS INTRAVENOUS BORTEZOMIB

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Background: Bortezomib plays a key role in the management of patients with multiple myeloma and has traditionally been administered intravenously. Subcutaneous administration is an alternative with a study by Moreau et al in 2011 demonstrating similar efficacy to intravenous use with an improved safety profile. The European Medicines Agency approved its subcutaneous administration in 2012 however there is little published data on its use in clinical practice.
Aims: Our hospital changed to subcutaneous use in April 2012. This study aimed to compare our experience of patients treated with subcutaneous *versus* intravenous bortezomib.
Methods: Patients treated with bortezomib between January 2011 and September 2013 were identified and their records retrospectively reviewed. Demographic details, side effects experienced, remission duration and survival data was collected. Statistical analysis was performed using GraphPad Prism©.
Results: 54 patients were treated with bortezomib; 28 by intravenous and 26 by subcutaneous route. The median age was 70.5 years. The majority of patients had received at least 1 prior treatment (89% subcutaneous; 90% intravenous) with 81% and 75% having received a previous IMiD. Patients received a median of 5.5 cycles in both groups with a similar cumulative dose administered (23.5mg/m^2 subcutaneous; 23.3 mg/m^2 intravenous group). 75% in the intravenous arm were treated with bortezomib alone or in combination with dexamethasone compared to 35% in subcutaneous arm where the remainder received a combination of bortezomib, cyclophosphamide and dexamethasone (VCD 54%) or bortezomib, adriamycin and dexamethasone (PAD 11%). 12% of subcutaneous group underwent autologous stem cell transplant *versus* 4% in intravenous group. The overall response rate (CR+VGPR+PR) was similar between the subcutaneous and intravenous groups (80% *versus* 71% respectively) with a median time to best response of 4 cycles in both (range 1-7). The median follow up was 12 and 24 months in subcutaneous and intravenous groups respectively with no significant difference in overall survival (1 year survival 84% and 78%, p=0.78) or relapse-free survival (median 13 and 6 months, p=0.28). The trend towards improved relapse-free survival may be due a higher number receiving autologous stem cell transplants in the subcutaneous arm. There were 50 adverse events recorded in the subcutaneous group compared with 43 in intravenous group with 10(20%) and 7(16%) grade 3-4 adverse events respectively. Of interest 50% of adverse events in the subcutaneous group occurred in those who received VCD therapy. 14% of adverse events required discontinuation of Bortezomib in both groups with 22% and 16% needing dose reductions in subcutaneous and intravenous groups respectively. 16 patients in both groups developed peripheral neuropathy (62% and 57%, p=0.84) with 3 (12%) *versus* 5 (18%) patients developing Grade 3-4 in the subcutaneous and intravenous groups. There were more Grade 3-4 haematological toxicities in the subcutaneous group (19% and 2%), however 4 of the 5 patients in the subcutaneous group were being treated with VCD or PAD
Summary and Conclusions: Our study demonstrated no significant difference

in outcomes between subcutaneous and intravenous bortezomib. We did not find the significant reduction in peripheral neuropathy with subcutaneous administration seen in previous studies however the safety profile was equivalent. Subcutaneous administration has obvious advantages in terms of patient experience and potential cost savings and should be considered a viable alternative to intravenous bortezomib.

PB1647

SMOLDERING AND SYMPTOMATIC MULTIPLE MYELOMA. EPIDEMIOLOGY IN THE NORTH AREA OF SANTA CRUZ DE TENERIFE: 1990-2009

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Background: Multiple myeloma (MM) is the second most common hematological malignance (after lymphoma), and constitutes 1% of all cancers. The incidence rate for MM is 5.8 per 100,000 per year.
Aims: Due to the high number of patients diagnosed with MM in Tenerife, and especially in La Palma, we wonder whether the incidence in our study area would be greater than that reported in the literature
Methods: We retrospectively reviewed the cases of all patients diagnosed with MM (smoldering and symptomatic) in our reference area, which includes the north of the island of Tenerife and the entire island of La Palma, (patients should have lived in this areas at least 2 years before of the diagnosis) between 1990 and 2009. We collected demographic data, laboratory variables, and treatment and response. We separate the cases according to the municipality, type of myeloma and year of diagnosis. We use the annual census of each municipality to estimate incidence.
Results: Of 409 patients (203 men and 206 women), 313 were symptomatic and 96 were smoldering (SMM) (29 low risk and 49 high-risk cases with 18 unclassified). The mean age was 68.4 years (± 0.568), with a range between 32 and 92 years. 62% of patients were> 65 years. The incidence of the total area in the last 15 years (more reliable data) is 5.6 cases per 100,000 citizens / year with an incidence of 7,53 in La Palma island and 5,14 in Tenerife island (p=0.0012). The highest incidence in Tenerife was in 2008 (8.29) and in 2002 in La Palma (15.99), although both islands the incidence trend is ever upward for decades (Figure 1A). Within the island of Tenerife, the towns of La Laguna and Tegueste have the highest incidence (8.64 and 5.10, respectively), while on the island of La Palma are Barlovento (12,05), Villa de Mazo (10,37) and Garafía (10,21). The latest available incidence obtained from the last five years is of 6.36; in Tenerife 5,89 and 8.39 in La Palma (p=0.0612). The median overall survival from diagnosis (OS) was 3.75 years±0.37 years and the median overall survival from treatment (OS_T) was 2.78 years±0.29 years. The median progression-free survival after treatment (SLP_T) was 1.35 years±0.10 years. The median time of progression to symptomatic MM from low risk SMM was 4.02 years±0.68 years, while for high risk SMM was 1.21 years±0.25 years. The OS_T of high risk SMM was not different from the symptomatic MM´s of novo (p=0.117). Multivariate analyses were performed using Cox's regression. Were clinically significant the following factors: A) At diagnosis: Durie-Salmon (Figure 1B), ISS, LDH, comorbidities and percent of plasma cell B) Related to treatment: response to the first line (Figure 1C) , transplantation and maintenance.

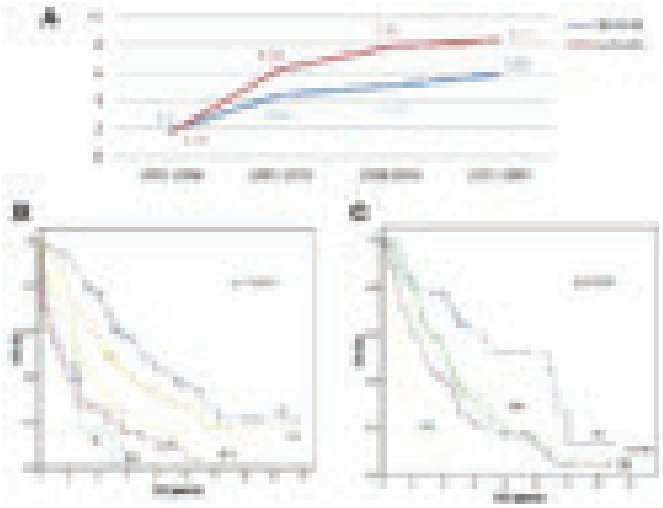

Figure 1.

Summary and Conclusions: The incidence of MM in our area is high, especially on La Palma island. The causes of this high incidence in some towns in the province should be studied in more detail. The high risk SMM evolves much faster to symptomatic MM and the survival, once treated, is similar to symptomatic. This finding suggests that high-risk smoldering myeloma should be assimilated in terms of prognosis to symptomatic myeloma.

PB1648

PHASE II TRIAL TO INVESTIGATE EFFICACY AND SAFETY OF BENDAMUSTINE, DEXAMETHASONE AND THALIDOMIDE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS AFTER TREATMENT WITH LENALIDOMIDE AND BORTEZOMIB

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Background: Despite the introduction of new and efficient drugs for multiple myeloma (MM) treatment, mainly bortezomib-based multidrug regimens, most patients relapse and lenalidomide based also provide only a temporary disease free state. After the third relapse the prognosis is usually dismal and new therapeutic approaches are needed.
Aims: The aim of this trial is to assess the tolerability and efficacy of bendamustine in association with dexamethasone and thalidomide (BDT) in relapsed/refractory patients after lenalidomide and bortezomib or who are ineligible to these drugs.
Methods: The primary endpoints of this multicenter phase II trial were the assessment of efficacy, in an intention to treat analysis, and toxicity. Treatment consisted of bendamustine (60mg/m2, d 1, 8, 15), dexamethasone (20mg, d 1, 8, 15, 22) and thalidomide (100mg, d1-28) repeated every 28 days for 6 cycles.
Results: Up to now 18 of the planned 30 patients were enrolled and completed at least the first therapy cycle. They underwent a median of 2 previous treatment lines (range 1-6). Median age was 60 years and most patients presented with an ISS-1 (61%), a good performance status (50%) and had a IgG kappa monoclonal component (33%). 4 patients completed the planned treatment and another 4 underwent ≥4 treatment cycles. Treatment was usually well-tolerated and overall only 4 patients experienced grade 3/4 hematologic toxicity. During 53 cycles, 5 treatment related serious adverse events (9%) were recorded (3 cases of pneumonia, 1 cerebral hemorrhage, 1 deep vein thrombosis and 1 pulmonary embolism), leading to death in 2 patients (4%). Of note note, no grade 3/4 neurologic toxicity has been observed. Up to now the ORR was 5/9 (55%): 3 very good partial remissions and 2 partial remissions. Other 2 patients had stable disease and 2 progressed during treatment after 2 and 4 treatment cycles, respectively and died (Figure 1).

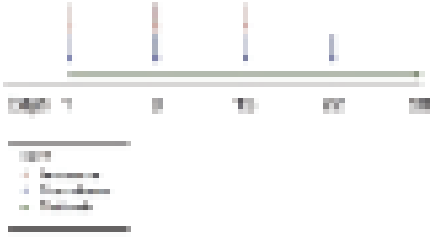

Figure 1.

Summary and Conclusions: In this interim analysis, BDT demonstrated to be an effective regimen for relapsed/refractory MM patients. The toxicity, as expected, in these heavily pretreated patients was not negligible, but manageable and no grade 3/4 neurologic side effect was observed.

PB1649

INTER-LABORATORY DISCORDANCE OF BETA-2 MICROGLOBULIN RESULTS: IMPACT ON THE VALIDITY OF THE INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA

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Background: Multiple myeloma prognostication is largely defined by the International Staging System (ISS)[1] using serum beta-2 microglobulin (β2M)

and albumin results. Local external quality assurance data showed substantial, method-dependant variations in β2M results.
Aims: We determined the variability of different β2M methods using patient samples, and whether this influences ISS prognostic scores.
Methods: β2M stability after freeze-thawing was established first. Specimens (n=21) were then sent frozen to four laboratories using different methods/instruments for β2M: Lab1, nephelometric (Beckman Coulter Immage); Lab2, chemiluminescent (Immulite 2000); Lab3, turbidimetric (Roche Cobas c502) and Lab 4 turbidimetric (Abbott Architect c16200). All laboratories use the same albumin method (bromocresol purple).
Results: Lab1 produced β2M results consistently higher than other laboratories (Figure 1): mean difference +1.55 mg/L (28.5%) (95%CI: 1.02 – 2.09, p<0.0001) compared to Lab2; +1.09 mg/L (20.1%) (95% CI: 0.78 – 1.41, p<0.0001) to Lab3; and +0.51 mg/L (9.3%) (95% CI: 0.29 – 0.73, p<0.0001) to Lab4. Compared to Lab1, ISS scores were classified one stage lower in 7, 5 and 3 patients by Lab2, Lab3 and Lab4, respectively. One patient could have been classified either stage I, II or III depending on which laboratory performed the tests. Albumin methods aligned well and had minimal impact on ISS score.
Summary and Conclusions: Results from different β2M methods are highly variable and harmonisation is required. This study has important implications on the validity of ISS staging for myeloma.

PB1650

EFFICACY OF LENALIDOMIDE-BASED AND BORTEZOMIB-BASED REGIMENS IN THE FIRST RELAPSE OF 144 MULTIPLE MYELOMA PATIENTS: A SINGLE CENTRE EXPERIENCE
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Background: The introduction of novel agents, such as proteasome inhibitors and immunomodulatory drugs, has substantially changed the treatment paradigm of multiple myeloma (MM). It is not yet clear whether some of the novel agents are significantly better than others for the first relapse of MM.
Aims: In this retrospective study, we have analyzed the outcomes of patients (pts) with the first relapse of MM treated with lenalidomide-based (L) regimens (n=72) or bortezomib-based (B) regimens (n=72) with aims to compare the efficacy of these therapies. Both treatment groups are comparable in baseline clinical parameters including age, clinical stages according to ISS and DS staging systems, the type of M-protein, presence of renal impairment and in the first-line therapy of MM.
Methods: Clinical stages according to ISS were the following: stage 1 in 32% of pts (46/144), stage 2 in 43% of pts (62/144) and stage 3 in 25% of pts (36/144). Renal insufficiency was presented in 19% of pts (27/144). Median age was 69 years (range: 49-83). Median follow-up from start of treatment was 32 months. Lenalidomide-based (L) regimens were used in 72 pts; lenalidomide+alkylating agent+ dexamethasone: 54 cases (75%), lenalidomide + dexamethasone: 15 cases (21%), alone lenalidomide: 3 cases (4%). Patients were treated with lenalidomide 25 mg daily (day 1-21, repeating of cycle on day +28); in cases with renal impairment the dose was 10 mg daily. Bortezomib-based (B) regimens were used in 72 pts; bortezomib+alkylating agent+ dexamethasone: 58 cases (81%), bortezomib+dexamethasone: 11 cases (15%), alone bortezomib: 3 cases (4%). Bortezomib was used in standard dose 1.3 mg/m^2 subcutaneously on days 1, 4, 8, 15. The cycle repeated on day 21-28 for up to 9 cycles or until progression. Median of L and B treament cycles was 5, range 1-9.
Results: In the lenalidomide-based group, overall response rate (ORR) was 56%, 10% of pts achieved the complete response (CR), 21% of pts were in very good partial response (VGPR), 25% of pts in partial response (PR), 8% of pts had minimal response (MR) or stable disease (SD) and 35% of pts had progression of disease. Median time to progression (TTP) from the start of relapse treatment was 18.4 months, median overall survival (OS) was 38.3 months. In the bortezomib-based group, overall response rate (ORR) was 51%, 13% of pts were in CR, VGPR was achieved in 16% of pts, PR in 22% of pts, MR or SD in 16% of pts, progression was observed in 33% of pts. Median TTP and OS from start of bortezomib treatment were 18.2 and 40.8 months, respectively.
Summary and Conclusions: The lenalidomide-based and bortezomib-based regimens are effective in treatment of the first relapse MM with ORR 51-56%. Medians of TTP and OS are similar for both treatment groups. According to our findings, there are no significant differences between treatment regimens containing either lenalidomide or bortezomib for the first relapse of MM.

PB1651

COMBINED USE OF HLC AND FLC IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHIES PATIENTS
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Background: International Myeloma Working Group guidelines recommend serum protein electrophoresis (SPEP) and serum free light chain (FLC) immunoassays with derived kappa/lambda ratios for diagnosis, prognosis and monitoring in multiple myeloma and monoclonal gammopathies. Heavy light chain (HLC) immunoassay can be used in screening, monitoring and risk stratifying of multiple myeloma and monoclonal gammopathies patients.
Aims: We have effected FLC plus HLC assays at diagnosis and after at regular intervals for monitoring of multiple myeloma disease or monoclonal gammopathies.
Methods: Beginning from July 2012 we have observed 150 patients in our Haematology Unit selected by the presence of monoclonal component at SPEP. We have evaluated HLC and FLC ratios on the basis of patients disease (Multiple Myeloma (MM), smouldering myeloma, Monoclonal Gammopathy (MGUS), Waldenström disease (MW).
Results: In many patients combined evaluation of HLC and FLC is useful for identification of CR an MDR in course of multiple myeloma monitoring. In several cases both tests are needed to exclude presence of minimal amounts of M-Ig. Persistent disease was indicated by an abnormal HLC ratio in 10/43 patients who achieved CR with normal FLC ratios. Somewhat contradictory is the observation of a normal HLC ratio in 15/48 IFE-positive patients achieving nCR or VGPR. Also we have observed several cases of double M-component in course of therapy for multiple myeloma with normal FLC ratio.
Summary and Conclusions: Combined evaluaton of HLC and FLC is useful for diagnosis, prognosis and monitoring of Multiple Myeloma and Monoclonal Gammopathies.

PB1652

THE COMPARISON OF BORTEZOMIB-CONTAING REGIMEN AND THALIDOMIDE-CONTAINING REGIMEN; SUPERIORITY OF BORTEZOMIB, DOXORUBICIN, AND DEXAMETHASONE THERAYPY IN NEWLY DIAGNOSED MYELOMA WITH RENAL IMPAIRMENT
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Background: Multiple Myeloma (MM) with renal impairment (RI) has had poor prognosis so far. Bortezomib-containing regimen (Bor-R) and thalidomide-containing regimen (Thal-R) have already been proven to be safe and effective in MM with RI. Bor-R such as bortezomib and dexamethasone (BD) therapies, and combination regimens of bortezomib with other agents are recommended in a consensus statement of International Myeloma Working Group (IMWG) for patients of MM with RI. However, only a limited number of reports are available regarding the comparative studies among Bor-R, Thal-R, and lenalidomide-containing regimen. Furthermore, the best Bor-R combination has not been demonstrated yet.
Aims: To clarify the best regimen for newly diagnosed MM with RI, we compared the recovery of renal function, response of MM, and toxicities between Bor-R and Thal-R.
Methods: Newly diagnosed MM with RI from January 2004 to December 2013 in National Hospital Organization Disaster Medical Center in Japan and Seoul National University Hospital in South Korea were enrolled in this study. Patients, MM characteristics, regimen, renal response, and MM response were obtained from medical charts. As for an initial therapy, Bor-R including BD therapy, Bor, doxorubicin, and dexamethasone (PAD, Takezako N, *et al.*, 14th IMW abstract P220), and Bor, melphalan, and prednisone (VMP), and Thal-R were enrolled. Supportive care including hydration and administration of bisphosphonates were allowed. Renal functions were measured by the simplified Modification of Diet in Renal Disease formula to identify RI in MM patients with stabilized serum creatinine levels, and best renal response was evaluated according to a consensus statement of IMWG for patients of MM with RI. Major renal response means either CR or PR. Best myeloma response during initial induction treatment was assessed according to IMWG response criteria.
Results: Sixty-three patients were enrolled in this study. Median age was 68 years-old, and Male/Female was 41/22. Of 63 patients, 56 cases were ISS >2. As for an initial therapy, 45 received Bor-R (BD=23, PAD=11, VMP=11), and 18 received Thal-R. Compared to Bor-R, younger age, better PS were statistically more common in Thal-R. Overall renal response and major renal response rate was 34/45 (75.6%), and 25/45 (55.6%) in Bor-R, and 10/18 (55.5%), and 7/18 (38.9%) in Thal-R (statistically not significant). However, day to best renal response of Bor-R was 14 days, whereas, it was 50.5 days in Thal-R (P=0.01). None of 2 in Thal-R, 3 of 5 in Bor-R who required dialysis became independent of the procedure. Myeloma response was not statistically significant between the 2 groups. Among Bor-R, younger age in PAD, better PS in VMP were statistically more common. Major renal response rate was superior in PAD (P<0.01), and days to best renal response of BD, PAD, and VMP were

14, 10, and 19 days, respectively. Furthermore, PAD was superior in myeloma response among Bor-R (P<0.01). On the other hand, hematologic toxicities were statistically severer in PAD. Regimen, sex, hypercalcemia, and myeloma response affected renal response in univariate analysis. On the other hand, multivariate analysis demonstrated regimen (P=0.01), and myeloma response (P=0.04) contributed to major renal response.
Summary and Conclusions: Bor-R, and Thal-R were effective in MM with RI. However, Bor-R might have earlier renal response. Furthermore, PAD was considered as best regimen among Bor-R from the view of higher renal response rate, earlier renal response, and myeloma response in newly diagnosed MM with RI.

PB1653

NOVEL MARINE COMPOUNDS WITH ANTI-MYELOMA ACTIVITY IN VITRO AND *IN VIVO*

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Background: The prognosis of patients with multiple myeloma (MM) has substantially improved during the last years- due to implementation of novel drugs into the treatment.
Aims: However, MM is still incurable and novel treatment strategies are needed.
Methods: Here, we tested novel substances for their anti-myeloma activities *in vitro* and *in vivo*. 11 new marine compounds with unknown activity against MM were applied in different concentrations to the MM cell lines OPM-2, NCI, U-266 and on primary multiple myeloma cells *in vitro*. Apoptosis was determined by FACS using annexin V FITC/ 7AAD-staining and GFP-ELISA. The compounds effective *in vitro* were tested further on PBMC's and on GFP-transgenic MM cell lines *in vitro* and *in vivo* (CAM assay).
Results: 6/11 compounds showed anti-myeloma effects *in vitro*. IC50 was <10[nM] for 4/6 effective substances, suggesting activity similar to Bortezomib. These compounds were also effective against purified human MM cells *in vitro* and exhibited anti-myeloma effects on GFP- transgenic OPM-2 cells *in vivo*.
Summary and Conclusions: New compounds with potential anti-myeloma effects *in vitro* and *in vivo* were identified.

PB1654

HEMODIALYSIS WITH HIGH CUT-OFF FILTERS AND CHEMOTHERAPY FOR MULTIPLE MYELOMA PATIENTS WITH ACUTE RENAL FAILURE

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Background: Acute renal failure (ARF) in multiple myeloma (MM) is present in about 10-15% of the cases, and the patient's survival depends on the recovery of their renal function. Cast nephropathy, caused by intratubular precipitation of circulating free light chains (FLC), is the most common cause. To avoid this, the early introduction of anti-myeloma treatment is necessary. One coadjuvant measure is the elimination of FLC by hemodialysis with high cut-off filters (HCO) (Theralite), which removes a huge amount of FLC.
Aims: This study assessed the combination of chemotherapy and hemodialysis with HCO on amount of free light chains, renal recovery and hematologic response in patients with MM and ARF.
Methods: We report 9 cases (8 M/ 1 F) of ARF and MM treated with this procedure. Average age was 60 years (42-71). Types: Bence -Jones MM: 6 (4 κ and 2 λ), IgG: λ: 2 and IgG: κ: 1. Average level of serum creatinine was 6,9 mg / dL (2,9-12,1). Average creatinine clearance (MDRD -6) was 9,9 mL/min/1,73m2 (4,51-22,3). Average proteinuria was 14,9 g /24 h (1-35). Average serum FLC (Binding -Site) was 7845 mg / L (1130-20200). Time between the onset of renal failure and the start of the hemodialysis with HCO was 38 days (5-90). FLC measures at the beginning and at end of treatment were conducted. The antiMM treatment was: 7 patients on Velcade (Bortezomib) - Dexamethasone, 1 patient on Velcade - Revlimid (Lenalidomide) - Dexamethasone, 1 patient on Velcade - Thalidomide - Dexamethasone and Revlimid - Dexamethasone.
Results: Average of hemodialysis with HCO was 12 (6-27). Average serum FLC after the procedure was 287 mg / L (17-790). Average final serum creatinine was 2,62 mg/dL (0,82 to 5,9), and creatinine clearance was 44,64 mL/min/1, 73m2 (9,54 to 95,4). Renal response was complete in 3 patients, partial in 3, minimum in 1 and failure in 2. Seven patients were dialysis independent. The hematologic response was: 4 RC, 1 VGPR, 2 PR, 2 MR. Two patients died of MM. Patients who did not recover renal function or only partially, had an average start of hemodialysis of 56 days, an average FLC level of 10067 mg/L, and a dialysis average of 16,7. Meanwhile, patients who achieved response had an average start of hemodialysis of 20 days, FLC level of 6056 mg/L, and a dialysis average of 7,3.
Summary and Conclusions: In our experience the combination of hemodialysis treatment with HCO combined with an antiMM treatment provides a satisfactory result (66% of the responses) in patients with MM and ARF. The predictive factors of response where the amount of serum FLC and the early introduction of hemodialysis with HCO.

PB1655

FACTORS PREDICTING RENAL RECOVERY IN MYELOMA PATIENTS PRESENTING WITH DIALYSIS DEPENDENT ACUTE KIDNEY INJURY

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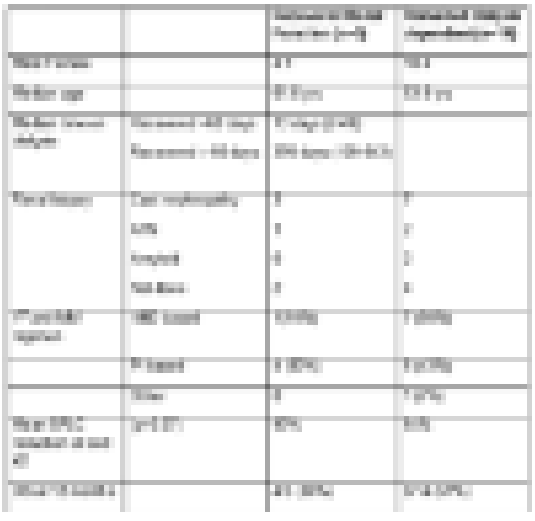
Background: Renal failure is a common complication of myeloma (MM) caused by toxic effects of high monoclonal free light chains along with other contributory factors. Up to 10% of MM patients present with dialysis dependent acute kidney injury (AKI). Early and sustained reduction in light chains leads to improved renal recovery, translating into improved survival and quality of life. The use of novel agents developed over the last decade has made this a realistic aim and the advent of serum free light chain (SFLC) monitoring has enabled the rapid assessment of disease response to treatment given their significantly shorter half-life than whole M-protein.
Aims: To identify factors associated with renal recovery in patients presenting with MM and dialysis dependent AKI.
Methods: Data were collected retrospectively for patients presenting to our centre, a district general hospital with a tertiary referral renal unit. We analysed those patients with a new diagnosis of MM presenting with AKI requiring dialysis. Adequate data were available for 19 patients presenting between 2010 and 2012.
Results: All patients had SFLC at diagnosis of >1000 mg/l (mean 8655mg/l, range 1200-34600, 74% kappa, 26% lambda). 8 patients had light chain only disease whilst the other 11 patients also had a paraprotein (IgG in all cases). Median age was 63 years, 14 male *vs* 5 female. Where recorded all patients were ISS Stage 3. 3 patients died within 60 days of diagnosis (16%). 4 patients underwent ASCT, two whilst still receiving dialysis but with a reduced dose of melphalan (140mg/m^2). 3/19 patients demonstrated renal recovery by 3 months (median time on dialysis 13 days, range 2-49). These patients all had a mean reduction in their SFLC of >90% by the end of cycle 2 of therapy (mean reduction 96% *vs* 64% for those who did not go on to recover renal function). 2 additional patients went on to recover renal function later. They had a reduction in SFLC levels by the end of cycle 2 of 92% and 89%, in one patient this was after a change in therapy from a thalidomide based regimen to bortezomib following deterioration of disease markers. Further comparison is made in Table 1. Patients who recovered renal function had a longer overall survival (OS) than those who did not (median NR *vs* 385 days, p=0.25). The OS in all patients at 12 months of 68% (13/19) compares favourably to a historical cohort from our centre who presented between 2000 and 2005 which had a 12 month OS of 45%.

Table 1.



Summary and Conclusions: Our "real life" data in an unselected group of

patients suggests that the use of proteasome inhibitors as the first anti-MM treatment and the speed of reduction in SFLCs predicts for renal recovery in MM patients presenting with AKI. However, this study was not sufficiently powered to determine whether these factors are significant or independent. We suggest that clinicians should undertake frequent monitoring of SFLC and consider a change in therapy if response does not occur quickly.
Overall survival at our centre in this patient cohort has improved with the advent of novel agents in comparison to historical studies.

PB1656

BORTEZOMIB, MELPHALAN, AND PREDNISOLONE COMBINATION CHEMOTHERAPY FOR NEWLY DIAGNOSED LIGHT CHAIN (AL) AMYLOIDOSIS: A SINGLE-CENTER EXPERIENCE IN KOREA

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Background: AL amyloidosis is a monoclonal plasma cell disorder associated with progressive end-organ dysfunction and poor survival outcomes. Bortezomib combination chemotherapy is rapidly active in AL amyloidosis with high rates of hematologic and organ responses.
Aims: We aimed to determine the efficacy and toxicity of bortezomib, melphalan, and prednisolone (VMP) as first-line chemotherapy in patients with AL amyloidosis who were ineligible for autologous stem cell transplant (ASCT).
Methods: We conducted a retrospective review of patients with newly diagnosed AL amyloidosis with or without multiple myeloma who were initially received a VMP regimen between 2011 and 2013 at a single center.
Results: For 19 patients included, the median age was 65 years (range, 42-74 years) and in 90% of patients had two or more organs; heart and kidneys were affected in 18 (95%) and 6 (32%) patients, respectively. Most of the patients was advanced stage AL amyloidosis (84% in 2004 Mayo Stage III and 92% in 2012 Mayo Stage III or IV). Sixteen (84%) patients had a hematologic response, including 7 (37%) complete response, within a median time to response of 1 month (range, 1-3 months). Cardiac and renal responses were seen in 44% and 40% of patients, respectively. At median follow-up of 8 months (range, 1-32 months) months, median progression-free survival and overall survival was 8 and 11 months, respectively. The most frequent grade 3-4 adverse events were thrombocytopenia, diarrhea, and pneumonia.
Summary and Conclusions: Our results suggest that VMP seems to be highly effective treatment for AL amyloidosis as a first-line treatment. Further investigation of VMP as treatment for AL amyloidosis is needed.

PB1657

PROGNOSTIC FACTORS FOR SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA PRESENTING WITH RENAL IMPAIRMENT

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Background: Renal impairment (RI) is a common complication at diagnosis in patients with multiple myeloma (MM). The presence of renal impairment is associated with an increased risk of early death.
Aims: This study examined the prognostic factors for survival outcomes in patients with MM presenting with RI.
Methods: Retrospective data for 104 patients who presented RI at diagnosis were analyzed. RI was defined as an estimated glomerular filtration rate (eGFR) ≤ 60 ml/min/1.73 m^2 using the simplified Modification of Diet in Renal Disease formula.
Results: Baseline median eGFR was 39.0 ml/min (range 5.1-59.8). Achievement of renal complete response (renalCR) was observed in 37.1% of patients. Predictors of overall survival were evaluated using logistic regression analysis which included age (> 65), eGFR<30ml/min, oliguria (< 500 ml/day), thrombocytopenia (platelet<100,000/mm^3) , achievement of renalCR, myeloma response (≥ partial response) and performance of autologous stem cell transplantation (ASCT). On multivariate analysis, oliguria at diagnosis (hazard ratio [HR]= 4.493, p=0.001), thrombocytopenia at diagnosis (HR= 2.666, p=0.003), and performance of ASCT (HR = 0.456, p=0.039) were significantly associated with overall survival.
Summary and Conclusions: In conclusion, an oliguria and thrombocytopenia at diagnosis were important predictors for poor survival in MM patients with RI.

PB1658

THE IMPACT OF HYPERGLYCEMIA ON RISK OF SEVERE INFECTIONS DURING EARLY PERIOD OF INDUCTION THERAPY IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: The association with hyperglycemia and infections during induction chemotherapy has been reported in a number of hematologic disorders.
Aims: We evaluated the incidence of hyperglycemia and its association to development of severe infection during early period of initial chemotherapy in patients with multiple myeloma (MM).
Methods: The records of 176 patients were retrospectively analyzed. Patients were stratified into three groups by World Health Organization criteria: eugylcemia, mild hyperglycemia and overt hyperglycemia.
Results: A total of 35 (19.9%) patients developed overt hyperglycemia (≥ 200 mg/dl) during induction therapy. Serious infections occurred in 32 (18.2%) of 176 patients and infection-related mortality within 2 months after treatment was 2.3% (4 patients). In a univariate analysis, overt hyperglycemia, poor performance status (≥ 2), International Stage System III, lymphopenia (< 500/μL), and elevated serum creatinine (≥ 2 mg/dL) were associated with serious infections. In multivariate analysis, only overt hyperglycemia (OR 5.188, 95% CI 2.046-13.155, p=0.001) and poor performance status (OR 4.105, 95% CI 1.588-10.611, p=0.004) remained significant. Furthermore, patients in the overt hyperglycemic group showed increased mortality compared to that in patients in the other groups (38.6 *vs.* 56.8 months, p=0.046).
Summary and Conclusions: This study demonstrates an association between hyperglycemia and serious infections during induction therapy in patients with MM.

PB1659

PERIPHERAL BLOOD ABSOLUTE MONOCYTE COUNT AS PREDICTOR OF OVERALL SURVIVAL IN MULTIPLE MYELOMA

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Background: The tumor microenvironment, including tumor-infiltrating lymphocytes and myeloid-derived cells, is an important factor in the pathogenesis and clinical behavior of different neoplasms.
Aims: However, the prognostic significance of peripheral monocytes in multiple myeloma (MM) was never assessed thus far.
Methods: In a retrospective unselected cohort including 120 patients diagnosed as having active MM at our institution over a 10-year period (2000-2010) we evaluated the prognostic impact of absolute monocyte count (AMC), absolute lymphocyte count (ALC) and lymphocyte/monocyte ratio (LMR).
Results: Median age of patients was 69 years (range, 41-86 years) and there were 64 males and 56 females. Patient distribution according to ISS was as follows: ISS 1, 75 cases; ISS 2-3, 45 cases. After a median follow-up time of 39 months (range, 2-160 months)53 patients died while median OS was 52 months. Looking at the prognostic impact of ALC,AMC and LMR different thresholds were set. While ALC or LMR failed to demonstrate any impact on clinical outcome of MMpatients in terms of overall survival (OS), a cut-off value of 0.450 x 109 for AMC, corresponding to 75th percentile, was strongly associated with OS. Survival curve according to AMC is presented below. As shown, median survival of patients with AMC<0.450 x 109 at the time of diagnosis was 91 months therefore significantly longer than median survival of patients with AMC ≥0.450 x 109 (34 moths) [HR, 2.67 (95% CI, 2.12-3.22); P=0.004]. No correlation could be found between AMC and ISS score (P=0.719), response to therapy (P=0.966) and comordidity index as assessed by ACE-27 score (P=0.135). In contrast, an inverse correlation between patient age and AMC characterized our cohort (P=0.04) (Figure 1).

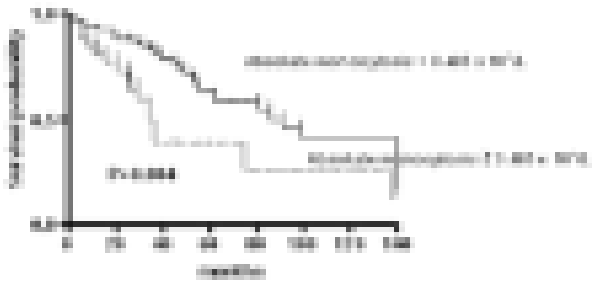

Figure 1.

Summary and Conclusions: In summary, AMC at diagnosis, as a simple index which reflects tumor microenvironment, predicts clinical outcome in MM patients.AMC isan objective, reproducibleand cost effective test, which can easily be obtained from standardblood count. Additional studies on AMC are warranted in MM patients to better understand the roles of monocytes in this disease.

PB1660

COMPARISION OF TREATMENT OUTCOMES BETWEEN TREATED WITH NOVEL AGENTS COMBINED CHEMOTHERAPY AND CONVENTIONAL CHEMOTHERAPY IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA IN SOUTH KOREA

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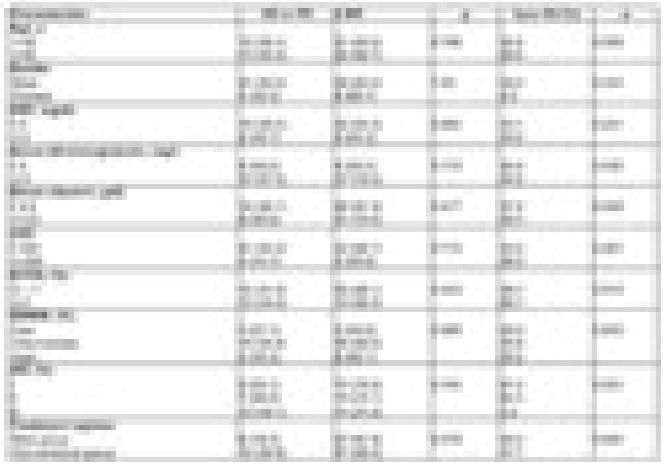
Background: Waldenström macroglobulinemia (WM) was defined as a distinct clinicopathologic entity characterized by bone marrow infiltration by lymphoplasmacytic lymphoma (LPL) and IgM monoclonal gammopathy. WM is very rare hematologic malignancy which has an overall incidence of approximately 3 per million persons per year, accounting for approximately 1% to 2% of hematologic cancers in USA. The incidence rates of WM are lower in South Korea than those in USA, which were documented about 0.3 per 1000,000 person-years according to data of National Cancer Information Center in South Koea. However, many reported studies presented the superior efficacy of chemotherapy combined novel agents including rituximab, bendamustine, fludarabine, bortezomib, lenalidomide, and thalidomide than that of conventional chemotherapy. But novel agents combined chemotherapy for patients with WM have been used restrictively in Asia and south Korea because of very low incidence and limitation of insurance. So until now, there were a few reports about clinical features, epidemiology, and cytogenetics of WM in Asia including south Korea. Moreover, there is no study about treatment outcomes in patients with WM treated by novel agents combined chemotherapy.
Aims: This unique study was planned for investigation of clinical features and comparision of treatment outcomes between novel agents and conventional chemotherapy.
Methods: A total number of 72 patients who newly diagnosed WM and received chemotherapy at the South Korea between January 2005 and December 2012 were enrolled retrospectively in the current study. We analyzed nationwide retrospective data from sixteen university hospitals in South Korea. We divided into new group which were included patients treated by novel agents combined chemotherapy and conventional group treated by conventional chemotherapy. We analyzed overall response rates and overall survival rates in two groups.

Table 1.



Results: The median age of patients was 65.5 years (range, 37-92 years) and male to female ratio was 5 : 1. Median follow up duration was 22.6 months. Clinical features including the incidence rates of hypervisocsity syndrome, B symptoms, splenomegaly, and extranodal involvement were 11.1%, 12.5%, 25.0%, and 40.3% in our study. The analyzed patients included new group (n=33, 45.8%), and conventional group (n=39, 54.2%). New group was shown slightly higher overall response rates than those treated with conventional group (81.8% *vs* 69.2%, p=0.219). The 5 years overall survival rates were not shown differences between two groups (new group *vs* conventional group; 44.0% *vs* 47.7%, p= 0.640) (Table 1).
Summary and Conclusions: In our study, clinical features of WM were shown similar characteristics compared with western data. Patients treated with novel agents combined chemotherapy were shown a trend of higher overall response rates. But overall survival rates were shown similar between two groups. Our results were not shown significant difference of treatment outcomes between novel agents and conventional treatment because of limitation of analyzed patients number and short follow up duration. So further studies are needed to confirm efficacy of novel agents in patients with WM.

PB1661

OUTCOME OF NEWLY DIAGNOSED SYMTOMATIC MULTIPLE MYELOMA IN PATIENTS AGED > 80 YEARS: SINGLE CENTRE EXPERIENCE

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Background: Multiple Myeloma (MM) is a plasma cell neoplasm typical of the elderly, with a median age at diagnosis of 65 years. It accounts for approximately 10% of all hematologic malignancies. The increase of median age in western countries has led to an increase in the incidence of this disease; in addition the introduction of novel agents, such as the immuno-modulatory drugs thalidomide and lenalidomide, and the proteasome inhibitor bortezomib, has considerably changed the therapeutic scenario both for young and elderly patients with MM.
Aims: The purpose of this study was to evaluate the outcome of newly diagnosed symptomatic multiple myeloma in patients aged ≥ 80 years.
Methods: We report a retrospective analysis of the outcome of 15 very elderly (≥ 80 years) patients (M/F: 11/4) diagnosed and treated at our Institute from January 2008 to January 2014. Median follow-up was 16 months (range 6-49) after the start of treatment to determine the characteristics of this subset of very elderly patients.
Results: Median age at diagnosis was 83 years (range 80-89) and PS was <2 in 12 cases (80%). One or two concomitant diseases requiring specific treatments were present in 10 patients (67%), and 3 or more concomitant diseases were present in 5 patients (33%). MM was IgG lambda in 7 patients, IgG k in 4 patients, IgA k in one case, IgA lambda in 1 case, and micromolecular in 2 cases. According to the International Staging System 8 patients were classified as III stage and 7 patients as II stage. Anemia (median value: 9.8 g/dL) was present at diagnosis in 11 patients (73.3%) and was the most frequent CRAB feature. Bone lytic lesions were present in 10 patients (67%) and zoledronic acid and ibandronic acid were used in 8 and 2 patients respectively. First line therapy was bortezomib/dexamethasone in 8 patients (53.3%), and melphalan/prednisone +/- thalidomide in 7 patients (46.7%). Hematologic toxicity was infrequent but usually weak/moderate (grades 1 & 2 on the WHO scale), and was resolved only with dose delays. Six patients received erythropoiesis-stimulating agents. Extrahematologic toxicity was observed in 6 patients (40%) and neuropathy was the most common adverse event for treatment. Seven patients (47%) had at least one disease progression since diagnosis and were therefore switched to second-line therapy. The median time to first disease progression was 10 months (range 5-28). 9 patients (60%) are still alive and continuing to receive treatment. Five patients died due to disease progression. One patient died due to urinary bladder cancer.
Summary and Conclusions: A study in a larger series of patients is warranted but our experience showed that no upper age limit should be applied for the administration of new drugs with multiple myeloma; these treatments could be offered to very elderly patients, including those with severe concomitant diseases.

PB1662

TREATMENT OF BENCE-JONES MULTIPLE MYELOMA WITH CHEMOTHERAPY WITH/WITHOUT STEM CELL TRANSPLANTATION

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Background: Bence-Jones myeloma multiplex is a progressive disease characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of incomplete immunoglobulins, containing only the light chain portion of the immunoglobulini.This type of myeloma occurs 15-20%.The mediana overall survival is approximately 4 years. Patient outcome in BenceJones myeloma has been remarkably improved due to the use of combination therapies including chemotherapy and stem cell transplantation.High-dose melphalan with autologus stem cell support has been an integral part of myeloma therapy for mor than 28 years.
Aims: A retrospective analysis of outcome of treatment Bence-Jones myeloma.
Methods: Since 1995. until 2013. we treated 60 pts (39 men and 21 female), average age 55,5 years (range 36-85). Regarding ISS score, the group included: ISS1 15 pts, ISS2 10 pts, ISS3 35 pts. Renal insufficiency was present in 35 pts. High risik pts was defined by the presence of following factors: b2M >5,5 mg/l,albumine<3,5gr/dl, CRP >6, high lactat dehydrogenase, renal failure, stage III.Pts treated with induction,consolidation and maintenance therapy.Conventional induction treatments were applied as following regimens:VAD(42),MP(9),CTD(3),PAD(2) and TAD(4).
Results: Conventional chemiotherapy introductory clinical response was achieved in 41pts (70%) (MR-5 pts, PR-15 pts,VGPR-21 pts), while in 19 pts (30%) established disease was resistant. Transplantation had been done with 33 pts (55%), while 27 pts (45%) were treated with conventional chemotherapy adjusted to the vital age and comorbiditity. In the group of pts with transplantation done tandem had been carried out with 11 pts and secondary SCT had been done in 4 relapsed pts. With 1 pts with tandem SCT allogenic(singen) SCT had been done. TRM is 3%. Maintenance therapy with Thalidomid had been done in 27 pts for 4-40 months, with IFN had been done in 4 pts for 6-12 months. Impact of high risk factors on outcome/PFS/OS was of no significance, except for elevated high lactat dehydrogenase values that are associated with rapid relapse. The presence of bone lesions associated with short PFS and with no difference in OS. In the group of transplanted no difference in OS with/without a response to primary therapy, but in the nontransplanted pts OS was significance shorter in patients who did not achieve a response.The transplanted patients had significantly longer PFS (mediana 14 months *vs* 8 months, p<0,05) and longer OS (mediana 50 months *vs* 20 months,p<0,001). 20 pts (33%) of treated pts are living, while 40 pts (67%) died. Univariate log. regres. analysis showed that non-transplant patients are 10,41 times more likely to terminate lethal compared to transplant patients (RR 10,41(95%C.I.43,47-2,52),p<0,001).
Summary and Conclusions: Our study showed ASCT is a more effective method of treatment of patients with BenceJones myeloma compared to the conventional chemotherapy, but the results are still unsatisfactory. Impact of high risk factors on outcome/PFS/OS was of no significance, except for elevated high lactat dehydrogenase values that are associated with rapid relapse. One of the major efforts to improve the results of intensive therapy and ASCT involves the integration of novel agents (proteasome inhibitors and immunomodulatory drugs) into the transplantation sequence.

PB1663

DIAGNOSTIC DILEMMAS AND TREATMENT DECISIONS: EXTRAMEDULLARY PLASMACYTOMA OF TESTIS AS A FIRST MANIFESTATION OF MULTIPLE MYELOMA – CASE REPORT

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Background: Overall data indicates that 65-71% of patients with multiple myeloma (MM) have some form of extramedullary plasmacytoma (EMP) as well. In available literature, only 10 cases of testicular EMPs presented with simultaneous bone marrow involvement, or a diagnosed MM. Due to small patient numbers and historical retrospective analyses over many decades, no firmly established criteria for treatment exists.
Aims: The aim of our case report is to present an extremely rare case of testicular extramedullary plasmacytoma (EMP), as first manifestation of multiple myeloma (MM) with focal osteolytic lesions, in terms of diagnostic dilemmas and personalized treatment approach.
Methods: We report a case of 68-year old male with testicular plasmacytoma as a first manifestion of MM. Eight months prior to diagnosis, a swelling of a left testicle occurred and was accompanied with scrotal pain. There was no abnormalities in the initial laboratory findings, except increased serum fibrinogen (5,3g/l), ß2 microglobulin (5,6mg/l), and erythrocyte sedimentation rate (100/1h). Ultrasound exam of testicular tissue showed enlarged left testicle with hypoechoic lesions. Thoracic an abdominal CT scan did not reveal pathological findings. Subsequent total left orchiectomy was performed, and immunohistochemical profile of testicular tissue (monoclonal CD38$^+$, CD138$^+$, lambda light chain$^+$ plasma cells) indicated diagnosis of EMP. Immunoelectrophoresis of serum and urine detected high level of monoclonal (M) IgG lambda protein in serum (54,9g/l), followed with discrete Bence-Jones proteinuria (0,1g/l). There was no plasma cell infiltration in the bone marrow biopsy. Furthermore, in terms of Fluorescent in-situ hybridization (FISH) analysis of tumor cells, there were no typical or high risk cytogenetic abnormalities specific for the plasma cell disorders like del13q14; t(4;14); t(14;16); or del17. Considering current infrequent EMP localization, IgG lambda M protein above 30g/l, and absence of bone marrow infiltration with plasma cells, FDG PET-CT was performed within differential diagnostics of EMP in contrast to MM. Surprisingly, PET-CT scan revealed few scattered osteolytic lesions, in right humerus, sacral bone and left femur.
Results: Unusually, first manifestation of underlying plasma cell disorder in our patient was development of testicular EMP, without systemic symptoms of MM. In terms of diagnostic dilemmas, following confirmation of EMP and absence of medullar involvement, presence of significant concentration of IgG lambda M protein in serum, indicated further exploration with FDG PET-CT. Consequently, existence of osteolytic lesions at right humerus, sacral bone and left femur confirmed diagnosis of IgG lambda multiple myeloma in IIIA CS (Durie&Salmon criteria), ISS score 3 with testicular EMP localization. Treatment with 4 of planned 6 cycles, according to the CTD regimen was applied, followed by the achievement of very good partial remission (Blade criteria) according to patient's laboratory findings after second cycle of treatment.
Summary and Conclusions: Testicular involvement in MM is extremely rare with incidence 0,6-2,7%. FDG PET-CT scan may be obligatory diagnostic method in a case of atypical EMP localizations, with a strong impact on the treatment decision in accordance to the final diagnosis and staging.

PB1664

THE ROLE OF MERTK IN THE PROGRESSION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) TO MYELOMA (MM)

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Background: The identification of reliable biomarkers that predict which patients will progress from MGUS to MM would greatly assist treatment decision-making. In longitudinal gene expression profiling experiments, examining the transcriptome of individual patients when first diagnosed with MGUS and then subsequently MM, we identified a number of differentially expressed genes associated with the MGUS to MM transition. MERTK (c-mer proto-oncogene tyrosine kinase) was one such gene, whose expression was lost during the transition from MGUS to MM.
In this study, we performed quantitative real-time PCR (Q.RT-PCR) and immunohistochemistry (IHC) of marrow trephine biopsies recovered from patients (n=10), when first diagnosed with MGUS and subsequently MM, to confirm that MERTK was lost as a function of disease progression. In addition, we examined the prognostic significance of the loss of MERTK expression
Aims: To investigate the role of MERTK in MM progression by mean of Q.RT-PCR and IHC
Methods: Trephine biopsies were recovered from 10 patients when first diagnosed with MGUS and subsequently MM (n=20 trephines). Serial 5 µm trephine section were stained with either a MERTK-specific antibody (Abcam, UK) or an antibody to the plasma cell marker CD138 (Dako, USA). The number of CD138+MERTK+ cells in each section was assessed by 3 pathologists blinded to the level of MERTK mRNA expression in each patient. The magnitude of change in MERTK expression from MGUS to MM of each paired patient sample was expressed as ΔMERTK (defined as the ratio of the overall MERTK in MM sample compared with that in MGUS sample). A ROC curve was used to identify the best cut-off value of ΔMERTK that resolved different clinical outcomes for our cohort of patients.

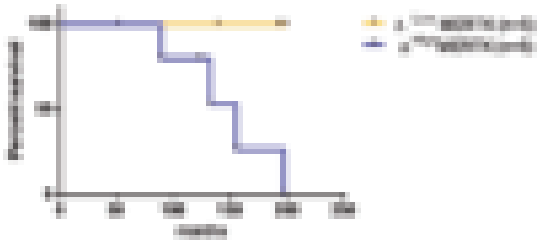

Figure 1. Kaplan-Meier curve of OS of MM patients transformed from MGUS with differential MERTK expression, p=0.33 Chi-square test.

Results: A consistent reduction in MERTK expression was observed in all patients as they progressed from MGUS to MM as evidence by a decrease in mRNA and protein expression (unpaired t-test, p=0.05). The magnitude of the

change in MERTK protein expression (ΔMERTK) in patients as they progressed from MGUS to MM varied (ΔMERTK range 0.25-0.98). A cut-off value of 0.51 for ΔMERTK was determined by ROC curve analysis and Δ^{high} MERTK was defined as <0.51 (*i.e.* greater reduction in MERTK expression from MGUS to MM). While not statistically significant, a trend towards better overall survival (OS = time of diagnosis of MGUS to death) was observed in MM patients with Δ^{low} MERTK (*i.e.* lesser reduction in MERTK expression from MGUS to MM) (Figure 1). The median OS for Δ^{low} MERTK was not reached while the median OS for Δ^{high} MERTK was 155 months from the diagnosis of MGUS. Although not statistically significant, patients with Δ^{high} MERTK had a shorter time to progression (TTP) (median 45 months) from MGUS to MM as compared to that of Δ^{low} MERTK (median 104 months).

Summary and Conclusions: The loss of MERTK expression that accompanied the transition from MGUS to MM was associated with a trend toward shorter TTP and OS in our cohort of MM patients. In view of the sample size, additional MGUS/MM paired samples are required to increase the power of study. In addition, further experiments are required to examine the biological role and prognostic value of MERTK in MM transformation.

PB1665

CYTOGENETIC IMPACT ON LENALIDOMIDE TREATMENT IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA: A REAL LIFE EVALUATION

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Background: Despite the progress obtained by the introduction of novel agents, treatment of relapsed/refractory multiple myeloma (rrMM) remains a clinical challenge. Long-term treatment aims to delay progression of MM, but there is concern regarding tolerance, especially in the non-study patient (pt) population. The mode of action of Lenalidomide (Len) as an immunomodulatory agent and the tolerability profile led to approval of the drug for continuous treatment until disease progression (PD) or unacceptable toxicity.

Aims: Due to the low number of reports assessing benefit and risk of long-term Len treatment in non-selected rrMM patients, we retrospectively analysed the long-term outcome in pts with rrMM treated with Len.

Methods: 91 pts were included in this study, treated with Len ± Dex for rrMM in the approved indication until PD or unacceptable toxicity. Median age was 70 years (range 42-85), median pretreatments were 2 (range 1–6). 40% of patients were relapsed and 60% refractory from previous therapy. 69% received prior treatment with bortezomib, 20% with thalidomide and bortezomib. High dose therapy was performed in 44%. Cytogenetic analysis and/or FISH was available in 76 pts (86%). According to time of stopping therapy for any reason (progression of disease or toxicity), patients were separated in three groups: Early Stop group (ES) (n: 23), when stop therapy occurred within 6 months from starting Len; Intermediate group (INT) (n: 23) for patients interrupting therapy between 7 to 24 months; and Long Runners group (LR) (n: 45), when patients were continuing therapy for more than 2 years.

Results: High risk cytogenetic, referring to the presence (alone or combined) of hypodiploid caryotype, del 13 in classical cytogenetic, del 17, FISH t(4;14), t(14; 16), was demonstrated in 29% of cases (53% ES, 43% INT, 21% LR). High risk cytogenetic and 1q21 gains were reported in 59% ES, 43% INT and 21% LR. Overall response rate (ORR) was 62% comprising 13 patients (14%) with VGPR and 13 patients (14%) with RC. Among the three cohorts, ≥ PR was demonstrated in 82% of LR (44% ≥ VGPR, with 20% RC), 82% of INT (26% ≥ VGPR, with RC 17%), and 4% (one PR) in ES. The median duration of treatment with Len was 20 months (range, 1-53 months). Overall, 35 patients (38%) experienced haematological (neutropenia and thrombocytopenia) and 39 (43%) non haematological adverse events (dyarrhea, rash) of clinical significance (grade 3-4) during Len treatment. Median PFS for the whole population was 2.8 years. The presence of 1q21 gains (p<0,0006), age (<65 *vs* >65) (p< 0,03), and response to therapy (ES *vs* INT *vs* LR) being statistically significant (p< 0,001). In multivariate analysis, significance was maintained for +1q21 (HR 4.105, p< 0.0001) and response to therapy (HR 0.370, p< 0,0021). OS for the whole population was 4.7 years and probability of two year OS was 74% (CI 95% 64-82%). +1q21 gains were demonstrated to negatively impact on survival (p< 0.001) maintaining its negative prognostic value in the Cox multivariate analysis (HR 2.41, p< 0.013).

Summary and Conclusions: Our study confirme that Len is safe and effective in patients undergoing long-term treatment in the setting of rrMM, leaving to a significant benefit in terms of PFR and OS for prolonged treatment. Analyzing the mechanisms impacting on the disease control mediated by Len therapy, our results suggest that 1q21 gains represent a detrimental feature in these patients, identifying a subset of patient poorly responsive to Len therapy.

PB1666

HIGH THROUGHPUT EX VIVO MONITORING OF DRUG SENSITIVITIES IN MULTIPLE MYELOMA COMBINED WITH CLINICAL TRIAL ASSESSMENT

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Background: Treatment for multiple myeloma (MM) patients with poor prognostic markers such as the t(4;14) translocation remains a major challenge. Despite the availability of novel agents such as bortezomib and lenalidomide, these patients may be poorly responsive and could potentially benefit from a personalized approach to treatment.

Aims: To identify novel treatments for MM, we established an *ex vivo* drug sensitivity and resistance testing (DSRT) assay assessing sensitivity of patient cells to hundreds of approved or investigational oncology drugs. In addition, genomic and transcriptomic profiling were performed to better understand disease progression and drug response mechanisms.

Methods: Bone marrow (BM) aspirates and skin biopsies were collected after informed consent from newly diagnosed (10) and relapsed (26) MM patients. CD138+ cells were isolated for analyses. The DSRT repertoire includes 306 small molecule compounds, pre-plated in 384-well plates over a 10,000-fold concentration range. CD138+ cells were added and cell viability measured after a 3-day incubation. Drug sensitivities of the CD138+ cells was determined and compared to sensitivities of healthy BM mononuclear cells to identify MM specific responses. CD138+ cell and skin biopsy DNA was analyzed by exome sequencing and CD138+ cell RNA analyzed by transcriptome sequencing.

Results: The index patient was a 56-year old male with MGUS IgA-kappa since 2011. CRAB criteria were met in June 2013 and the patient was included in the Finnish Myeloma Group-MM02 trial, consisting of three induction cycles of lenalidomide/bortezomib/dexamethasone (RVD) plus randomized mobilization (Cy 2g/m2 + G-CSF 5µg/kg *versus* G-CSF 10µg/kg) followed by ASCT and then lenalidomide maintenance starting three months after ASCT. Molecular cytogenetic/FISH analysis indicated t(4;14) (FGFR3conIGH x 2) (90%), +++1q21 (CKS1B x 5) (80%), del (13q14)/-13 (13s319 x 1,13q34 x 2) (30%), (13s319 x 1,13q34 x 1) (60%). BM samples were taken for DSRT and profiling prior to treatment initiation. In agreement with the t(4;14), RNA sequence analysis revealed high gene expression levels for *FGFR3* and *MMSET*. Six compounds known to have activity against FGFR3 were tested in the DSRT assay: dovitinib, sorafenib, nintedanib, AZD4547, AZD1480 and NVP-BGJ398. The cells showed sensitivity to nintedanib and AZD1480 at high nM levels, EC50 778 nM and 568 nM, respectively, but lacked sensitivity to the other FGFR3 inhibitors (EC50 > 10 µM). The cells, however, were selectively sensitive to the BCL-2 inhibitor ABT-199 (EC50 42.5 nM), which correlated with high *BCL-2* gene expression levels. Of the RVD drugs, the cells showed very good sensitivity to bortezomib (EC50 3.1 nM), little sensitivity to lenalidomide (EC50 9.3 µM) and no sensitivity to dexamethasone (EC50 > 10 µM). With the described treatment, the patient achieved sCR/immunophenotypic remission with minimal residual disease of 0.003-0.0004% by ASO-RQ-PCR of the clonal IgH gene rearrangement before lenalidomide maintenance.

Summary and Conclusions: While t(4;14) leads to deregulated *FGFR3* expression, this may not necessarily predict for sensitivity to FGFR3 inhibitors. Recent studies suggest that MMSET is the pathogenic driver in t(4;14) MM and may therefore be a better target for treatment. Comprehensive assessment by combined *ex vivo* drug testing and deep molecular profiling may help quickly identify alternative optimal treatments such as ABT-199 for the index patient. In addition to this present patient, additional data on other newly diagnosed patients will be presented.

PB1667

RESPONDING PATIENTS SHOW DURABLE RESPONSES TO BENDAMUSTINE IN DOUBLE REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: Bendamustine - an alkylating agent with purine-analogue like activities - shows activity in both de novo and relapsed/refractory MM patients.
Aims: The BHS MM study group initiated a study from February till December 2012 and included 20 patients with a prior history of relapse after both bortezomib and lenalidomide treatment. Other inclusion criteria were the absence of end-stage renal disease, a correct residual marrow function and the absence of plasma cell leukemia.
Methods: Twenty patients with a median age of 69 years (52y-83y) received bendamustine until disease progression. The median number of bendamustine cycles infused was 4 (1-8). Patients were heavily pretreated (mean number of prior regimens was 5, ranging from 3 to 8). Eleven patients received at least one autologous stem cell transplantation. Responses were assessed by the treating physician.
Results: The overall response rate (according to EBMT criteria) was 45% (1 very good partial response and 8 partial responses), 2 patients showed a minor response, 3 patients a stable disease and disease progression was described in 6 patients. The progression free survival (PFS) for the whole population was 90 days, but responding patients had a PFS of 133 days (compared to 60 days in non-responding patients, p=0.001). Overall survival was 350 days for responding patients compared to 137 days for non-responding patients (p=0.0006). The toxicity was mainly haematological with gr III-IV cytopenia in 50% of patients. One patient presented a septicaemia and another one a CMV infection (Figure 1).

Figure 1. Overall survival for responding *vs* non-responding.

Summary and Conclusions: In this heavily pretreated patient population, survival rates for patients responding to bendamustine salvage therapy were encouraging. We believe that well-selected double refractory patients might benefit from bendamustine as salvage treatment, but further prospective clinical trials are needed in this situation.

PB1668

USEFULNESS OF HEAVY-LIGHT CHAIN IMMUNOANALYSIS IN MULTIPLE MYELOMA PATIENTS WHO UNDERWENT ASCT. A NEW LEVEL OF RESPONSE?

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Background: In the last years the world of Myeloma patients has evolved, now we have many new drugs under investigation accompanied by new techniques for assessment. Currently the freelight chain immunoassay (FLC) (Bindingsite, Birmingham, UK) is part of the mandatory assessment for response according to IMWG, but the Heavy-Light-Chain (HLC) immunoassay is still under investigation with promissory results and developing a new category: immunoparesis. It seems like the recovery of uninvolved HLC (immunoparesis recovery – IR-), is the result of the immune system reconstitution.
Aims: To analyze the immunoparesis recovery (IR) in patients with MM who underwent Autologous Stem Cell Transplantation (ASCT) and correlate with outcomes.
Methods: A retrospective chart review was performed with all consecutive secretory MM patients who underwent ASCT in our institution between May 2011 and Oct 2013. Clinical, demographical and laboratory assessment before ASCT and 8-12 weeks after and outcomes were registered.
Results: A total of 42 patients were registered. Median follow-up 14 months. MF ratio: 19/23 (54.8/45.2%), mean age 58.5 y.o. (40-70). Immunoglobulin Subtype: IgG-Kappa: 38.1% (16), IgG-Lambda: 23.8% (10), IgA-Kappa: 14.3% (6), IgA-Lambda: 9.5% (4), Bence-Jones-Kappa: 4.8% (2), Bence-Jones-Lambda: 9.5% (4). Durie-Salmon Stage: IA: 14.3% (6), II-A: 31% (13), III-A: 40.5% (17), III-B: 11.9% (5), missing-data 1 case. After induction therapy (see table 1) response assessment were: minimal response: 7.9% (3), Partial Response (PR): 52.4% (22-of them 2 with IR), VGPR: 11.9% (4), SR: 9.5% (4), SR+IR: 11.9% (5), missing data: 4 cases. After ASCT, evaluation reveals 11 patients who achieved IR (8 with SR, 1 VGPR and 2 PR) the distribution according response were: stable disease: 5.2% (4), PR: 41% (16), VGPR: 28.6% (7), SR: 14.7% (6), SR+IR: 20.5% (8), missing: 3 cases. During the follow-up, a total of 16 patients who achieved at least PR after ASCT had relapsed or progressed, 11 from PR group, 4 from VGPR and 1 from SR group. Only 2 from the group of IR after ASCT (1 PR and 1 VGPR), IR were a predictor of good outcome (p= 0.032). The mean PFS was 20.7 months (16.2-25.2), median 20 months (13.7-26.3), for patients who achieved IR was 26.7 months with median not reached. The OS from ASCT was: 31.4 months (27.9-34.8).
Summary and Conclusions: Even with a small cohort, we believe the HLC analysis is a useful assessment to define patient under risk of relapse. More studies are warranted for define the role of these tests in MM patients.

PB1669

FREE LIGHT CHAIN ESCAPE REFLECT A RESISTANT RELAPSE IN MULTIPLE MYELOMA (MM), ACCOMPANIED BY DECREASED SERUM TGF-BETA1 (TGFB1); CLINICAL CORRELATIONS

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Background: Free light chain escape (LCE), defined as the occurrence of sFLC increase with stable or falling monoclonal immunoglobulin (M-Ig) concentrations was shown to reflect disease aggressive evolution in MM and to eventually reflect molecular myeloma cells changes or reveal clonal cell competition. However microenvironmental factors usually also contribute to disease evolution.
Aims: As TGF-b1, a pleiotropic cytokine that normally down-regulates B-cell proliferation and modulates Ig production, was found increased in the serum of MM patients with a better outcome, we aimed to study its levels in parallel with the prevalence and clinical impact of LCE in a series of MM patients.
Methods: We studied 234 symptomatic MM patients diagnosed and followed in our hospital. 29%, 42%, 29% and 22%, 30%, 48% were in Durie-Salmon and ISS stages I, II, III and 1, 2, 3 respectively. MM type was IgG in 138, IgA in 49, light chain only in 40, IgD in 4, non-secretory in 3 and IgM in 1 patients. As all patients were symptomatic, they all had received at least 1 treatment line (median 3). Serum TGF-b1 measurements were performed retrospectively by ELISA according to the manufacturer's instructions in frozen sera drawn at diagnosis or relapse and at the time of LCE in 10 patients.
Results: Eighteen patients presented LCE (7%). The median number of treatment lines received prior LCE was 5. Nine out of 18 LCE patients were asymptomatic and in a good performance status at the time of LCE onset and relapse would not have been obvious in the absence of free light chain (FLC) serial follow-up measurements. The median time from LCE to last follow-up or death was 2 months. 6/18 LCE patients had previously received high dose treatment and ASCT while all had received new drugs. Median TGF-b1 levels at the time of LCE was 8882 pg/ml as compared to 44196 at diagnosis or previous relapse (p<0.01).
Summary and Conclusions: LCE represent a rare form of relapse that corresponds to a very aggressive disease behavior, probably due to the emergence of a new resistant clone. In order to observe the phenomenon, regular follow –up with quantitative Ig and FLC measurements is needed. The low TGF-b1 levels observed at LCE may reflect escape from a residual immune surveillance allowing the emergence of new clones.

PB1670

FUNCTIONAL AND SEROLOGICAL RESPONSE IN PARAPROTEINAEMIC NEUROPATHY TO A STANDARDISED CHEMOIMMUNOTHERAPEUTIC REGIMEN

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Background: Paraproteinaemic neuropathy (PPN) is a rare disorder associated with MGUS with evidence of a pathogenetic role for the paraprotein. Many cases are associated with the presence of antibodies to myelin associated glycoprotein (MAG) and some have an underlying lymphoproliferative disorder (LPD). The natural history of the disease is of slowly progressive neurological deterioration, existing treatments have poor efficacy, and none have been proven to alter outcome. Single agent Rituximab failed in an RCT to achieve the primary endpoint

and single agent chemotherapy has produced variable results with toxicity in very small numbers of patients due to the rarity of the disorder.
Aims: The aim of the study was to evaluate the potential for combination chemoimmunotherapy similar to that used successfully in haematological malignancies to produce functional and serological responses in patients with PPN.
Methods: 12 patients with IgM PPN (7 with MGUS all of whom had anti-MAG antibodies and 5 with underlying Lymphoplasmacytic Lymphoma) were treated with a standardised chemoimmunotherapeutic regimen, comprising six cycles of Rituximab 375mg/m^2, Cyclophosphamide 750mg/m^2 and five days of Prednisolone 50mg/m^2 every 21 days, together with strict anti-microbial prophylaxis (*i.e.* 'R-CVP', with the omission of Vincristine).
Patients were assessed at baseline with bone marrow biopsy and CT scans to identify underlying LPD and with standardised neurological assessments including electrophysiology, functional MRC and Overall neuropathy limitation scores (ONLS) together with paraprotein and Anti-MAG antibody quantitation. Assessments were repeated at 3 months and 12 months post treatment.
Results: All 12 patients had an IgM paraprotein, 7 (all of whom were anti-MAG positive) had IgM MGUS and 5 had underlying lymphoplasmacytic lymphoma. At 3 months post completion of treatment, IgM paraprotein levels had fallen in all 12 patients (mean at baseline 6.24g/L, sd 4.4g/L, mean at three months post treatment 3.08g/L, sd 2.3g/L). 4/5 patients with underlying LPL achieved complete remission and the other partial remission as assessed by BM biopsy and CT scans. Functionally, the ONLS improved in 50% patients and was static in 42%. 75% of patients improved their sensory sum score, 50% by at least two points. MRC sum score of muscle power improved in 42% and remained static in 50%. Electrophysiological studies demonstrated improvements in upper limb parameters in 50% of cases and were static in the other 50%. 12 month post treatment follow up data available for 7 patients, suggests that their early response to treatment has been sustained with no functional or serological relapses to date. Furthermore 86% patients have shown falls in anti-MAG antibody titre with a mean reduction of 53% suggesting sustained immunological improvement. The treatment was generally well tolerated. There was no treatment related mortality. One patient had a mild haemorrhagic cystitis and another had influenza pneumonitis both with complete resolution.
Summary and Conclusions: This chemoimmunotherapeutic protocol (R-CP) has shown striking functional and serological responses in a cohort of patients with IgM related neuropathy in the context of a disease with a progressive neurological disabling trajectory. Patients with underlying LPD have also achieved remission and those with anti-MAG antibodies have shown ongoing fall in levels. There have been no major complications. These encouraging early results suggest the need for further evaluation in the context of a randomised clinical trial.

PB1671

PERCENTAGE OF URINARY ALBUMIN EXCRETION AND SERUM FREE LIGHT CHAIN REDUCTION AREIMPORTANT DETERMINANTS OF RENAL RESPONSE IN MYELOMA PATIENTS WITH MODERATE TO SEVERE RENAL IMPAIRMENT

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Background: Renal impairment (RI) is a major cause of morbidity and mortality in patients with multiple myeloma (MM) and approximately 50% have renal impairment and 20% of patients have acute renal failure depending of its definition. Although reversal of renal dysfunction significantly affects prognosis of MM with RI, there is no reliable test for predicting reversibility of RI in MM patients.
Aims: We postulated that MM with high albuminuria reflects glomerular disease that is difficult to reverse. We examined the impact of urinary albumin excretion percentage on renal recovery in MM patients with RI.
Methods: We retrospectively analysed 259 patients admitted to our hospital form April 2000 to September 2013.Clinical variables and laboratory data that may affect myeloma treatment response were extracted. RI was defined as an estimated glomerular filtration rate (eGFR), which was measured before treatment ≤50 ml/min/1.73 m2 by the simplified Modification of Diet in Renal Disease formula. Maximal renal response was evaluated according to the recently proposed "Criteria for the Definition of Renal Response to Antimyeloma Therapy" from IMWG working group. Patients with preexisting severe renal impairment (creatinine≥2.0 mg/dL) due to causes other than MM were excluded from the study. Extracted clinical data related to myeloma and RI included age, sex, complete blood count, urinalysis, serum protein electrophoresis, serum free light chains (FLC), serum albumin, β2-microglobulin, serum creatinine, urinary protein concentration, and urine protein electrophoresis. Reduction of involved FLC (iFLC) was checked at day 12 and at 21 after the start of anti-myeloma therapy. Percentage of urinary albumin excretion was calculated by protein electrophoresis pattern on admission. The results were examined for relationship to renal response by odds ratio (OR) using logistic regression analysis.
Results: Moderate to severe renal impairment (estimated glomerular filtration rate, eGFR≤50mL/min/1.73m2) was observed in 118 patients (46%) and renal responses of CRrenal, PRrenal, MRrenal, and no response were obtained in 42 (36%), 16 (14%), 13 (11%), and 47 (40%) patients, respectively. We examined the association between subsequent any renal response and the degree of iFLC reduction on days 12, and 21. Receiver operator curves (ROC) were constructed to determine the best cut-off% of iFLC reduction for renal recovery. From the curve, cut off point of% iFLC reduction at day 12 was identified as 81.8% with specificity 93%, sensitivity 49%, and area under the curve (AUC) of 0.73. Similarly, 95.5% reduction was identified with specificity 92%, sensitivity 49%, and AUC of 0.73 on day 21. Although renal recovery was significantly better in patients with age ≤70, myeloma response ≥VGPR, involved FLC (iFLC) reduction from baseline ≥80% on day 12, ≥95% on day 21, and urinary albumin ≤25% on univariate analysis, only reduction of iFLC ≤95% on day 21(OR: 6.26, p =0.01) and urinary albumin ≤25% (OR: 3.55, p =0.01) remained significant on multivariate analysis.
Summary and Conclusions: Urinary albumin excretion ≤25% and reduction of iFLC ≤95% on day 21 were considered as positive predictors for favorable renal response in patients with moderate to severe renal impairment. Our data will prove the important information for selecting the myeloma patients with renal impairment who will benefit from intensive antimyeloma therapy and mechanical removal of FLC.

PB1672

STRINGER RESPONSE WITH IMMUNOPARESIS RECOVERY AFTER BORTEZOMIB-BASED THERAPY IN MULTIPLE MYELOMA AND RELAPSE. USEFULNESS OF HEAVY-CHAIN-LIGHT ASSESSMENT

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Background: Bortezomib has proven to be one of the major advances on multiple myeloma (MM) therapy. Actually is part of the first-line standard of care therapy for patients with MM. Multiple strategies have been developed to try to predict response or improve the assessment of response and follow-up of MM patients, current International Myeloma Working Group (IMWG) criteria of response included immunophenotype and immunoparesis analysis. The Heavy Lite^{TM} and Free Lite^{TM} (Bindingsite, Birmingham, UK) permit a separate quantification of the kappa- and lambda-bounded amounts of a given immunoglobulin analysis (HFC) and the free light chains kappa or lambda amount quantification (FLC) and both are excellent tools for immunoparesis assessment.
Aims: To analyze the usefulness of immunoparesis analysis by Heavy-Lite^{TM} and Free-lite^{TM} in patients who receive bortezomib-based therapy in our institution.
Methods: A retrospective chart review was performed including the patients diagnosed of IgA or IgG secretory MM who received therapy with bortezomib even for relapsed as on first-line therapy. General clinical characteristics, schedules of therapy, number of cycles, response to therapy and relapse were recorded. For analysis only patients with at least 4 cycles of bortezomib based regimen and HLC and FLC assessment at the end of therapy or between 8-12 weeks after autologous stem cell transplantation (ASCT). Period of study: June 2004 to December 2013.
Results: At the end of study a total of 78 MM patients received bortezomib-based therapy, of them 73 complete 4 or more cycles and were included in the analysis. Male/Female ratio: 34/39, mean age: 67.3 years old (46-81), therapeutic schedules were: bortezomib-prednisone: 2 (2.7%), bortezomib-dexametasone: 36 (49.3%), bortezomib-melfalan-prednisone: 21 (28.8%), bortezomib-dexametasone-lenalidomide: 9 (12.3%) and bortezomib-talidomide-dexametasone: 2 (2.7%), bortezomib-adriamicine-dexametasone: 2 (2.7%), bortezomib-cyclophorphamide-dexametasone: 1 (1.4%). Around 70% of patients received at least 6 cycles of therapy. Immunoglobulin Myeloma type: IgAL: 20.5% (15), IgAK: 16.4% (12), IgGK: 50.7% (37) and IgGL: 12.4% (9). A total of 75.3% (46) presented an abnormal HFC ratio at diagnosis, analysis of immunoparesis before therapy revealing 79,4% (58) affected patients; a total of 76.7% (56) registered an abnormal FLC ratio at diagnosis. Response to therapy were as follows: a stringent complete response (SR) were achieved by 27.4% (20) of cases (10 associated with IR), very good partial response (VGPR): 4.1% (3), partial response (PR): 56.2% (41) patients and not-response/progressive-disease: 12.4% (9) patients. At the time of post-therapy evaluation, 35.6% (26) of patients had normalized the FLC-ratio, 20.5% (15) of the SR and, 1,4% (1) of VGPR and 10.9% (8) of PR. Normalization of HFC-ratio only occurs on patients on SR and VGPR: 20.6% (13). About immunoparesis analysis only 23.3% (17) patients presented immunoparesis recovery (IR) at the end of therapy, 16.4% (12) of SR patients and the rest on VGPR (2) and PR (4) patients. After a median follow-up of 29 months (9-94) 63% (46) of patients had relapse, mean progression free survival time (PFS) for all patients was 28.5 months (22.6-34.4) median PFS 26 months; for patients who achieve SR was 32.5 (26.6-38.5); the association of SR with IR was related to a less tendency to relapse and need of therapy, 9/10 patients who achieved this status are not-relapsed.
Summary and Conclusions: In our cohort, the patients who achieved a SR with a normalization of immunoparesis shows a clear tendency to less incidence of

relapse; probably this reflect a better response with not only an undetectable monoclonal protein but a normalization on immune system. Even in small cohorts, the immunoparesis recovery analysis through HFC quantifications seems to be an useful tool to determine a new level of response. More investigations on this field are warranted.

PB1673

A THOROUGH EVALUATION TO RULE OUT THE PRESENCE OF PLASMACYTOMAS IS OF IMPORTANCE IN PATIENTS WITH NEWLY DIAGNOSED SYMPTOMATIC MYELOMA

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Background: Myeloma is a neoplasm of plasma cells that causes painful, bone-destructive lesions. It may present as a solitary lesion (plasmacytoma), or involve multiple sites (multiple myeloma, MM). Solitary plasmacytomas of bone or extraosseous plasmacytomas, depending on the tissue of origin, develop in isolation without systemic manifestations of MM. Some patients with MM has additional tumor masses that can affect various organs and worsen prognosis. The incidence of osseous and extraosseous plasmacytomas in MM is 7% at diagnosis and about 17% at the end stages of the disease.
Aims: To investigate possible correlations between laboratory findings and clinical outcome in patients with newly diagnosed symptomatic myeloma.
Methods: Twenty-seven adult patients (median age 56 years; male 15, female 12) with newly diagnosed MM were included. The diagnosis of active MM was made according to standard diagnostic workup (history and physical exam, serum albumin, quantitative immunoglobulins, beta-2 microglobulin, serum and urine electrophoresis, immunofixation, and free light chain assays, bone marrow (BM) aspiration and biopsy, BM multicolor flow cytometry (MFC) analysis of plasma cell (PC) population, plasmacytoma immunohistochemistry (IHC), BM routine cytogenetic and FISH). The entire patients had skeletal survey and one of the additional methods: computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET)/CT scan. MM staging assessed using Durie-Salmon and International staging systems. All the patients had primary bortezomib-based therapy. Responses were investigator-assessed and based on EBMT and IMWG response criteria.
Results: Eleven patients (40.7%) had plasmacytomas at diagnosis, which were revealed using CT, MRI, and PET/CT (4, 5, and 2 cases, respectively), six of them (55%) had no plasmacytomas at skeletal survey. 7/11 patients (64%) had symptomatic plasmacytomas. CT (7), MRI (7), and PET/CT (2) did not reveal plasmacytomas in 16 patients with bone lesions. Ten patients (37.0%) had osseous plasmacytomas, and 6/10 (60.0%) had multiple (≥2) osseous plasmacytomas. One patient had extraosseous (both breasts) plasmacytomas. 9/11 (81.8%) patients manifested with signs of vertebral column instability or spinal cord compression were operated on: 5/9 (55.5%) - before initiation of primary therapy, and 4/9 (44.5%) - between treatment cycles. During follow-up period (2 years after diagnosis) one patient was diagnosed as having new osseous plasmacytoma, which was successfully operated on. MFC was performed on 27 (100%) consecutive BM aspirates: CD38 and CD138 was expressed in all cases (100%), CD19 – 9 (33.3%), CD56 overexpression – 11 (40.7%), CD117 – 9 (33.3%). Patients' PC phenotype detected on BM MFC and results of plasmacytoma IHC were identical. 10/11 (90.1%) patients with plasmacytomas showed no CD117 overexpression on PC (results of BM MFC and IHC assays), whereas one patient was not assessed. At a median follow-up of 35 months 2-year overall survival was 77.9% (plasmacytomas at diagnosis), and 93.7% (without plasmacytomas) (*p>.05*).
Summary and Conclusions: Our study shows that the presence of plasmacytomas correlate with the absence of CD117-expression in patients with newly diagnosed symptomatic myeloma. The newly diagnosed patients with MM require a very thorough evaluation including CT, MRI and/or PET/CT (preferable diagnostic tool) to rule out the presence of plasmacytomas, especially in patients with CD117 negative PC patterns. Further studies are needed to confirm this proposal.

PB1674

COMPARISON OF RESPONSE DURATION (RPD) AFTER THALIDOMIDE, BORTEZOMIB AND LENALIDOMIDE ADMINISTRATION TO MULTIPLE MYELOMA (MM) PATIENTS; A SINGLE CENTER EXPERIENCE

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Background: With the introduction of novel drugs, mainly Thalidomide, Bortezomib and Lenalidomide and the enrichment of MM therapeutic armamentarium, patients' survival has improved because they produce qualitative responses accompanied by a prolonged response duration (RPD) and because a new treatment is usually more potent than the re-administration of an old one. On the meanwhile, numerous next generation new drugs are on their way. It is generally accepted that RD is longer after first response and steadily shortens with subsequent treatment lines, as new resistant clones may appear. It is however unknown if new treatments can produce long-lasting responses after multiple relapses.
Aims: To investigate RPD produced by new treatments after initial and multiple relapses.
Methods: 229 symptomatic MM patients, followed and treated in our hospital were studied; 22%, 30% and 48% of them were in ISS stages 1, 2 and 3 respectively. MM type was IgG in 62%, IgA in 22%, light chain only in 13%, IgD in 2% and non-secretory in 1% of patients. They received a total of 718 treatment lines (median 3), including various treatment combinations with or without new drugs. However, only double combinations are evaluated here in order to avoid bias. One hundred and thirty three patients received Bortezomib-Dexamethasone (VD), 81 Thalidomide-Dexamethasone (TD), and 90 Lenalidomide-Dexamethasone (RD). Median survival of the whole cohort was 48 months. Comparisons between RPD observed according to drugs administered and lines were evaluated by the chi square test.
Results: Considering all treatment lines together, patients receiving new treatments achieved better quality of response and RPD ($p<0.01$ both). As expected, TD and VD treatment produced longer RPD before than after 3rd relapse (p=0,002), while RPD did not differ among the first three treatment lines. Of the 3 drug combinations, only VD produced valuable responses at re-administration, although of shorter duration. Interestingly RD produced sustained responses with no difference in longevity at any line administered; thus median RPD after RD administration in 55 patients at 2nd and 3rd line was 10,29 months (range 1-52,2) while it was 13,21 months (1-31.48) in 35 patients treated after 3rd relapse.
Summary and Conclusions: We confirmed that better response rates and RPD were observed with novel drugs compared to conventional ones. Furthermore, we showed that sustained RPD were observed with all TD, VD and RD until 4th relapse, while RD was able to produce equally long-lasting RPD before and after 3rd relapse suggesting that the drugs ability to restore the microenvironment may delay the occurrence of new clones in responding patients, thus encouraging its administration upfront.

PB1675

IMPACT OF CUMULATIVE BORTEZOMIB DOSE ON RESPONSE TO TREATMENT AND PATIENT SURVIVAL

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Background: Multiple myeloma (MM) is an incurable plasma cell neoplasm. Newer agents in MM such as bortezomib have improved the clinical outcome of patients (pts) with this disease. Recently, it has been suggested that a higher cumulative bortezomib dose may be associated with improved response rates and even survival.
Aims: To determine if the cumulative bortezomib dose has an impact on disease response and patient outcome.
Methods: We performed a unicentric, retrospective cohort study, reviewing the clinical data of all MM pts treated with bortezomib-containing regimens between January 2012 and December 2013. The regimens included: bortezomib-cyclophosphamide-dexamethasone (VCD) in transplant-eligible pts, bortezomib-dexamethasone (VD) and bortezomib-melphalan-prednisone (VMP) in transplant-ineligible pts. We compared the planned bortezomib dose with the real delivered bortezomib dose, examined toxicities and causes for patients decreasing dose/cycles and studied the response rate, progression free survival (PFS) and treatment free interval. PFS was calculated using the Kaplan Meier method and curves were compared by log-rank test. Chi-square was used to test for differences in the proportions of responses obtained in each group.
Results: We analysed 92 pts, 59 males; median age at start of bortezomib regimen was 65 yo (min 37; max 89); main subtypes were IgGK 40, IgGL 18 and IgAK 13; ISS 1 in 34 pts and ISS 3 in 27 pts; bortezomib containing regimens were VCD in 55 pts, VMP in 19 and VD in 18; bortezomib was given intravenously (iv) in 42 pts, subcutaneously (sc) in 38 and 12 pts started iv and changed to sc during treatment. Median line of bortezomib containing regimen was 1 (range 1-8). The median number of planned cycles was 6 but only a median of 4 were performed. The median planned dose of bortezomib was 31.2 mg/m^2 (min 20.8; max 52) but the median delivered dose was 20.8 mg/m^2 (min 1.3; max 46.8). The main reasons to decrease bortezomib doses were thrombocytopenia (23 cases), polyneuropathy (19 cases) and infection (16 cases). Only patients who received a full dose of bortezomib achieved sCR (n=3). From the 11 pts achieving CR/sCR, only 2 had bortezomib dose reductions; from the 21 pts achieving VGPR, 3 had bortezomib dose reductions; from the 35 pts achieving PR, 6 had bortezomib dose reductions and from the

12 non-responders, 6 had dose reductions. We found a significant association between bortezomib doses delivered and response rate (p=0.017): the relative risk of achieving any response between patients with and without bortezomib dose reductions is 0.32 (CI95% 0.15 - 0.72). With a median follow-up of 8.4 months, PFS is associated with cumulative bortezomib dose, as pts who needed bortezomib dose reductions greater than 20% seem to have a worse PFS compared to pts treated with the planned bortezomib dose (median 23 months for pts with bortezomib dose reductions and not reached for pts without dose reduction). Median treatment-free interval was significantly longer in pts without bortezomib dose reductions (median not reached) compared to pts who needed dose reductions (median 3.4 months, p=0.0042).
Summary and Conclusions: These data suggest that a higher cumulative dose of bortezomib, reflecting prolonged treatment duration and lack of drug interruption/reduction (dose intensity), is associated with improved response rates, better PFS and longer treatment free interval.

PB1676

PRETREATMENT LYMPHOPENIA AND POOR PERFORMANCE STATUS ARE RISK FACTORS OF SEVERE BACTERIAL INFECTION IN PATIENTS WITH MULTIPLE MYELOMA DURING CHEMOTHERAPY WITH BORTEZOMIB CONTAINING REGIMENS

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Background: Infection is the leading cause of morbidity and mortality in patients with multiple myeloma.
Aims: The aim of this study is to identify the risk factors associated with the development of severe infections in patient with multiple myeloma during chemotherapy with bortezomib-containing regimens.
Methods: A total of 98 patients with myeloma who were treated with bortezomib-based chemotherapy between 2006 and 2012 were examined. Using the logistic regression method, a variety of factors were analyzed for the development of severe bacterial infections (SBI) at each 427 courses.

Figure 1. Probability of severe bacterial infection according to the number or three risk factors (lymphopenia, poor performance status, early course [≤2 courser] on treatment) at each courses.

Results: Median age of patients was 62 years and 40.6% (30/98) of patients treated with bortezomib-containing chemotherapy as first-line treatment. The SBI was developed in 57% (56/98) of patients and 19% (81/427) of bortezomib courses. Of 81 SBI episodes, 42 (53%) episodes were clinically documented infection, 30 (37%) episodes were microbiologically documented infections, and 9 (11%) episodes were fever of unknown origin. The most common type of infection was pneumonia (60%), and only 6 episodes were febrile neutropenia. In univariate and multivariate analysis of the development of SBI by 98 patients, we could not find significant factors other than poor performance status (Hazard Ratio [HR] 5.365, 95% Confidence Interval [C.I.] 2.004-14.364, *P* =.001). However, in univariate analysis of the development of SBI by each 427 courses, poor performance status (ECOG ≥2) (*P* <.001), early courses of therapy (≤2 courses) (*P* <.001) and pretreatment lymphopenia (absolute lymphocyte count<1.0 × 10^9/L) (p=.043) were associated with increased risk of SBI at each courses. Multivariate analysis confirmed poor performance status (ECOG ≥2) (HR 3.920, 95% C.I. 2.305-6.666, *P* <.001), early courses of therapy (≤2 courses) (HR 2.782, 95% C.I. 1.633-4.740, *P* <.001) and pretreatment lymphopenia (HR 1.728, 95% C.I. 1.016-2.937, p=.043) as risk factors for development of SBI during the chemotherapy with bortezomib containing regimens. The probability of SBI at each course was 5.1% in courses with no risk factor, 14.9% with 1 risk factor, 23.9% with 2 risk factors and 59.5% with 3 risk factors (*P* <.001, Figure 1). In patients who were eligible for autologous stem cell transplantation (ASCT), 14 of 18 patients (77.8%) who didn't experienced SBI received ASCT, but only 6 of 26 patients (23.1%) who experienced SBI received ASCT (*P* <.001). Among these transplant-eligible patients, the patients who experienced SBI showed a significantly shorter median overall survival than patients who didn't experienced SBI (11.2 month *vs* not reached, p=.016) as well as progression free survival (6.0 months *vs* 24.4 months, p=.015). The multivariate cox analysis demonstrated that the experience of SBI was the only statistically significant factor for inferior progression free survival in patients who were eligible for ASCT (HR 2.806, 95% C.I. 1.174-6.705, p=.020).
Summary and Conclusions: In conclusion, patients in the courses with these risk factors should be more closely monitored for the development of bacterial infection and be considered to use prophylactic antibiotics.

PB1677

MONITORING OF MINIMAL RESIDUAL DISEASE (MRD) IN PATIENTS WITH MULTIPLE MYELOMA (MM) BEFORE AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

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Background: According to criteria recommended by IMWG the effect of treatment of MM patients is evaluated not only by clinical, morphological, immunochemical parameters, but also by the absence of clonal plasma cells in the bone marrow in immunohistochemistry and immunofluorescence studies.
Aims: Monitoring of MRD in MM patients at the stage of high dose consolidation.
Methods: We screened 32 patients with MM (m -17, w -15), age 41-65 years (M-58). Phase of the disease after induction of bortezomib - containing regimens: CR- 12, VGPR-8, PR -12. Stem cell mobilization (SCM) included cyclophosphamide 4g/m^2 plus G-CSF 5 μg/kg/day. Prior ASCT conditioning included melphalan 200mg/m^2. After ASCT CR-19, VGPR-13. Bone marrow samples were examined by multiparameter flow cytometry (MFC) before SCM, 2-6 months after ASCT by using BD FACSCanto II flow cytometer. Myeloma plasma cells (PC) were determined according to aberrant antigens expression CD38/CD138/CD45/CD19/CD56/CD117/CD28 (A.C. Rawstron's protocol 2006). The analysis was based on at least 1000 000 CD45+ events. MRD(-)<0.01%, near-threshold amount myeloma PC was determined in amount of 0.01-0.04% residual cells.
Results: The examination of 12 patients in CR, achieved by the induction treatment, MRD (-) was detected in 6 (50%). Near-threshold amount of myeloma PC defined in the other 6 patients with CR and 1 patient with VGPR. After ASCT immunochemical CR diagnosed in 19 patients , among of them MRD (-) observed in 7 cases (37%) , while the remaining 12 patients showed near-threshold value myeloma PC.
Summary and Conclusions: MFC is the method of choice for accurate quantitation of residual myeloma PC in MM patients at different stages of therapy. Recognition of MRD after ASCT indicates the advisability of the appointment maintenance therapy.

PB1678

RESPONSE TO INDUCTION TREATMENT ADAPTED SELECTION OF MOBILIZATION REGIMEN IN MULTIPLE MYELOMA: THE G-CSF ALONE VERSUS HIGH-DOSE CYCLOPHOSPHAMIDE PLUS G-CSF REGIMEN

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Background: There is no guideline for choice of mobilizing regimen for autologous stem cell transplantation (ASCT) in multiple myeloma although both of G-CSF alone regimen and cyclophosphamide plus G-CSF regimens are recommended by International myeloma working group and used most commonly.
Aims: This study was designed to evaluate the efficacy and transplant outcomes of response to therapy adapted selection of mobilization regimen.
Methods: A total of 156 patients who diagnosed with MM were admitted for peripheral blood stem cell mobilization using cyclophosphamide plus G-CSF or G-CSF alone between September 2006 and September 2012 from 7 institutions in Korea were analyzed retrospectively. The patients (N=62) who received G-CSF alone for mobilization in complete response (CR) or very good partial response (VGPR) status and the patients (N=65) who received HD-CY + G-CSF for mobilization in response less than VGPR were classed as the 'Response adapted group' (N=127). Besides, we classified the 'Random CY group' as an intended control group (N=58) with same patient composition of disease status at mobilization of half-CR + VGPR (N=29) and half-less than VGPR (N=29).
Results: In the response adapted group, the patients received G-CSF alone (G-CSF group) and the patients received HD-CY + G-CSF (CY group) were

comparable for age, sex and clinical prognostic features at diagnosis as well as previous therapies except the percentage of plasma cell in bone marrow at mobilization (CY group 4.0% *vs.* G-CSF group 0.7%, P<0.001). The total quantities of CD34+ cells collected per patients were 6.6 x10^6/kg in G-CSF group and 12.7 x10^6/kg in CY group (P <0.001). The rate of successful mobilization (≥4 x10^6/kg) tend to be higher in CY group but had no statistical difference (79% and 91%, P=0.064). Of note, requisite mobilization (≥2 x10^6/kg) was achieved in most patients of similar proportions in both groups without significant difference (97% and 97%, P>0.999). Duration of hospitalization for mobilization was longer in CY group (9 days *vs.* 17 days, P<0.001) and the treatment-related toxicity was greater in this group: 34 patients (37%) developed fever requiring antibiotics during the neutropenic period after HD-CY and 51% and 52% of patients required red cell and platelet transfusion support, respectively. After ASCT, median time to neutrophil engraftment was 10 days for G-CSF group and 11 days for CY group (P=0.004) despite the quantities of infused CD34+ cells were higher in CY group than G-CSF group (6.2 x 106/kg *vs.* 4.5 x 106/kg, P<0.001). Transplantation outcomes were compared between the response adapted group and the random CY group. The patients in two groups were comparable with each other in their demographic, clinical, and laboratory characteristics. VGPR + CR rate and CR rate tended to higher in the response adapted group than the random CY group (77.5% *vs.* 69.6% and 60.8% *vs.* 51.8%, respectively) without statistical significance. At a median follow-up of 34 months after ASCT, there were no differences in adjusted overall survival rate and progression-free survival rate (P=0.6 and P=0.635, respectively) (Figure 1).

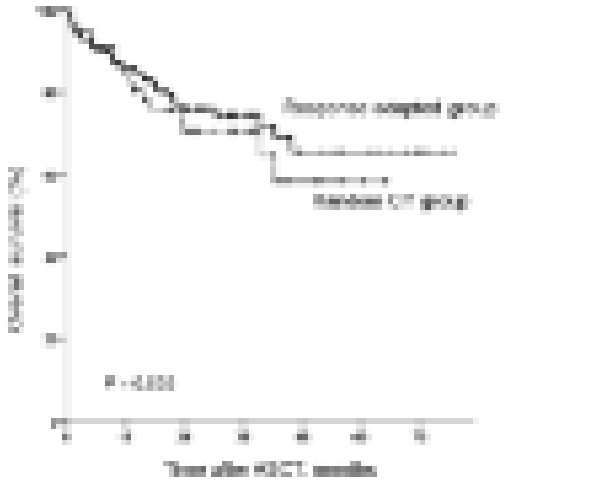

Figure 1.

Summary and Conclusions: These data suggest that the requisite CD34+ cell collections for the transplantation can be achieved with both regimens of HD-CY plus G-CSF and G-CSF alone in most MM patients. Furthermore, G-CSF alone as mobilization regimen was not only cost-effective with less toxicity, but it showed satisfactory outcomes after transplantation in the good responders to previous therapy. We suggest that risk adapted choice of mobilization regimen is appropriate and mobilization with G-CSF alone is preferred in the MM patients who achieve a good response to induction treatment.

PB1679

CYTOGENETIC RISK GROUP BY FISH IS THE MOST IMPORTANT INDEPENDENT PROGNOSTIC FACTOR TO PREDICT OUTCOMES AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA PATIENTS WITH EXTRAMEDULLARY DISEASE

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Background: Cytogenetic abnormalities are considered to have an important prognostic value in multiple myeloma (MM), helping to identify high-risk patients. Fluorescence *in situ* hybridization (FISH) is more sensitive than conventional cytogenetics for recognizing chromosomal changes. Several FISH-detected abnormalities have been associated with inferior prognosis, including deletion of chromosomes 17 and 13 and t(4;14)(p16.3;q32). However, it has been paucity data about prognostic significance of FISH abnormalities for autologous stem cell transplantation (ASCT) in MM patients with extramedullary plasmacytoma (EP).

Aims: Aim of study is to determine the prognostic impact of FISH data for ASCT in MM patients with EP.

Methods: Total forty-nine cases, whose FISH data of the bone marrow samples at diagnosis were available, were collected retrospectively.

Results: The t(11;14) cytogenetic abnormalities were seen in seven of 38 patients (14.3%). Del (13q) and t(4;14) were found in ten (20.4%) and three (6.1%) of 39 patients, respectively. No t(14;16) and del (17p) cytogenetic abnormality was detected in 38 patients. Concomitant presence of t(11;14) and del (13q) was seen in two cases and with t(4;14) in one case. Combined t(4;14) and del (13q) abnormalities was observed in one case. Three-year PFS rate was 39.5%±11.9% in patients without del (13q), 35.5%±11.1% in those without t(11;14), and 31.0%±10.0%, respectively. On the other hand, all patients were relapsed within three years after ASCT in those with del (13q) (p=0.001), t(11;14) (p=0.003), and t(4;14) (p=0.006), respectively. Patients with del (13q), t(11;14), and t(4;14) had lower OS rates than those without del (13q) (3-year OS rate, 37.5%±17.1% *vs.* 88.2%±8.0%; p=0.002), t(11;14) (3-year OS rate, 50.0%±20.4% *vs.* 80.4%±8.9%; p=0.022), and t(4;14) (3-year OS rate, 0% *vs.* 78.5%±8.6%; p=0.058). Patients without t(14;16) revealed comparative 3-year PFS (32.0%±8.5%) and OS rates (72.6%±7.9%) compared to those patients without del (13q), t(11;14), or t(4;14). Based on these FISH results, we make new FISH prognostic risk groups. Of 27 patients who had four FISH data at diagnosis, patients who were positive more than one of four FISH assigned to high-risk group (*n* = 4), those who were positive one of four FISH assigned to intermediate-risk group (*n* = 8), and patients with all negative FISH data assigned to low-risk group (*n* = 15). Median PFS of high- and intermediate-risk groups showed 28 months and 13 months, respectively. In high-risk group, median PFS duration was only 4 months (*P*<0.001). OS duration in patients with low-risk group did not reach to median value until the time of data collection. Median OS duration was 42 months in intermediate-risk group and 9 months in high-risk group (p=0.001).

Summary and Conclusions: It was suggested that FISH risk group was associated with the most important independent prognostic factor for PFS (HR, 12.228) and OS (HR, 4.528) in MM patients with EP who underwent ASCT.

PB1680

THE FREQUENCY OF GENETIC ABNORMALITIES AND THEIR IMPACT ON SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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Background: During the past decade, considerable progress has been made in our understanding of the disease process and factors that influence outcome. Overall survival in myeloma has improved significantly in with the emergence of thalidomide, bortezomib, and lenalidomide. Cytogenetic analyses, including fluorescence *in situ* hybridization (FISH), have contributed to a better understanding of the clinical implications of these chromosomal abnormalities in myeloma. Stratification of patients into various risk groups based on the chromosomal markers is being utilized by many centers for prognostic counseling, selection, and sequencing of therapy approaches.

Aims: To estimate the incidence of genetic abnormalities (GA) and their impact on overall survival (OS) in patients with multiple myeloma.

Methods: The OS was prospectively assessed in 188 patient (median age 61 years, range 28 - 93; male: female ratio – 1:1.4) with newly diagnosed multiple myeloma (NDMM). The incidences of GA were determined in all patients. Cytogenetic studies were performed on bone marrow samples using standard GTD-method. Metaphase FISH analyses were performed according to the manufacturer's protocol with use of DNA probes: LSI 13(RB1)13q14, IGH/CCND1, IGH/FGFR3, LSI TP53 (17q13.1) (Abbott).

Results: The frequency of GA in NDMM was 30.8% (58/188): 2.0% (3/152) - by conventional karyotyping, 37.1% (49/132) – by FISH analyses and 5.5% (6/108) – using both methods. GA (two aberrations) were detected in 6.4% (12/187) patients, complex abnormalities (>3 aberrations) – in 3.7% (7/187) patients. No GA were detected in 130 patients (69.2%). The frequencies of GA were similar in patients <65 years (36/121) and in patients >65 years (22/67) (29.8%, and 32.8%, respectively; *p>.05*). The incidence of GA in patients stratified according to ISS did not different significantly, but was higher in patients with ISS III at diagnosis (ISS I (n=29) – 27.6%, ISS II (n=32) – 21.9%, ISS III (n=50) – 48.0%) (*p>.05*). The estimation of the incidence of GA did not reveal any significant differences in NDMM patients depending on the type of secreting immunoglobulin: IgG (38/113) – 33.6%, IgA (10/31) – 32.3%, IgD (0/1) – 0%, Bence-Jones (3/10) – 30.0%, and non-secreting myeloma (1/5) – 20.0% (*p>.05*). Translocation t(11;14) was detected in 16.0% (30/188) of NDMM patients, del13q14 -13.8% (26/188), t(4;14) – 4.2% (8/188), del17p13.1 – 3.7% (7/188). Isolated t(11;14) was detected in 10.1% (19/188) of patients, del13q14 – 8.0% (15/188), del17p13.1 – 1.6% (3/188), t(4;14) – 0.5% (1/188). The median OS in standard-risk patients (normal

karyotype, t(11;14), hyperdiploidy) was 70 months (n=150), intermediate-risk (t(4;14), del13, hypodiploidy) – 47 months (n=25; p=*.42*), high-risk (del17, complex karyotype) – 45 months (n=13; p=*.19*) (Figure 1).

Figure 1. OS depending on molecular classification and risk stratification.

Summary and Conclusions: GA are independent prognostic factors in patients in multiple myeloma. The median OS was higher in standard-risk than in intermediate- and high-risk patients. FISH analysis increase the frequency of detection of highly specific genetic markers and should be utilized for decision making, selection, and sequencing of therapies in patients with NDMM.

PB1681

LONG TERM SURVIVORS MM: MANY THANKS TO THE IMMUNE SYSTEM?

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Background: MM is a plasmacellular malignancy associated with an high incidence of morbidity and mortality. Prognosis depends on a variety of factors including patient's characteristics (age, comorbidities, cytogenetics) and the old Durie-Salmon and the newer ISS (International Staging System) staging system. These prognostic systems identify patients having a better, worst or intermediate prognosis. In fact, since median survival of the best stage is about 70 months, we cannot talk about good prognosis. Over the last decade new drugs such as IMIDs and proteasome inhibitors has been introduced in treatment and favourably increased OS and PFS of MM patients but unfortunately this disease still remains an incurable disease. Numerous defects of the immune system have been described in MM, although the relative clinical importance of these remains elusive. We have examined B, T lymphocytes and neutrophils subset of 15 patients whose diagnosis was made more than 10 years ago (9 pt 13 years, 3 pt 11 years, 3 pt 10 years) by flow citometry analysis. In addition we have performed some functional experiments to evaluate the neutrophils function. Patient samples were compared with healthy donors (10), newly diagnosed multiple myeloma patients (30) and MGUS patients (20). We have observed that profile of neutrophils functional activity, the number of CD4+/CD25+CD127low/Foxp3+ T regulatory cells, the number of the recently described *exhaustive T lymphocytes and* CD19+/CD24hi/CD38hi B regulatory cells was more similar to healthy donors or MGUS patients and was different from newly diagnosed MM patients. Our data indicate a particular behaviour of immune system in long term MM patients survivors, suggesting another point of view in the evaluation of MM.
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Methods: We have examined B, T lymphocytes and neutrophils subset of 15 patients whose diagnosis was made more than 10 years ago (9 pt 13 years, 3 pt 11 years, 3 pt 10 years) by flow citometry analysis. In addition we have performed some functional experiments to evaluate the neutrophils function. Patient samples were compared with healthy donors (10), newly diagnosed multiple myeloma patients (30) and MGUS patients (20).
Results: We have observed that profile of neutrophils functional activity, the number of CD4+/CD25+CD127low/Foxp3+ T regulatory cells, the number of the recently described *exhaustive T lymphocytes and* CD19+/CD24hi/CD38hi B regulatory cells was more similar to healthy donors or MGUS patients and was different from newly diagnosed MM patients.
Summary and Conclusions: Our data indicate a particular behaviour of immune system in long term MM patients survivors, suggesting another point of view in the evaluation of MM.

PB1682

CONFOCAL MICROSCOPY IS USEFUL FOR THE DEFINITION OF THE BORTEZOMIB RELATED NEUROPATHY IN MULTIPLE MYELOMA PATIENTS

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Background: Painful sensory peripheral neuropathy (PN) represents the main dose-limiting toxicity of Bortezomib (Bor). Confocal microscopy (Confoscan 4, Nidek), allowing a detailed visualization of the cornea which is the most innervated tissue especially with small sensitive fibers, is used for early identification of diabetic sensory PN.
Aims: To evaluate corneal nerve sub-basal plexus in Multiple Myeloma (MM) patients (pts) treated with Bor using Confoscan 4 in order to define its possible role in the study of Bor - related neuropathy.
Methods: Corneal nerve sub-basal fibers length and number, grade of tortuosity according to Oliveira scale and Nidek index and beadings' density were evaluated with Confoscan 4. Confoscan 4 was performed in 22 MM pts under treatment or previously treated with Bor and 8 healthy controls. The distribution and the characteristics of corneal nerve sub-basal fibers of controls were similar to those of healthy population reported in the literature. In details in our controls median fibers length was 1197 µm, median fibers number 7/ field, median beadings' density 58/mm, median tortuosity grade according Oliveira scale 0.3 and according Nidek index 2.1. MM pts had a median age of 66 years (range: 44-79) and healthy controls of 63 years (range: 58-67). 16 MM pts were receiving Bor at time of evaluation (11 as first therapy line, 5 relapsed) and 6 received Bor previously; median time from the last dose was 7.5 days (range: 1 day – 35 months). Median number of Bor received doses was 16.5 (range:3-80). 9/22 pts (40.9%) presented PN at time of evaluation: 4 grade I (45%), 3 grade II (33%), 2 grade III (22%).
Results: Compared to healthy controls MM pts showed a significant reduction in terms of length and number of corneal fibers (median length 552 µm in MM *vs* 1197 µm in control p<0.001; median number 4/field *vs* 7/field p<0.001), a higher grade of tortuosity with both methods (1.5 *vs* 0.3 according to Oliveira scale, p<0.001; 3.9 *vs* 2.1 according to Nidek index p<0.001) and increased beadings' density (75/mm *vs* 58/mm, p=0.001). All MM pts have fibers' alterations even in absence of a clinical evident PN. There were not statistically significant differences in fibers' length and number among pts symptomatic and asymptomatic for PN (length 554 µm *vs* 549 µm, p=0.86; number 4/field *vs* 3/field, p=0.661). Tortuosity and beadings' density were higher in symptomatic pts, but this difference did not reach a statistical significance (tortuosity 2 *vs* 1.5 according to Oliveira, p=0.29; 4.2 *vs* 3.8 according to Nidek p=0.81; beadings' density 78/mm *vs* 73/mm p=0.27) probably due to the small number of pts studied. Patients under treatment with Bor showed a higher beadings' density with respect to those off therapy but this difference was not significant (76/mm *vs* 66/mm, p=0.14).
Summary and Conclusions: Confoscan 4 can be a useful tool for the detection of Bortezomib induced neurological damage in Multiple Myeloma patients. Prospective studies are needed to evaluate if it could also help physicians in early detecting neurological symptoms emergence.

PB1683

PULMONARY FUNCTION TEST CAN BE USEFUL TO PREDICT SEVERE PULMONARY COMPLICATIONS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BORTEZOMIB

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Background: Bortezomib is a proteasome inhibitor indicated for the treatment of Multiple Myeloma patients. Among extrahematologic adverse effects, neuropathy and gastrointestinal symptoms are the most commonly reported, whereas severe pulmonary complications have been described rarely.
Aims: To describe a possible relationship between the administration of bortezomib and the development of pulmonary complications and evaluate the predictivity of pulmonary function test (PFT).
Methods: Between January 2012 and December 2013, 115 pts affected by MM were treated with bortezomib-based therapy in our Department. Three pts developed severe pulmonary complications related to bortezomib therapy. The characteristics of the three pts are described in Table 1. All three pts had a previously untreated syntomatic myeloma, no history of pulmonary diseases and normal chest X-ray before bortezomib treatment. PFT showed medium-marked reduction of DLCO and restrictive abnormalities. After administration of bortezomib (range: 2-6 days) the three pts presented similar clinical symptoms: fever and acute impaired oxygenation followed by respiratory failure. Radiologic

manifestations were similar including: bilateral pulmonary infiltration, interstitial pneumonia and pleural effusion (Figure 1). Bacteriological blood and urine samples were negative in all pts. Bronchoalveolar lavage were performed in all of them, only the third one was positive for Influenza B virus. Pt 2 underwent computed tomography (CT)-guided percutaneous transthoracic needle biopsy and the pathology report described focal fibrosis. In Table 2 are summarized details of severe pulmonary complications of the three cases. Due to the negative etiological report and the lack of response to broad-spectrum antibiotics high dose intravenous corticosteroid, methylprednisolone, was started. Only one pt needed endotracheal intubation and mechanical ventilation. We have seen clinical, gasometric and radiological improvement in few days in all pts.
Results: After recovery all pts were treated with no bortezomib-based therapy and two of them received autologous stem cell transplant without pulmonary complications.

Table 1.

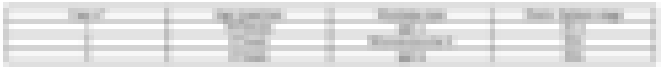
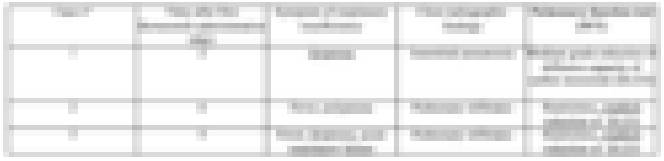


Table 2.



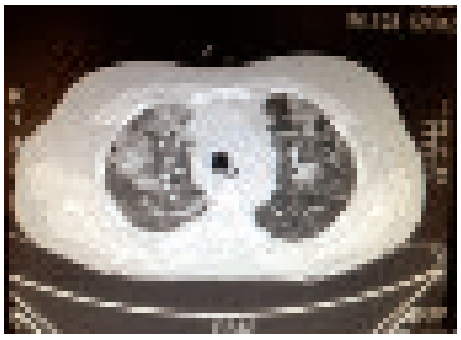


Figure 1.

Summary and Conclusions: This report underlines that bortezomib may cause serious lung injury as described in literature. PFT showed to be a useful diagnostic test before bortezomib-based treatment to identify patients at risk of respiratory failure and requiring second level investigations such as chest CT and pneumological evaluation.

PB1684

MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA WITH BENDAMUSTINE

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, that produces both single- and double-strand breaks in DNA, and shows incomplete cross resistance with other alkylating drugs, proved to be effective either in relapsed or refractory and in new diagnosed MM as single agent or combined with steroid and has also additive/synergistic activity with bortezomib.
Aims: Here we evaluate the efficacy and tolerance of bendamustine in combination with bortezomib-dexamethasone in patients with relapsed and refractory multiple myeloma, whose prognosis is severe, so that there is a strong need for new options for the management of these patients. A retrospective analysis of patients with relapsed and refractory MM, who had received bendamustine as salvage therapy, has been performed.
Methods: 24 patients, 13 males, 11 females, with advanced multiple myeloma, treated with several lines of treatments, and refractory to all the lines previously performed, received a schedule Bendamustine-based. Median age at diagnosis was 63.2 years (range 39-82) while age at start of treatment was 66 years (range 48-83), and median number of prior lines of treatment was 6.3 (range 4-8). ISS was equally distributed, and cytogenetic characteristics were evaluable in 9 patients, only two of whom had cytogenetic abnormalities, and in particular one of them had del13q and in the other one was observed t(11;14). All the patients had previously been treated with schedule containing bortezomib and lenalidomide, while 90% of them had been treated with melphalan, 77% with cyclophosphamide and 34% with anthracyclines, and 30% had also received radiotherapy. 58% of patients had undergone at least to a single autologous stem cell transplantation. Last treatment before bendamustine was a bortezomib-based regimen in 39%, an IMIDs- based regimen in 49% (a combined bortezomib/IMIDs-based regimen in 27%), while 12% of patients had received other chemotherapies. All patients were relapsed and refractory to last therapies received.
Results: Only patients completing at least two two courses of Bendamustine were considered for analysis. A total of 87 cycles was administered (median 4.3, range 2-9). In 91% of patients bendamustine was variously associated to bortezomib (66%), or IMIDs (25%) and only in 8% it was combined only with dexametasone. In our schedule, Bendamustine was given, at a median dose of 90 mg/sqm (range total dose : 120-180 mg) on day +1 and +2 every 28 days. After a median follow-up of 6.1 months, median OS from diagnosis was 57.3 months, while median OS from start of Bendamustine was 6.7 months (range 2-19 months). 11/24 patients died for progressive disease. 2/24 patients died for other causes (one for cardiovascular disease and the other one had a gastric cancer). Grade 3 transfusion-dependent anemia occurred in 36% while in 53% grade 3 neutropenia occurred. We observed no severe extrahematologic toxicity, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG uniform response criteria, 15 out of 24 evaluable patients achieved a partial response after a median time of 2.4 months with an overall response rate of 62.5%. In particular, for 3 patients of this study, Bendamustine treatment was a bridge to second autologous stem cell transplantation, and for one patient for allogenic stem cell transplantation, after having achieved a PR.
Summary and Conclusions: In conclusion, Bendamustine has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to almost all available therapeutic resources, and in particular cases it could be considered as a bridge to a second autologous or to allogeneic BMT.

PB1685

EFFECTIVENESS OF HAEMODIALFILTRATION WITH HIGH-CUT-OFF MEMBRANE IN PATIENTS WITH MULTIPLE MYELOMA AND RENAL FAILURE, A SINGLE CENTER EXPERIENCE

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Background: Acute kidney failure in multiple myeloma (MM) occurs in 12%>20% of patients and is a poor prognostic factor for patient survival. Recent studies have shown that dialysis with a High-Cut-Off membrane (HCO) removes free light chains (FLC) and is being an effective treatment for acute renal failure that could be a determining factor for the survival.We have retrospectively evaluated the effectiveness of HCO membrane in the recovery of renal function in addition to a standard anti myeloma treatment.
Aims: Effectiveness of haemodialfiltration with High-Cut-Off membrane in patients with multiple myeloma and renal failure.
Methods: From January 2010 to April 2013, 13 patients were diagnosed of MM with severe acute renal failure (ARF). Population: 8 female/5 male, median age: 67.7 years (40-83), Bence-Jones MM: 8, IgG:3, IgD: 1, IgA:1. Creatinine: 8.26 mg/dl (3.61-21.3), glomerular filtration rate, Cockcroft-Gault: 9.4 ccs/min (2.9-19.7). All patients were treated with bortezomib (1.3 mg/m2 days 1-4, 8-11) and dexamethasone (40 mg, days 1-4, 9-12) 2 patients received conventional haemodialysis (CH) and 11 with HCO membrane.
Results: Number of HCO haemodialysis sessions: 6 (1-12). Average Cr post treatment: 1.37 mg/dl. Renal response: CH: stable: 2. HCO: complete: 2, partial: 8, minor: 1. Dialysis Independence: Global: 12/13 (92.3%), HCO: 100%, CH: 50%. Time to renal response (days): minor: 17.5, partial: 59.3, complete: 210. MM response: sCR: 6, CR: 3, PR: 4. Number of chemotherapy cycles: 6 (4-8) The 3 patients with <65 years could receive an autologous hematopoietic stem cell transplant. After a median follow up of 27 months, relapse rate was: 4/13 (30.7%) and OS: 100%.
Summary and Conclusions: The combination of antimyeloma treatment plus HCO haemodialysis is a highly effective treatment for acute renal failure due to myeloma nephropathy, with a 100% rate of dialysis independence. In our series, only 2 patients remained with stable renal disease (1 with dialysis dependence), both of them treated with CH.

PB1686

I-FISH DETECTION OF THE MOST FREQUENT CHROMOSOMAL ABNORMALITIES IN SELECTIVE CD138+ PLASMA CELLS IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM), is characterized by the accumulation of Plasma Cells (PC) in the Bone Marrow (BM) and like other hematological malignancies has both normal and clonal neoplastic cells.

Aims: Cytogenetic studies in MM are hampered by the low proliferative capacity of PC. In order to detect the chromosomal abnormalities by Interphase Fluorescent *In situ* Hybridization analysis (I-FISH) it is important to perform the analysis on the PC.
Methods: Enrichment of PC by CD138 positive selection prior to I-FISH is one way to enhance diagnostic sensitivity. We used a panel of FISH probes to detect numerical and structural chromosomal aberrations (for chromosomes 1, 13, 14 & 17) by I-FISH analysis. The analysis was performed on whole BM samples (routine r-FISH) and on CD138 + BM cells separated with magnet beadsseparation for subsequent I-FISH analysis (ms-FISH) from the same 15 patients with MM.
Results: In this study the purity of the PC separation ranged between 51% to 91%. The ms-FISH is considered to be the reference standard method and detect all the chromosomal abnormalities tested (100% sensitivity). Comparing r-FISH with ms-FISH, we have demonstrated that all the patients had detectable chromosomal abnormalities after PC separation. In 8 out of 15 samples the percentage of PC was less than 10% and the percentage of chromosomal abnormalities in both r-and ms-FISH was under the cut off limit (10%) for FISH detection of chromosomes 1, 13 and 17. Interestingly, all the samples were positive for chromosome 14 (cut off limit: 1%). One sample with 10% PC, was also positive for the chromosome 13 deletion by ms-FISH. All the six samples with more than 10% of PC presented chromosomal abnormalities in both r-FISH and the ms-FISH, but the presence of these anomalies was higher and statistically significant after PC separation. We calculated that the ms-FISH detection was highly sensitive and may replace the r-FISH in confidence level of 90% for chromosome 1 and 17; 95% for chromosome 13 and 99% for chromosome 14 (wilcoxon signed-rank test).
Summary and Conclusions: In our experience the CD138+ magnetic separation was effective. The most frequent chromosomal abnormality in all the cases investigated was chromosome 14 (IGH) and can be used as a screening test. We thereby determine that r-FISH can be used only in the cases of high percentage of PC in the whole BM. Since, the majority of the MM cases had low number of PC in the BM aspirates we recommend to isolate the PC first, and then to perform the ms-FISH on the targeted cells.

PB1687

MYD88 MUTATIONS ARE CORRELATED WITH 6Q DELETION IN KOREAN PATIENTS WITH WALDENSTROM MACROGLOBULINEMIA

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Background: Waldenstrom Macroglobulinemia (WM) is a malignant lymphoplasmo-proliferative disorder with IgM monoclonal gammopathy. The genetic changes associated with WM are not fully elucidated. Recently, whole genome study revelaed MYD88 L265P mutation as the key mutation in WM.
Aims: The aim of this study is to find out MYD88 L265P mutation status of Korean WM patients.We also investigated cytogenetic aberrations using conventional G-banding and fluorescence *in situ* hybridization (FISH), and analyzed their correlation with MYD88 L265P mutation status.
Methods: A total of 22 patients diagnosed with WM according to consensus recommendations from the Second International Workshop on WM was enrolled. Conventional G-banding and interphase fluorescence *in situ* hybridication (FISH) targeting 6q21, *IGH*, *RB1*, *TP53*, 1p25/1q32, and CEP 9/CDKN2A were performed using bone marrow (BM) aspirates. Of 22 WM patients, 16 patients were subjected for MYD88 mutation study and *MYD88* mutation was detected by Sanger sequencing.
Results: Of 22 patients, 5/18 patients (28%) showed cytogenetic aberrations by G-banding study. The incidence of 6q21 deletion was 17% by conventional G-banding and 33% when analysed by FISH. By FISH, 9/21 patients (43%) showed cytogenetic aberrations; 6q deletion in 7/21 (33%) patients and *IGH* rearrangement in 4/21 (19%) patients. Two patients had both 6q deletion and *IGH* rearrangement, and 2 patients had only *IGH* rearrangement without 6q deletion. Of 16 patients, 11 (69%) patients presented L265P mutation of *MYD88* gene. MYD88 mutation significantly associated with presence of 6q deletions (*P* value 0.053). Among patients with 6q deletion, all the 5 patients for whom sequencing was possible, *MYD88* mutation was found. The MYD88 L265P mutation was also associated with high lymphocyte burden in BM biopsy. There was no significant differences in other clinical characteristics and laboratory findings including peripheral blood lymphocytosis, and overall survival according to *MYD88* mutation status.
Summary and Conclusions: We first report the high frequency of MYD88 L265P mutation in Korean WM patients. We observed significant association of MYD88 L265P mutation (on chromosome 3p22) and 6q deletion.

PB1688

BENDAMUSTINE-BASED-TREATMENT FOR HIGHLY TREATED PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: Bendamustine is a nitrogen mustard that was developed as a bifunctional molecule with alkylator and antimetabolite properties. Compared with other frequently used alkylating agents, bendamustine induces higher rates of DNA double-strand breaks and is not cross-resistant with melphalan and several other cytotoxic drugs. Clinically, bendamustine has already been tested in patients with relapsed/refractory myeloma (MMRR). Based on these properties, we started using Bendamustine (combined or as single-agent), in patients with MMRR despite several previous treatment lines, in an attempt to control disease activity.
Aims: Our aim is to present a sample of 18 patients (from Nov´2009 to Oct´2013) with relapsed/refractory Multiple Myeloma (MMRR) treated at our institution with Bendamustine - based regimens. 11 women and 7 men, median age 58 years at the beginning of the treatment (range 43-79). The characteristics of the patients at the time of diagnosis were: ECOG ≥3 in 27,8%; types of MM: IgG 67%, IgA 11% and light chain of 22%; with state of ISS I in 39% of the instances, II in 16% and III in 45% of them. Bone lesions were presented in 70% of study population, those being >4 in 50% of the cases and plasmacytomas in 33,3%. Median previous lines received was 4 (with a 2-7 interval), including autologous-SCT in 4 patients (22%). 94% of the patients had received Bortezomib based regimen (VD 83% and VMP 44%) , all of them had received IMID's (Lenalidomide 94% and 22% Talidomide) and alkylants were administered in a 22% of patients. At the beginning of the treatment with Bendamustine 4 patients (22%) presented renal impairment (creatinine ≥ 2mg/dL) and 13 (72%) showed alterations in the free-light-chain ratio.
Methods: Response to treatment was assessed according to the IMWG criteria and the adverse events according to the National Cancer Institute´s Common Terminology Criteria for Adverse Event (3.0).
Results: Patients received a median of 3 cycles of Bendamustine (B) – based treatment (range 1-8) with a median dose of Bendamustine of 60mg/m2 (range 30-100) administered on days 1 and 2 of each cycle. Used treatment schedules were Bendamustine as single-agent in 3 patients, B+Prednisone in 9 patients, B+Dexametasone+Bortezomib in 5 patients, B+Lenalidomide in 1 patient. After a median of follow up of 6 month (range 2-13), the global response rate (≥ PartialResponse) according to the IMWG criteria was 33% (1 CR, 3 VGRP, 2 PR), with a 28% Stable Disease and 39% of PD. The average time until progression was 2.5 months (range 1-10). Grade 3-4 hematologic adverse events (AE) were observed as follows: anemia 28%, neutropenia in 28% and thrombocytopenia in a 33% of the patients. Non-hematologic grade 3-4 AE resulted in 22% patients with infection.
Summary and Conclusions: Multiple myeloma remains an incurable disease in most cases. In spite of the multiple lines previously received, Bendamustine is effective and well-tolerated in patients with MMRR. Based in our experience we would recommend early use of Bendamustine – based regiments (no later than third line or in combination with Bortezomib even earlier if there is evidence of renal impairment). More studies are required to establish and optimize its use.

PB1689

INTRAVENOUS SWITCHING OF BORTEZOMIB CAN OVERCOME RESISTANCE AFTER SUBCUTANEOUS ADMINSTRATION IN MULTIPLE MYELOMA PATIENTS

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Background: Subcutaneous bortezomib (SC) is reported as noninferior in efficacy to intravenous bortezomib in multiple myeloma (MM) patients and is associated with a lower incidence of neuropathy. SC administration of bortezomib is comparable with the IV route, in terms of overall systemic availability and pharmacodynamic activity, similar toxicity profiles, and similar response rates. As expected, the time required for SC administration to be absorbed is longer than for the IV route and accordingly the mean plasma concentration values are significantly lower for SC administration, and the median time to reach the final plasma concentration is longer. Nevertheless, the 20S proteasome inhibition seems similar in both routes of administration, and the longer time required for SC formulation to be absorbed results in a less-pronounced initial spike in proteasome inhibition.
Aims: To test if MM patient resistant to the SC injection can have a disease response with the IV administration of bortezomib due to the higher peak values of plasma concentrations in the IV formulation.
Methods: Thirty patients (median age 69, range 56-82) consecutively

presented in a time period of one year (January 2013-January 2014) at a single center received bortezomib SC injections at MM relapse. Type of immunoglobulin was: 18 IgG (12 kappa ;6 lambda), 5 IgA (3 kappa; 2 Lambda), 5 BJ (3kappa, 2 lambda), 2 NS. ISS Stage was: I (n=16), II (n=7),III (n=7). Cytogenetics on bone marrow selected CD138 plasma cells was done. Patients had received a median of 3 previous lines of therapy (range 2-6). Six patients were bortezomib naive, while 24 had received bortezomib previously.
Results: VMP was administered in 5 patients, VCD in 16 , Vel-dex in 7, VTD in 1, PAD in 1. Patients received a median of 5 cycles (range 2-7). Overall response rate (ORR) was 76%. Responses were: 4 CR, 2 VGPR, 17 PR, 7 NR.We swicthed SC injections in the 7 NR patients. They received a median of 2 cycles of bortezomib and dexamethasone day 1,4,8,11(range 1-5). 3/7 patients showed a response (2PR, 1MR, 4 NR). In the responders response was seen after the first cycle. Responders received IV bortezomib until progression. A median of response of 3 months was seen. The 2 patients achieving PR were previously treated with 3 lines of therapy and 6 lines of therapy; they were bortezomib naive at the SC administration. Addition of a third drug (melphalan) did not recover response at disease progression. Data will be presented about response kinetics and duration.
Summary and Conclusions: IV administration of bortezomib in MM patients previously resistant to SC injections should be considered. It could be hypothesized that higher peaks of the drug in the IV administration can overcome resistance in few patients. Further studies are warranted to confirm these findings.

PB1690

INCIDENCE OF INFECTION ACCORDING TO INTRAVENOUS IMMUNOGLOBULIN (IVIG) USE IN AUTO-HCT RECIPIENTS WITH MULTIPLE MYELOMA
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Background: According to the guidelines for preventing infectious complications among HCT recipients, administration of intravenous immunoglobulin (IVIG) is only indicated if the patients had severe hypogammaglobulinemia (ie, serum IgG level<400 mg/Dl). Although IVIG is not routinely recommended, many centers still use IVIG during post-HCT period.
Aims: In this study, we evaluated and compared the incidence of infection according to the post-HCT IVIG use in multiple myeloma (MM) patients with autologous HCT.
Methods: A total of 162 MM patients who received auto-HCT between Jan 2008 and Jun 2013 were retrospectively reviewed. Defining infectious events was based on the physicians' judgement at the time of recording a medical chart. Infectious events were counted from the date of discharge after auto-HCT to the first date of next treatment for the recurred/progressive disease, or to the last follow-up date for the patients without further treatment.
Results: The median age was 54.5 (23-67), and DSS at diagnosis was stage I in 23 patients (14.3%), II in 30 (18 6%), and III in 108 (67.1%). After auto-HCT, 53 of 162 patients (32.7%) experienced 103 infectious events. Upper respiratory tract infection was most common (n=31, 30.1%) and pneumonia (n=27, 26.2%) and herpes zoster (n=15, 14.6%) came next. Among the identifiable organisms causing respiratory tract infection, influenza virus (n=10) and pneumococcus (n=9) were predominated. Incidence of infection was not statistically different according to the IVIG use (34.8% in IVIG (-) *vs* 31.3% in IVIG (+), p=0.631). Incidence of infectious events requiring hospitalization and multiple infectious events showed no difference between the groups (p=0.147, p=0.156). Respiratory tract infection was occurred in 15 (22.7%) in no IVIG group and 16 (16.7%) in IVIG group (p=0.335). Although not statistically significant, patients receiving IVIG had a trend towards less lower respiratory tract infection (15.2% in IVIG (-) *vs* 6.3% in IVIG (+), p=0.062).
Summary and Conclusions: In auto-HCT recipients with MM, incidence of infectious events after transplantation was not different according to the prophylactic IVIG use. Potential benefit of IVIG for prevention of lower respiratory tract infection may be considered, which needs further evaluation.

PB1691

THE RELATION BETWEEN INITIAL PET FINDINGS AND CLINICAL PARAMETERS IN MULTIPLE MYELOMA PATIENTS
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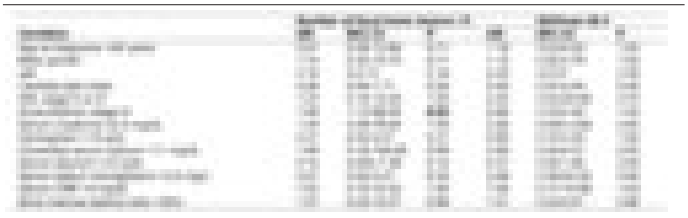
Background: In a recent study, F-fluorodeoxuglucose (FDG) positron emission tomography (PET) in multiple myeloma (MM) patients was evaluated and a positive correlation with clinical parameters was reported. Moreover, it was found to be a predictor of outcome. However, the present data is still not enough for routine usage of FDG-PET in our daily practice beyond simply detecting the involvement of bone and extramedullary tissues.
Aims: We aimed to investigate the correlation between baseline FDG-PET findings and clinical parameters in our MM patients.
Methods: We conducted a retrospective review of our MM patients' files. As a total of 385 patients' data who were followed at our out-patient clinics from January 2005 to december 2013 were analysed. Among those, 48 patients who were chemotherapy naive were found to have baseline FDG-PET evaluation. We compared the initial clinical characteristics of these patients with their PET findings: the number of focal bone lesions (FLs) and the maximum standardized uptake value (SUVmax). Relationship between the above mentioned initial disease characteristics and FDG-PET parameters were analysed by chi-square test.
Results: A total of 48 patients (23 male) were included for analysis. The median age was 62 years (range 55-72). At diagnosis the median hemoglobin level was 10.5 g/dL (range 9.3-11.5) and 29.2% of patients had anemia. The proportion of patients with hypoalbuminemia, hypercalcemia and azothemia were 15%, 5% and 5% respectively. Median serum CRP was 4 mg/L (range:3-10.5) and 9% of patients had CRP more than 5 mg/L. Among patients with secretory disease (97.9%) IgA, IgG and light chain only disease were 8.3%, 62.5% and 27.1%. The median amount of bone marrow plasma cells were 40% (range 25-60) and 25% of patients had more than%30 plasma cells in the bone marrow. At presentation median serum beta-2 microglobulin was 3.5 mg/L (range: 2.5-5.0) and 20% of patients had international staging system (ISS) stage 3. The ratio of Durie-Salmon (DS) stage 3 disease was 43.8%. Regarding baseline FDG-PET findings, 41 (85.4%) patients had lesions on imaging. Of these FDG-PET positive patients, 17 (54.8%) had more than 3 FLs. The median SUVmax for these FLs was 6.5 (range: 4.9-10) and 6 patients had a SUVmax value greater than 6.5. Briefly, a positive relationship between DS stage 3 and number of FLs on PET was found. However, there was not any other statistically significant relationship considering others (Table 1).

Table 1. Relationship between the number of focal bone lesion, SUVmax value and baseline characteristics.



Summary and Conclusions: A statistically significant positive correlation was found between baseline FDG-PET findings and DS stage in MM patients. So, our findings partially support the evidence in the literature and it seems that imaging helps staging disease at the time of initial diagnosis. There is need for further studies.

PB1692

DOES IT MATTER THE TYPE OF MYELOMA? CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF IGA AND IGD MYELOMA
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Background: According to the type of M protein, patients (pts) with IgG multiple myeloma (MM) are the most common, representing ≈60% of cases. IgA and IgD myeloma occurs in 20% and 2%pts respectively.
Aims: The aim of study was to analyse clinical and biochemical features of pts with IgA and IgD myeloma in a view of the appropriate personalized treatment approach.
Methods: The study included 70 newly diagnosed MM pts with IgA (64pts, 30 male/34 female, mean age 58yrs, range 35-79) and IgD myeloma (10pts, 5 male/5 female, mean age 55yrs, range 31-78yrs). According to the clinical stage, distribution of IgA myeloma was: I 6pts (9,4%), II 24pts (37,5%); III 34pts (53,1%); and IgD: II 2pts (20%), III 8pts (80%). Regarding ISS score, within IgA myeloma predominate pts with ISS2 and ISS3 (30pts, 73,2%), while there was higher incidence of pts ISS1 (4pts, 66,7%) with IgD myeloma. Renal impairment existed in 20pts (27%; IgA MM: 17pts, 26,6%, and IgD MM: 3pts, 30%). Thalidomide based combinations were applied in the majority of pts (52pts, 70,2%). High-dose treatment and autologous stem cell transplantation were applied in 15pts (22,9%).
Results: Both of the groups were characterized with notable 24h proteinuria (IgA: mean value 1,26gr/l, SD=2,25, median 0,28gr/l, range 0,04- 8,70gr/l ; IgD: mean value 1,05gr/l, SD=1,57, median 0,22gr/l, range 0,06- 4,02gr/l), and significantly higher incidence of the LDH elevation above 500U/l in pts with IgA

myeloma (p <0,001). Similarly, there was higher incidence od LDH elevated above 500U/l in pts with IgD myeloma, but without statistical significance (R= 0,075, p= 0,669). Of notice, there was significantly higher incidence of extramedullary disease (p <0,008), and plasma cell leukemia (p <0,001) in pts with IgD myeloma. Treatment response (CR/VGPR/PR/MR) was achived in 57pts (77,1%; IgA MM: 49pts, 76,6%; IgD MM: 7pts, 70,0%) with median duration of 12m (range 8- 110m). Median overall survival (OS) for the whole group was 35m (range 1- 170m). There was no significant diference between pts with IgA and IgD myeloma in duration of TTP (median 10m *vs.* 20m; Log Rank =0,694, p =0,405), and OS (median 35 *vs.* 43m; Log Rank =0,048, p =0,826).
Summary and Conclusions: Clinical features that characterizes IgA and IgD myeloma are notable 24h proteinuria, significant LDH elevation, and occurrence of the extramedullary disease and plasma cell leukemia in patients with IgD myeloma which indicates the following aggressive course of disease, and necessity of the intensive first-line treatment.

PB1693

HAEMOSTATIC DISORDERS IN NEWLY DIAGNOSED MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is associated with many hemostatic disorders, leading to thrombotic complications. The most frequently thrombosis is registered in patients with MM during the first year after diagnosis. Without prophylaxis thrombosis' rate can achieve 34-58%.
Aims: The aim of the study was to determine the frequency and characteristics of hemostatic disorders in patients with MM and to define the adequacy of anticoagulant and antiaggregant prophylaxis during induction therapy.
Methods: Between March 2012 and May 2013 25 patients (13 male and 12 female) at the age of 29-72 (median 54 years) with newly diagnosed multiple myeloma were included in a prospective study. Stage I was diagnosed in 2 patients, stage II in 9 patients, stage III in 13 patients according to Durie-Salmon, and in 9, 11 and 5 patients according to International Staging System respectively. Hemostasis investigation was performed with Activated Partial Thromboplastin Time (APTT), factor XIIa dependent time of fibrinolysis, D-dimer level. Integral methods of hemostasis investigation were used: thrombin generation assays (principle parameter was endogenous thrombin potential, ETP), thromboelastography and a new method of a spatial fibrin clot growth registration – thrombodynamics (TD). Induction therapy included courses PAD and VCD. All patients received heparin (500 ED/hour twenty-four-hour infusion) or acetylsalicylic acid (100 mg per day) as thromboprophylaxis. Statistical analysis was done with multiple linear regression method.
Results: Hypercoagulation was detected in 17 (68%) patients with at least one of the used methods. However APTT was normal in all patients. Five patients had a factor XIIa dependent time of fibrinolysis elongate. In 11 patients D-dimer levels were elevated. D-dimer concentration had a statistically significant correlation with ETP (Pearson correlation coefficient R: 0,65; p=0,01) and factor XIIa dependent time of fibrinolysis (Pearson correlation coefficient R: 0,63; p=0,01). Inverse relationship was established between paraprotein level and optical density of fibrin clot measured by TD (Pearson correlation coefficient R: 0,65; p=0,01). There was one episode of thrombosis in a patient 71 years old after he discontinued the use of acetylsalicylic acid on his own in the setting of prolonged immobilization.
Summary and Conclusions: Increased readiness for thrombogenesis was found in 68% of patients with newly diagnosed MM. Acetylsalicylic acid 100 mg per day or 500 ED/hour twenty-four-hour heparin infusion was found to be adequate thromboprophylaxis. Possible meaning of such risk factors as elderly age and prolonged immobilization was shown.

PB1694

VALIDITY OF STRINGENT COMPLETE RESPONSE IN ROUTINE MANAGEMENT OF MULTIPLE MYELOMA PATIENTS TREATED WITH THE NOVEL AGENTS
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Background: Normalization of serum free light chains (sFLC) ratio in patients with Multiple Myeloma (MM) achieving complete response (CR) may define a deeper degree of response after therapy than that defined by the CR criteria. The stringent CR (sCR) requires normalization of sFLC ratio and absence of clonal plasma cells in bone marrow in addition to the criteria for CR (Negative immunofixation of serum and urine, disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow).
Aims: The aim of this study is to evaluate the prognostic utility of sCR in patients newly diagnosed with MM treated with novel agents in the routine practice.
Methods: Twenty three patients with MM (10 IgG MM, 5 IgA MM, 2 IgD MM and 6 Bence Jones MM) achieving CR after therapy with Bortezomib/ Dexametasone were included in this study. Disease Free Survival (DFS or time after treatment where disease remains stable) was estimated by Kaplan-Meier method and compared by log-rank tests. Cox proportional hazard analysis was performed for multivariate analysis. Serum free light chains were measured by turbidimetry (Freelite) in a SPA PLUS analyzer (The Binding Site Group Ltd, Birmingham, UK) and immunofixation was performed in a HYDRASYS (Sebia, FR) analyzer.
Results: The median follow-up of the patients was 18 months (range 14-31 months). Eleven patients achieved sCR (48%; 7 IgG MM, 2 Kappa Light Chain MM and 2 Lambda Light Chain MM) and 12 patients (52%; 5 IgA MM, 3 IgG MM, 2 IgD MM and 2 Lambda Light Chain MM) achieved CR. During the period of study there were 8 relapses, six (2 IgA MM, 2 IgD MM, 1 IgG MM and 1 Lambda Light Chain MM) in patients achieving CR and two (1 IgG MM and 1 Kappa Light Chain MM) in patients achieving sCR. The median DFS for patients achieving CR was 18 months and not reached for those achieving sCR. Patients achieving CR had a DFS rate of 24% compared with 75% for sCR (p=0.022). Results showed that achieving a sCR was an independent prognostic factor for survival (HR = 6.57; 95% CI, 1.09-39.80; *vs* CR; p=0.039).
Summary and Conclusions: The presence of an altered sFLC ratio suggests the existence of a persistent clonal population that is secreting small amounts of monoclonal protein. Our results indicate that sCR represents a deeper response state compared with conventional CR which translates into a longer DFS. Despite the small cohort, analysis of sFLC ratio was able to identify a group of patients with more favorable prognosis and support its inclusion in the response criteria for MM patients treated with novel agents.

PB1695

A SEQUENTIAL AND NOT OVERLAPPED USE OF NEW DRUGS FOR INDUCTION THERAPY OF MULTIPLE MYELOMA: A PROPOSAL OF A COMBINED TREATMENT TO INCREASE THE COMPLETE REMISSION AND OVERALL SURVIVAL RATES
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Background: Multiple Myeloma (MM) remains an incurable disease despite intensive therapy such as high-dose melphalan and autologous stem cell transplantation (ASCT). In multivariate analysis, we know that achieving a complete remission (CR) before ASCT is a significant independent variable for OS and TTP and maintenance therapy is an important option to protract PFS. Contemporary use of new drugs increases the overall response rate (ORR) and stringent complete remission (sCR) rate, but it's unclear the impact on PFS, TT retreatment, extra-bone disease incidence and 2nd line therapeutic strategies.
Aims: According this assertion, we planned a preliminary monocentric study to evaluate the results of a global intention to treat comprehensive of induction, transplantation, maintenance and further lines of treatment at relapse. It is based on a sequential therapy without the contemporary use of new class of drugs; it aims to obtain the same results of standard care, without the cumulative toxicity by overlapping use of new drugs, so to permit to reach again at least the VGPR at relapse,in good clinical condition for a possible second transplantation.
Methods: From September 06 to June 2011 we enrolled 24 pts (7 M, 17 F) with symptomatic and untreated MM; median age was 53 years (34 – 64); M-protein was measurable in 17 pts and 12 pts showed IgG type , 5 pts IgA; disease was classified as non secretory in 2 pts and micromolecular in 5 pts; ISS score was 1 in 15 pts, 2 in 5 pts and 3 in 4 pts . Patients underwent to VALD regimen (Vincristine 2 mg, Liposomal Adriamicine 40 mg/m2 on day 1 and Dexametasone 40 mg day 1-4) for 3 cycles (day 1 -21); 15-30 days after last VALD, pts started 3 courses bortezomib regimen (1.3 mg /m2 on day 1,4,8,11) and 15 days after last dose pts received Cyclophosphamide 4g/m2 and G-CSF for stem cell harvesting. After 1-2 months pts underwent to autologous stem cells transplantation with Melfalan 200 mg/ m2-based conditioning regimen. Finally, patients started maintenance therapy with Bortezomib 1.3 mg/m2 (day 1-21) for two years and Interferon α 1500000 UI/twice at week until progression.The refractory or relapsed pts received Lenalidomide based second-line therapy.
Results: the OS study shows 84% pts (20/24) alive, with a median follow up of 35 months (0-83 months); 17% (5/24 pts) died (2 for pneumonia and 3 for myeloma progression). ORR to first-line therapy was 79% (19/24 pts) , with sCR, VGPR, PR rate of 29,41,9% respectively; 21% (5/24 pts) had a stable disease and early underwent to the Lenalidomide-based second line therapy. 16% (4/24 pts) relapsed with a median of 24 months and underwent to the second-line therapy.The 2-years progression free-survival is 71% .50% of refractory and relapsed pts are alive and 60% of these achieved at least the

second VGPR. Toxicity was low and it mainly consisted of neutropenia (15%, WHO grade I-II) and mild peripheral neuropathy (20%, WHO grade I-II).1 pt showed an extra-bone disease at relapse (CNS).
Summary and Conclusions: Our study demonstrates how is possible to obtain analogous response in front of standard care in term of OR, PFS and ORR even without the overlapping the new drugs,moreover not yet officially allowed at the time of our experience. 1 pt (5%) showed an extrabone relapse, in contrast to 15-20% of cases in standard care;60% of the relapsed and refractory pts obtained again a new CR by short treatment with LenDex, with very limited toxicity, so to be able to program a second autologous or an allogeneic transplantation.

PB1696

PLEURAL EFFUSION AS CLINICAL FEATURE OF MYELOMA EXTRA-MEDULLARY INVOLVEMENT

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Background: Emergence of extramedullary disease (ED) is a poor prognostic event in patients (pts) with multiple myeloma (MM). Extramedullary masses may either arise from bone (EM-B) or develop in extra-osseous organs (EM-S). Pleural involvement represents a very uncommon site of EM-S.
Aims: To describe the prevalence and outcome of MM pts developing pleural involvement among a cohort of 456 patients consecutively observed in our Centre between 2000-2010.
Methods: Following approval by our institutional Ethic Committee, we reviewed clinical and haematological data of 456 MM patients consecutively observed in our Department between 2000-2010 in order to identify pts with pleural extramedullary disease either at diagnosis or during follow-up.
Results: In our cohort, prevalence of EM-S was 14% (65 pts out of 456). Among these pts with EM-S, 16 (24.2%) developed pleural involvement. Pleural involvement was observed most frequently at relapse (10 of 16 patients, 87.5%). Two patients (12.5%) had pleural involvement at diagnosis. Pts characteristics at diagnosis were: male gender 62.5%, median age 58.6 years (range 37.1-65.5 years), myeloma type IgG/IgA/IgD/light chain respectively in 50%/18.8%/6.2%/25% (8,3,1 and 4 pts), prior MGUS in 33% (2 pts), ISS stage I-II and III respectively in 15.4 and 84.6%, extensive bone disease (>3 sites) 81%; median haemoglobin, creatinine, calcium, LDH and beta2microglobulin were respectively 11.2 g/dl, 0.87 mg/dl, 9.3 mg/dl, 339 mU/ml and 6310 mcg/l. At the time of first pleural involvement, all pts had B2M higher than normal (median value 7130 mcg/l, range 3030-43582); median hemoglobin, creatinine, calcium and LDH were respectively 10.4 g/dl, 1.1 mg/dl, 9.2 mg/dl and 273 mU/ml. Pleural involvement was the unique extramedullary site involved at diagnosis in both two pts. Pts with pleural involvement at diagnosis had extramedullary relapse located in different sites (1 pts at lung, 1 pts at costae). At relapse, pleural involvement was often associated with multiple extramedullary locations. In detail, 4 pts (28%) had single pleural involvement, 7 pts (50%) had two extramedullary sites involved, 3 pts (22%) at least three; concomitant paravertebral masses was detected in 50% (7 pts) and 31.3% (3 pts) had costal masses. Other concomitant extramedullary locations were plasma cell leukemia, lung and skin, (each in 2 pts) and lymph nodes, breast, mandible, muscles, testis, and oropharynx (each in 1 pts). In pts developing pleural localization during follow-up, 64% were treated with new drugs and 64% with autologous stem cell transplantation. Median lines of therapies number before pleural locations was 3 (range 1-9). Pts with pleural involvement at diagnosis had a significantly worse outcome with respect to pts with different EM-S (13 *vs* 40 months, p=0.049). Instead, OS of pts developing pleural localization during follow-up was similar to that observed in pts with other EM-S (1.8 *vs* 2.1 years , p=0.094).
Summary and Conclusions: Pleural involvement was a rare isolated myeloma presenting feature with a very dismal prognosis. At relapse, pleural involvement is often associated with other extramedullary localizations, especially paravertebral and paracostal masses; pts with pleural involvement at relapse had similar outcome to pts developing other EM-S.

PB1697

EPIDEMIOLOGICAL, CLINICAL, BIOLOGICAL AND IMAGING CHARACTERISTICS OF MULTIPLE MYELOMA OVER A PERIOD OF 13 YEARS

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Background: Multiple myeloma (MM) or Kahler's disease is a malignant hematological disease due to a monoclonal tumoral proliferation of abnormal plasmocytes, located essentially in the bone marrow & producing a complete or no complete monoclonal immunoglobulin. The MM diagnosis is mostly easy by confronting the clinical, imaging, biological and cytologic criteria. In Algeria, the MM is the third most frequent malignant hematological disease with an incidence of 0.63/100.000
Aims: This retrospective study includes 495 patients diagnosed over a period of 13 years (from January 2000 to December 2012).
Methods: The diagnosis is based on: clinical findings, blood cell count, myelogram (a plasmocyte infiltration quantitatively ≥ 10%), trephine biopsy if normal myelogram, & immune-biochemical tests (protein electrophoresis & immune-electrophoresis or immune-fixation of serum proteins, creatininemia, calcemia & β_2 microglobulin). Full skeleton imaging (skull, cervical, dorsal & lumbar vertebrae, pelvis & chest) to screen for bone lesions. The tumoral mass & the prognosis are estimated using the Salmon & Durie staging & the International Scoring System (ISS) used since September 2005.
Results: 260 male and 235 female (sex-ratio : 1.1)). The average age was 60 years (29-89), 250 patients (50%) ≤ 60 years et 53 patients (11%) ≤ 40 years. Smoking was found in 70 (14%) pts, 05 pts have previously presented plasmocytoma , one patient followed-up for a monoclonal gammapathy, & one l patient had HIV disease . Familiar cancer history found in 40 pts (8%) . the most frequent appealing symptom was the pain in 298 pts (60%) , anemia form revealed MM in 75 cases (15%), a plasmocytar tumoral mass in 8 pts. The average delay to diagnosis was 4 months (0 – 48 months). The clinical exam objective an ECOG (Eastern Cooperative Oncology Group) > 2 in 231 pts (47%), 66 patients were painless (13%) & spine compression signs were present in 43 patients (9%). The average Hemoglobin was 9g/dl (≤ à 8g/dl in 231 patients), a calcemia > 120 mg/ l in 89pts (18%), serum creatinine > 20 mg/l in 141 pts (28%). The B2-migroglobulin dosing was performed in 211 patients (42%) including 94 patients (44%) having a B2-migroglobulin > 5.5mg/l. The monoclonal peak was found in 414 cases (84%): IgG in 244 pts (55%), IgA in 111 pts (25%) and IgD in 5 pts. Two patients presented however a biclonal peak & one presented an IgM peak. Seventy cases with light chain MM, 11 with non-secretory disease and 52 no determined . The light chain kappa in 270 pts (62%) and lambda in 165 pts (38%). The average peak level of monoclonal protein was 43 g/l (10-129), with a peak ≥ 50 g/l in 158 pts (38%) and ≥70g/l in 53pts (13%). The average medullary plasma cell was 30%, ≥ à 50% in 122 pts (25%) and less than 10% in 40 pts (9%), motivating the use of bone marrow biopsy in 39 pts. Diffuse bone lesions in imaging were present in 435 (88%), with fractures in 23 pts (5%) & a spine compression in 46pts (09%). The stage III of the Salmon-Durie staging system was reached by 470 pts (95%), stage II 12 pts and stage I :13 pts. The ISS was established in 211 pts (42%): I : 59 pts, II : 57 pts, III : 95pts.
Summary and Conclusions: We found in our department that the number of patients doubled in time: from 1994 to 2005 (12 years) = 216 pts (18 pts/year) and from 2000 to 2012(13 years) = 495 pts (38pts/year). Fifty percent were relatively young ≤ 60ans, and increase diagnosis delay ≥ à 4 months for 244 pts(50%) explain that almost of pts are at stage III

PB1698

TREATMENT OF MULTIPLE MYELOMA IN PATIENTS ELIGIBLE FOR THE AUTOLOGOUS STEM CELL TRANSPLANTATION: MATTER OF CHOICE, RESPONSE AND EFFICACY

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Background: Flood of novel agents dramatically changed the course and prognosis of multiple myeloma (MM), previously considered as uncurable, devastating disease. Following drug developement, dilemma regarding the role of high-dose treatment (HDT) and autologous stem cell transplantation (ASCT) during first remission, was imposed.
Aims: The aim of study was to analyze efficacy of the HDT and ASCT during first remission, after different induction therapies, and to single out treatment factors of the impact on the overall survival (OS) of newly diagnosed MM patients (pts) eligible for the ASCT.
Methods: The analyzed group consisted of 93 newly diagnosed MM pts (44 male/ 49 female, mean age 55 yrs, range 36-65) eligible for the ASCT. IgG myeloma was diagnosed in 57pts (61,2%), IgA in 16pts (17,1%), IgD in 3pts (3,2%), light chains in 15pts (16,1%), and non-secretory in 2pts (2,2%). According to the clinical stage (CS, Durie-Salmon), patients were distributed as follows: II 23pts (24,7%); III 70pts (75,3%). Regarding ISS score, the group included: ISS1 29,4%; ISS2 30,3%; ISS3 40,3%. Renal impairment was present in 16pts (17,2%). According to the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) distribution was as follows: 0-1 78pts (83,9%); ≥3 15pts (16,1%). Regarding applied treatment, patients were divided in three groups: CTD induction followed by HDT and ASCT (CTD+ASCT) in 31pts,

33,3%); VAD+ASCT in 27pts (29,0%); and CTD induction without HDT and ASCT in 35pts (37,7%). Conventional CTD chemotherapy without ASCT was applied in patients with HCT-CI ≥3.
Results: Patients treated with CTD+ASCT had significantly longer period of remission (median 24,3; range 10-42m, p<0,005) despite the lack of difference in the overall response (CR+VGPR+PR; χ^2 2,953, p= 0,079) between these three groups. Furthermore, patients treated with HDT+ASCT had significantly longer overall survival (OS) in comparison to the patients treated with standard CTD chemotherapy without ASCT (CTD+ASCT median OS 72,4m *vs.* VAD+ASCT median OS 48,2m *vs.* CTD median OS 35,8m, Log rank 7,813, p=0,02). In the model that included following factors: 1) Achievement of CR; 2) ASCT; 3) Remission duration ≥12m; and 3) treatment with thalidomide, only remission duration ≥12m was of significant impact on the overall survival (χ^2 10,841; p= 0,014).
Summary and Conclusions: Intensification of treatment with high-dose therapy and autologous stem cell transplantation strengthen with agents of novel mechanisms of action is of strong impact on the course of disease and overall survival in MM patients. Achievement of the long, sustainable remission ≥12m is of major significance for the overall survival of patients with multiple myeloma.

PB1699

EXTRAMEDULLARY INVOLVEMENT OF CENTRAL NERVOUS SYSTEM IN MULTIPLE MYELOMA: REPORT OF 4 CASES

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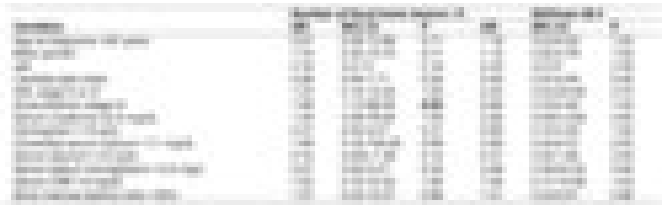
Background: Involvement of central nervous system (CNS) is a very rare event, described in about 1% of patients with no more than 200 cases reported in the literature. The involvement of CNS can occur as leptomeningeal myelomatosis and/or, less often, as intracranial masses. There are no predictory factors of such event, which is commonly associated with very poor prognosis. Median overall survival is of few months and no standard treatment is proposed.
Aims: to report 4 new cases of a very rare event
Methods: From 2010 until 2013, 268 newly diagnosed MM patients were observed in our Institution. None of our patients presented CNS localization at the time of diagnosis. On the other hand, during we observed 4 cases of CNS relapse during follow up.
Results: Main characteristic of these patients at diagnosis are summarized in Table 1.

Table 1.



Response to first line therapy was very good partial remission in patients n. 1, 3 and 4, while in patient n. 2 after first cycle second line therapy with intermediate doses of cyclophosphamide was needed to reach remission. Time from diagnosis to intracranial relapse was 24 months, 6 months, 18 months, and 11 months for patient 1 to 4, respectively. CNS localization was the first relapse for all patients except n. 1 who was at her third relapse. In all patients CNS involvement was the only manifestation of disease, none of them showing increase in monoclonal component or bone marrow plasmocytosis. Examination of cerebral spinal fluid was performeded in 3 patients (case n. 2, 3 and 4) because of ingravescent parestesias and paraparesis with pain. Examination was positive for the presence of monoclonal plasmacells, while MRI of spinal column and brain showed localization at the spinal column in case 3 and 4 and was completely negative in case n. 2. In patient n. 1 diagnosis of extramedullary myeloma was made after biopsy of one of 4 intracranial lesions mimicking meningioma detected by MRI. Symptoms were sudden letargia and confusion. Cerebral spinal fluid examination was negative. Treatment was intratecal methotrexate and aracytin twice a week in patient n. 2, 3 and 4. Patient n. 1 died one month after diagnosis due to progressive disease, and only a cycle with bortezomib and liposomial doxorubicine was done. Patient n. 2 died 1 month after diagnosis for pneumonia, without any other treatment. Patient n. 3, after intratecal therapy, underwent EDAP treatment for 2 cycles and then first ASCT. She is waiting for second ASCT and is alive after 10 months in complete remission. Patient n. 4, after 3 weeks intratecal treatment with negativization of cerebral spinal fluid, underwent systemic consolidation treatment with EDAP but died because of infection (Figure 1).

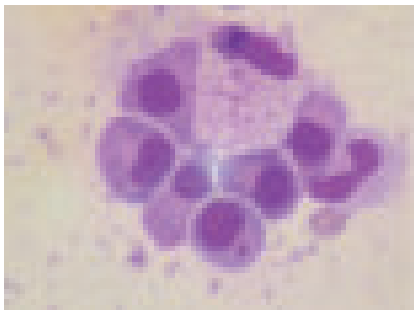


Figure 1.

Summary and Conclusions: As remarked above CNS involvement in multiple myeloma has a very poor prognosis. Some successful treatments are reported with new drugs.Our patients had all been previously exposed to bortezomib, and 2 of them to lenalidomide too, and relapse had occurred relatively quickly during the follow up. When eligible to therapy, we treated them with “old” drugs, and the only patient still alive at the time of writing underwent two courses of classical chemotherapy followed by ASCT.We propose that if alternative experimental treatment is not available, chemotherapy still can play a role in such cases.

PB1700

Abstract withdrawn

PB1701

PREDICTIVE FACTORS FOR SUCCESSFUL PERİPHERAL BLOOD STEM CELL MOBILIZATION IN PATIENTS WITH MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE

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Background: Autologous stem cell transplantation with high dose chemotherapy is considered the standard of care in patients with transplant eligible multiple myeloma patients . It is generally agreed that a minumum dose of 2.5 x 10 ^ 6 / kg CD 34 + cells is necessary for successful engraftment.
Aims: Our purpose of the study is to analyze predictive factors for successful peripheral blood stem cell collection in patients with multiple myeloma retrospectively.
Methods: 55 consecutive patients with multiple myeloma who proceeded to peripheral blood stem cell collection between March 2012 and February 2014 were analyzed retrospectively. There were 34 men and 21 women with a mean age of 58 (range 43-71) years. 14 patients (%25) had at least one prior autologous peripheral stem cell tranplantation history. 18 patients received recombinant human granulocyte-stimulating factor (G-CSF) alone. 4 patients who failed to mobilize with G-CSF alone were also received intermediate dose cyclophosphamide (4 gr/m²) with G-CSF. Total 41 patients were received cyclophosphamide plus G-CSF. At mobilization 17 patients (%30) were in complete response (CR), 7 patients (% 13) in very good partial response (VGPR), 20 patients (%36) in partial response (PR) and 11 (%20) patients in stabile (SD) or progesive disease(PD) . Mobilization failure was defined as the inability to collect 2.5 x 10 ^ 6 / kg CD 34 + cells following the collection.
Results: The rate of mobilization failure was%27 (15 of 55 patients). Mobilization failure was% 92 in patients with prior transplantation and% 5 in patients without transplantation (p<0.001). Mobilization failure rate was% 63 in patients with stabile disease or progresive disase prior to mobilization and%18 in patients with at least partial response (p: 0.028). Mobilization failure rate was%17 in patients with CR,% 14 in patients with VGPR,% 20 in PR but the difference was not significant (p:0.085) . Mean age was 61±6 in patients with mobilization failure *versus* 56±7 in patients who mobilized successfully (p: 0.034). 3 of 4 patients who failed to mobilize with G-CSF were mobilized successfully with cyclophosphamide plus G-CSF. 28 of 41 (%68) patients who received cyclophosphamide with G-CSF had febrile neutrophenic episode, and 14 (%34) patients had bacteremia during the febrile episode. Neither febrile episode nor bacteremia was associated with mobilization failure (p>0.05). 9 of 15 patients who failed to mobilized received plerixafor. 7 of 9 (%78) patients mobilized successfully with plerixafor. 1 patient died during mobilization with cyclophosphamide plus G-CSF due to pneumonia and respiratory failure.
Summary and Conclusions: The most significant predictors of mobilization

failure in patients with multiple myeloma was prior autologous stem cell transplantation. Achievement least PR before mobilization was an important predicting factor but CR and VGPR was not necessary for successful mobilization. Our data also indicate that plerixafor can rescue patients with mobilization failure especially patients with prior transplantation .

PB1702

CYBORD IS AN ACTIVE, WELL TOLERATED, COST-EFFECTIVE INDUCTION REGIMEN IN NEWLY DIAGNOSED MULTIPLE MYELOMA – A SINGLE CENTRE EXPERIENCE

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Background: Background: Based on evidence from clinical trials there is a growing consensus that a three drug regimen involving at least one novel agent (proteasome inhibitor or immunomodulatory drug) should be used as initial induction in patients with newly diagnosed multiple myeloma (NDMM), usually for 3-4 cycles, prior to harvesting stem cells and proceeding to autologous stem cell transplantation (ASCT). Three drug regimens achieve deeper responses both before and after ASCT and this has been shown to translate into superior progression free survival (PFS). CR rates with the three drug regimens prior to ASCT are reported to be in the range of 20-30% with correspondingly high rates of very good partial response (VGPR) (50-60%). One such regimen is the combination of bortezomib with cyclophosphamide and dexamethasone (CyBorD). The published overall response rates (ORR) from phase II trials with CyBorD are approximately 90%, with at least 60% of patients achieving VGPR. At our center, CyBorD has been used as front line therapy since 2008.
Aims: In this report we analyse our experience using CyBorD regimen as initial therapy in transplant eligible patients.
Methods: Methods: We retrospectively analysed clinical and laboratory records for 31 NDMM patients treated with CyBorD at our institution between 2008 and 2013, all of whom subsequently proceeded to ASCT. The standard protocol consisted of bortezomib 1.3 mg/m^2 i.v. twice a week, cyclophosphamide orally at a dose of 300 mg/m^2 weekly, and dexamethasone orally of 40 mg daily given in 4 days long blocks weekly. Since November 2009 we have also used weekly CyBorD.
Results: Results: The median age was 57 years (range from 45 to 65), including 24 males and 7 females. According to ISS, 45% patients were classified as stage I, 5% stage II, and 50% as stage III. The ORR was 90% post cycle I and increased to 96% post cycle IV. At least VGPR was observed in 13% of patients after cycle 1 and 56% after cycle IV. Transplantation had improved the responses and 79% had at least VGPR post transplant *versus* 63% prior, and 24% patients achieved CR post ASCT in comparison to 16% prior ASCT (Figure 1). The therapy was well tolerated. Haematological adverse events were acceptable and no patient required significant dose reduction or experienced treatment delay due to haematological toxicity. Importantly, no patients developed ≥ grade 3 peripheral neuropathy and no patients required dose reduction or discontinuation of bortezomib due to neurological complications. Stem cell mobilisation was performed using a combination of cyclophosphamide 1.5g/m^2 and G-CSF. All patients mobilized successfully without requirement for plerixafor. The medium CD34+ yield after 2 collection days was 8.2 x10^6/kg (range 1.8 -19.4). The PFS at 2 years was 75% and 64% at three years of follow up. At 2 years post transplant, the patients achieving at least a VGPR had a longer median PFS in comparison to patients with PR (91% *vs* 76%, p=NS). The cost analysis has shown that on average, the drug only cost of 4 cycles of CyBorD was 18 000€. This compares to 38 000€ for 4 cycles of bortezomib, lenalidomide, dexamethasone (VRD).

Figure 1.

Summary and Conclusions: Conclusions: Our data confirm the safety and efficacy of the CyBorD protocol and its applicability to routine clinical practice outside of large academic myeloma centres. CyBorD is an active, well tolerated and cost effective option for patients with NDMM, which could be an ideal backbone for the incorporation of new modalities, such as monoclonal antibodies in induction therapy. A randomized comparison with other triple drug regimens are required to fully establish its place in the treatment of newly diagnosed multiple myeloma.

PB1703

RESULTS OF AUTOLOGOUS STEM CELL TRANSPLANT IN MULTIPLE MYELOMA PATIENTS WITH RENAL FAILURE

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Background: Renal failure is observed in 20% of multiple myeloma (MM) patients at diagnosis. Many of these patients are considered ineligible for autologous hematopoietic stem cell transplantation (auto-HSCT) because of a high-risk of treatment-related toxicity.
Aims: The goal of our study is to evaluate the feasibility and efficacy of auto-HSCT in patients with MM with renal failure at the time of transplant, to analyze the reversibility of renal failure and to determine the overall survival (OS) and progression-free survival (PFS) after auto- HSCT.
Methods: Between January 1993 and November 2012, 256 patients with MM underwent auto-HSCT at our institution. At the time of transplant, only seven patients have renal failure which is defined as serum creatinine≥ 177 µmol/l. All patients received induction therapy with Dexamethasone + Thalidomide (3 cycles). Peripheral blood stem cells (PBSC) were mobilized and collected following granulocyte colony-stimulating factor (G-CSF) alone (n=5) or Cyclophosphamide and G-CSF (n=2). Conditioning regimen before autologous consisted of Melphalan 100mg/m2 (n=6) or 140mg/m2 (n=1). Response criteria were used according IMSG.
Results: The median age was 52years (45- 55yrs). Sex-ratio was 2.5. Six patients had Durie and Salmon stage III. Monoclonal componement type was light-chain lambda in 3 cases, complete Ig in 4 cases. At the time of transplant, at least a partial response was achieved in 4/7 patients [CR (n=3); PR (n=1)] and 3 patients were in response failure. Median serum creatinine and cratinine clearance (CrCl) at the time of transplant were 249µmol/l (range; 186 - 349) and 31 ml/mn (range; 16 - 40) respectively. No patient was dialysis-dependent before transplant. The median number of CD34+ infused was 4.24x106/kg (range; 2.08-7,5). The median times to absolute neutrophil count (ANC ≥ 0.5x 109/l) and platelet recovery (≥20 109/l) were 11 days (range;11-15) and 12 days (range;12–15) respectively. The median inpatient stay was 19 days (range; 17-33). We did not observe a treatment-related mortality. Renal function recovery was observed in 3patients, and it's defined as an increase in CrCl by at least 25% compared to the baseline. The average time of renal function recovery was 5months (range; 1-6months). Median serum creatinine and CrCl after transplant were 145 µmol/l (range;103- 306) and 44 ml/mn (range; 17- 75) respectively. Median survival and PFS was 30 months and 14 months respectively. At the latest news and after a median follow-up of 24.5months (range; 6.5-102months), 2 patients were in CR, 4 patients were in progression with associated worsening of renal function in two of them, and 1 patient was not evaluable.
Summary and Conclusions: Auto HSCT is feasible in patients with MM and renal failure with acceptable toxicity and with a temporary significant improvement in renal function in approximately one-half of the transplanted patients.

PB1704

HEAVY/LIGHT CHAINS PAIRS (HLC) AS AN ALTERNATIVE METHOD FOR MONITORING AN IGA MULTIPLE MYELOMA PATIENT

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Background: The quantification of heavy/light chains pairs (HLC) by the new immunoassay Hevylite is based on the recognition of epitopes spanning the junction of the immunoglobulin´s heavy and light chains. This assay can identify separately the different light chain types of each immunoglobulin class: IgGK, IgGL, IgAK, IgAL, IgMK and IgMK. Of particular interest and novelty is the possibility to quantify separately both isotypes of the tumor related immunoglobulin. In this clinical case we show the utility of the quantification of HLC IgAK, IgAL and IgAK/IgAL ratio as a method to monitoring an IgAK Multiple Myeloma.
Aims: To present the utility of heavy/light chains pairs (HLC) in the monitoring of patients with Multiple Myeloma.
Methods: SPE were peformed on CAPILLARYS 2^{TM} (Sebia), the

monoclonal component were identified by IFE on HYDRASYS™ (Sebia), serum inmunoglobulins (IgA, IgG and IgM) were measured by nephelometry on BNII nephelometer (Dade Behring). Heavy/Light chains pairs and serum free light chains were measured by turbidimetry on a SPA PLUS Analyzer (The Binding Site).
Results: We present the rare case of 12 years old boy that was diagnosed of IgAK Multiple Myeloma ISS III stage [hypercalcemia (16.6 mg/dl), increased IgA (4449 mg/dl) and total proteins (12.6 g/dl), normocytic anemia (9.5 g/dl of hemoglobin), altered ratio of serum free light chains (free kappa=219 mg/dl, free lambda=1.01 mg/dl, ratio=216.83) and osteolytic bone lesions (punched-out lesions in skull and vertebral compression)]. At diagnosis (Day 0) the serum proteinogram (SPE) shows a well-defined monoclonal large peak in the gamma region (4.34 g/dl correspond to monoclonal component) and identified by immunofixation as IgA Kappa. The IgA HLC ratio (IgAK=66.604 g/l, IgAL=6.302 g/L, ratio=10.57) identified clonal disease IgAK at diagnosis too. The patient began treatment with Bortezomib, Cyclophosphamide and Dexamethasone and the monoclonal protein was monitorized by SPE, IFE and HLC. During the treatment, the monoclonal protein was decreasing with reduction of the peak in SPE and the HLC ratio remained altered confirming the presence of the monoclonal protein. The monoclonal component IgAK was decreasing due to the good response to the treatment. At day +58 (after 4th cycle of chemotherapy) there was a little peak in SPE (0.18 g/dl of monoclonal component), with positive IFE and altered ratio HLC (IgAK=3.566 g/l, IgAL=0.664 g/l, ratio=5.37). At day +68 the SPE was negative but the HLC ratio remained altered (IgAK=3.566 g/l, IgAL=0.664 g/l and ratio=5.37) confirming the existence of monoclonal protein that it was verified by IFE. At day +131 (end of 5th cycle of chemotherapy) the SPE, HLC and IFE were negative confirming the absence of monoclonal protein due to the good response to the treatment. At the end of the treatment (day +184) after six cycles of chemotherapy the patient achieved a status of complete remission with negative immunofixation, <5% of plasma cells in bone marrow and normal HLC pairs and ratio.
Summary and Conclusions: The monitorization of IgA MM requires the measures of SPEP, IFE and total IgA. The use of the HLC IgAK, IgAL and their ratio IgAK/IgAL presents itself as an alternative method with high sensitivity for monitoring these patients, particularly *in situ*ations where traditional techniques show limitations (*e.g.* low concentracions, interference of other serum proteins, strong polyclonal background). The high sensitivity of the determination of HLC allows typing monoclonal component providing equivalent information to the immunofixation, with the added value of reporting a quantitative value.

PB1705

COMPARATIVE EFFICACY OF SUBCUTANEOUS AND INTRAVENOUS ADMINISTRATION OF BORTEZOMIB IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Bortezomib is currently the basis of combined regimens as newly diagnosed and relapsing forms of multiple myeloma (MM). The main side effect of bortezomib therapy is the development of peripheral neuropathy (PN) , impairing the quality of life of patients. Results of a multicenter international study MMY- 3021 confirmed a significant reduction in the incidence of peripheral neuropathy by subcutaneous administration of bortezomib compared with intravenous in patients with previously untreated multiple myeloma. This opens new perspectives for the use of bortezomib-containing regimes in patients with high risk of PN and lack of peripheral venous access.
Aims: to compare the efficacy and tolerability of standard combination regimens MM with subcutaneous and intravenous administration of bortezomib in patients with newly diagnosed multiple myeloma.
Methods: 27 patients with MM analyzed. The average age of patients was 68 years. Bortezomib in 12 patients were injected subcutaneously (s.c) and at 15 - intravenously (i.v) as part of combination regimens of standard chemotherapy (PAD, CVD, VMP). The effect of the treatment was assessed using standardized international criteria EBMT. Toxicity assessment was performed using the criteria of the National Cancer Institute of the United States (the National Cancer Institute Common Toxicity Criteria - NCI CTC), version 3-0 (Cancer Terapy Evaluation Program, Depatment of Health and Human Services, December 2003).
Results: the overall response rate (ORR) after 4-6 courses of chemotherapy in patients with s.c and i.v introduction was 83% and 80%. CR + nCR was recorded in 25% and 26.7%, PR in 58% and 53.3%, respectively. PN was observed in 33% and 46% of patients on s.c and i.v introduction. Peripheral neuropathy 3rd degree awarded in 8% and 20% , respectively. It should be noted that in 58% of patients in the group of subcutaneous administration of bortezomib marked cutaneous manifestations (1-2 degrees) in the form of congestion in an injection formulation, which does not require modification of the dose were stoped and independently. Gastrointestinal toxicity, preferably 1-2 degrees registered in 26.7% and 40%, respectively. Manifestation of hematologic toxicity most frequent thrombocytopenia and anemia were noted in 25% *vs* 33,3% and 16,6% *vs* 33,3% , respectively. Intensity data of adverse events consistent with grade 1-2 toxicity scale , which did not require adjustment of therapy and modification dates of drug administration .
Summary and Conclusions: with equal effectiveness of subcutaneous administration of bortezomib in patients with newly diagnosed MM has a better tolerability compared with intravenous that helps to optimize therapy and improve the quality of life of patients with MM.

PB1706

BORTEZOMIB-CYCLOPHOSPHAMIDE-DEXAMETHASONE IS A GOOD INDUCTION TREATMENT FOR YOUNG PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Based on evidence from clinical trials there is growing consensus that a three-drug regimen involving bortezomib, one steroid (dexamethasone or prednisone) and one immunomodulatory drug (thalidomide or lenalidomide) or an alkylating agent (melphalan or cyclophosphamide) should be used as induction treatment in young patient with newly diagnosed myeloma before autologous stem cell trasplantation (ASCT). One of these regimes is the combination of bortezomib, cyclophosphamide and dexamethasone (VCD). This scheme has been analyzed in several trials with very different results. Complete remission (CR) rate before ASCT ranges from 10% to 47%, with a very good partial response (VGPR) or better rate up to 61-65%. We have used this regimen in recent years as induction treatment in young patients with newly diagnosed myeloma.
Aims: The aim of this study is to analyze our experience in the response rate and tolerability to VCD, outside clinical trials, in young patients with multiple myeloma.
Methods: We retrospectively analyzed 22 young patients with symptomatic myeloma candidates for ASCT, who have received VCD as induction treatment between 2010 and 2013. Characteristics of the disease at diagnosis, as well as response and tolerability parameters were recorded.
Results: The mean age at diagnosis of these 22 patients was 56 years (range 42-65), with a predominance of males (69%). The distribution of myeloma type was as follows: 19 secretory myeloma (including two cases of Bence-Jones myeloma), 2 oligosecretory myeloma and 1 case of plasma cell leukemia. A significant proportion of poor-prognosis cases were included: 36% of patients had ISS stage 3, 27% had hypercalcemia at diagnosis, 23% had elevated LDH and 18% had poor prognosis cytogenetic [del 17p, t(4;14) or t(14;16)]. All the patients received 42-day courses of bortezomib 1,3 mg/m^2 (days 1, 4, 8 and 11 and 22, 25, 29 and 32); dexamethasone 40 mg (days 1-4 and days 22-25); and cyclophosphamide between 500 and 750 mg/m^2 (days 1 and 22). Number of courses ranged from 2 to 6 depending on the response. A CR was achieved in 8 patients (36%), including two patients with strict CR. The VGPR or better rate was 64% (14 patients) and a partial response or better rate of 86% (19 patients). Only three patients (14%) could be considered refractory to this treatment because of progressive disease or stable disease. From 19 patients who achieved at least PR, eleven have received an ASCT and have been subsequently evaluated, with a post-ASCT CR rate of 36%. Of the responders patients with measurable disease, main reduction of M component occurred within the first course and ranged from 40% to 95%. Concerning tolerability, VCD scheme is generally quite well tolerated. Nine patients (41%) required dose reduction of bortezomib, principally because of peripheral neuropathy but without stopping treatment. Two patients required dose reduction of cyclophosphamide because of myelotoxicity.
Summary and Conclusions: In our experience, VCD is a very good induction treatment with CR rates before ASCT similar to other three-drug induction regimes like VTD or VRD. This scheme is generally quite well tolerated. The main advantage of using VCD as induction treatment is that we can reserve immunomodulatory drugs (IMiDs), like thalidomide or lenalidomide, for subsequent relapses of the disease.

PB1707

OUTCOME OF MULTIPLE MYELOMA IN YOUNG PATIENTS UNDER 45 YEARS: ABOUT 41CAS.

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Background: Multiple myeloma (Kahler's disease) is a hematological malignancy due to tumor proliferation of monoclonal plasma cells in the bone

marrow, characterized by its clinical and biological polymorphism. The average age of onset of this malignancy is > 60 years. Multiple myeloma can also affect younger patients, 2.8% of cases are diagnosed under 40 years of age. Herein we present the characteristics and the outcome of 41patients (pts) aged under 45 years at diagnosis

Aims: a period of 12 years (2000-2011), between 444 patients (pts) with MM diagnosed 41 pts (9%) are under 45 years: 25 male and 16 female (sex ratio: 1.6). The median age was 41 years (32-44).Seventeen pts (4%) <40years old. Three pts had history of solitary plasmacytoma. The most commun sign is pain syndrome: 32 pts (78%), anemia syndrome: 6 cases (15%) and plasma cell mass: 2 pts.

Methods: Three pts had history of solitary plasmacytoma. The most commun sign is pain syndrome: 32 pts (78%), anemia syndrome: 6 cases (15%) and plasma cell mass: 2 pts. The median time to diagnosis was 3 months (0-48). ECOG 1-2: 20 pts (49%), the hematological parameters is characterized by a median Hb: 8g/dl (3-15), hyper calcemia ≥ 120 mg /l: 8 pts, serum creatinine ≥ 20 mg /l: 12 pts. Determination of β2 microglobulinemia is done in 13 pts (32%) with a rate ≥ 5.5 in 6 pts. The serum monoclonal peak was found in 32 cases (78%), the median peak: 40g /l (15-110) with a peak ≥ 50 g /l: 16 pts (50%), which is represented by an IgG: 21 pts (65%), IgA: 7 pts (22%), IgD: 1 pts, 09 cases of MM light chains, non-secreting: 2 pts and unspecified type in 1 pt. The median plasmacytosis in a marrow 35% (10-85). The PBJ is made in 37 pts (90%), positive in 19 pts (50%). Diffuse bone lesions are present in 37 pts (90%) , fractures in 2 pts and spinal cord compression in 7pts (17%). According to Salmon-Durie stage: III: 40 pts (97%) with 13 pts (32%) stage B, stage II: 1pt. International Staging System (ISS) used in 13pts (32%) I: 5pts, II: 2 pts, III: 6pts. The treatment used was: VAD (vincristine, adriamycin and dexamethasone) for 37 pts; TD (Thalidomide-Dexamethasone) for 3 pts and CTD (Cyclophosphamide-Thalidomide-Dexamethasone) for one pt. In December 2012, the median follow-up was 54 months(22-129).

Results: Nine pts are no evaluable because early death after 1-2 cycles. Thirty-two pts are evaluable, 28 pts received VAD (12 failures and 16 responses: 5 CR and 11 PR). Among patients in response, 12 pts were intensified with autologous stem cell transplantation after induction: 6 pts are alive in CR, 2 pts alive in PR and 4 pts died with a median survival of 49 months (19-129) and 4 pts without high dose chemotherapy and autologous stem cell cell transplantation died with a median survival of 5 months (4 -12). For the 12 treatment in failure : 2pts are alive (1 CR, 1 failure) and 10 pts died with a median survival of 10 months (3-66). Three pts received TD (1 CR, 2 PR) followed by autologous transplantation: 2pts are alive in CR (43.47 months), and one died (34 months). One patient treated with CTD grafted in CR is alive (22 months).

Summary and Conclusions: The prognosis of multiple myeloma patients remains poor, less than 5% of patients remain alive more than 12 years .The young's form disease is becoming more common with an aggressive clinical form and unfavorable evolution despite the introduction of new drugs.

Myeloproliferative neoplasms - Biology

PB1708

CALR-MUTATED OR TRIPLE-NEGATIVE (JAK-2 AND MPL) IN ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS

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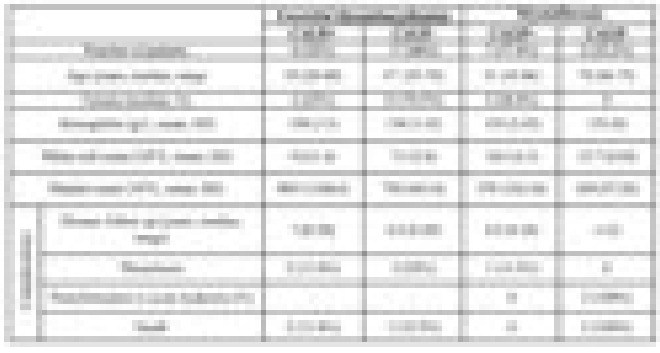
Background: Myeloproliferative neoplasms (MPN) are chronic myeloid cancers that manifest as expansion of one or more myeloid lineages, characterized by the overproduction of mature blood cells. The first insight into the molecular cause came when the somatic *JAK2 V617F* mutation was identified in the majority of patients with polycythemia vera (PV) and in a subset of patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF). Subsequent studies identified *JAK2 exon 12* mutations in patients with PV and mutations in the gene encoding thrombopoietin receptor (*MPL*) in a subset of patients with ET and MF. Recent studies have identified additional novel mutations, in the gene encoding calreticulin (*CALR*) in patients with ET or PMF without *JAK2* or *MPL* mutations. The frequency described in these patients was 70-84%. For the moment, 36 different mutations (all deletions, insertions, or both) have been labelled. Understanding the molecular basis for ET and PMF in patients without *JAK2* mutations is the most relevant focus in the area of myeloproliferative neoplasms and efforts must be aligned in this direction.

Aims: The main purpose of the study is to identify *CALR* mutations and to analyse the impact in patients with ET and MF without *JAK2* or *MPL* mutations retrospectively and evaluate the influence of clinical and biological features.

Methods: All myeloproliferative neoplasms were classified in accordance with the 2008 World Health Organization criteria. The fragment analyses of *CALR* exon 9 mutations were sized on a 3100 xl Genetic Analyzer (Applied Biosystems), and results were analysed using the Gene Mapper software version 4.0 (Applied Biosystems). Samples for 3 different mutations were Sanger sequenced for confirmation and determination of the exact type of *CALR* mutation. Samples were screened by qPCR for the presence of *JAK2 V617F* and *MPL W515L/K* mutations.

Results: *CALR* exon 9 mutations were analysed in 36 patients with myeloproliferative neoplasms: 27 with ET and 9 with MF were diagnosed in our centre, in the period Jan-1985 to Dec-2013. *JAK2* mutated and *CALR* wild type was detected in 2 patients with TE and 1 MF. A relevant number of patients with mutation *CALR JAK2* and *MPL* negative was found in MF and patients with TE (77.8% and 32%, respectively). Most frequent mutations were type 1 (del 52bp) and type 2 (ins 5bp). In TE, type 1 represented the 62.5% and type 2 the 37.5%. In MF, type 1 was 57.14%, type 2 28.6% and a new deletion of 31bp was detected, sequenced and not described yet. Clinical data can be observed in Table 1, without being able to draw conclusions due to heterogeneity of groups.

Table 1.



Summary and Conclusions: A high frequency of mutations in patients with *CALR* + MF double negative (77.8%) was observed in the present study, with a value similar to the described in the literature, it was not the case for ET, accounting a lower frequency (32%). A higher platelet count in ET cases CALR + was also confirmed, however no clinical differences were observed. Even more, the importance in detecting new non-described indel must be remarked. Different clinical courses were described in these patients, a larger study in order to consolidate these results and detect possible new mutations seems necessary, since was more indolent than that in patients with the *JAK2 V617F* mutation.

PB1709

PREDISPOSITION TO MYELOID NEOPLASIAS – THE ROLE OF OXIDATIVE STRESS GENES POLYMORPHISMS

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Background: The pathogenesis of myeloid neoplasia is complex and involves genetic, epigenetic and molecular alterations. Excessive production of Reactive Oxygen Species (ROS) and/or a decrease in antioxidant pathways can lead to an imbalance state termed oxidative stress (OS), that contribute to cell damage, apoptosis and ineffective hematopoiesis. OS has been observed in several hematopoietic malignancies and some evidences suggest a role of OS in etiology and pathogenesis of hematological neoplasias, namely in Acute Myeloblastic Leukemis (AML), Chronic Myelomonocytic Leukemia (CMML), Myelodysplastic Syndromes (MDS) and Myeloproliferative Neoplasias (MPN), including those Philadelphia (Ph)-positive as Chronic Myelogenous Leukemia (CML). The genetic variability in oxidative stress related genes might contribute to the differences in individual susceptibility to oxidative damage since it can lead to increase in ROS production or to decrease in its elimination.
Aims: In this context, we investigated the influence of SOD1, SOD2, COX2, CAT and NADPH *oxidase p22 phox*polymorphisms, as a risk factor for myeloid neoplasia development.
Methods: This study enrolled 277 patients diagnosed with myeloid neoplasia (65 AML, 10 CMML, 101 MDS, 101 MPN) and 150 healthy controls. The genetic polymorphisms of SOD1 (A251G), SOD2 (Ala16Val), COX2 (G-765C), CAT (C-262T) and NADPH oxidase *p22 phox* (C242T) were assessed by RFLP-PCR. The strength of association between polymorphisms and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%).
Results: Our results show a lower *wild type* allelic frequency of SOD1 (90%), CAT (80%), OGG1 (18%) and NADPH *oxidase p22 phox* (67%) polymorphism and a higher variant allelic frequency of SOD2 (52%) and COX2 (79%) in myeloid neoplasia patients, compared to controls. In these patients the predominant genotype was AA (90%), CT (52%), GG (60%), CC (69%), CC (80%) and CC (48%), respectively for SOD1, SOD2, COX2, CAT, OGG1 and NADPH *oxidase p22 phox*. Besides that, individuals with COX2 and NADPH *oxidase p22 phox* CC genotype and with TT genotype of CAT have an increase risk for hematological neoplasia development about 2,54-fold (CI95% 1,05-6,28; p=0,038), 1,85-fold (CI95% 1,22-2,82; p=0,0005) and 2,03-fold (CI95% 1,10-3,74; p=0,0021), respectively. Besides that, individuals with NADPH *oxidase p22 phox* CC genotype have an increase risk about 1,81-fold for MPN development (CI95% 1,05-3,1; p=0,038), especially CML, where the risk is 2,36-fold (CI95% 1,04-5,40; p=0,032). Moreover, the TT genotype of CAT show and increased risk for AML, MDS and Ph^- MPN development of 11,53-fold (CI95% 1,53-87,2; p=0,004), 3,93-fold (CI95% 2,02-10,01; p=0,041) and 5,39-fold (CI95% 1,58-18,47; p=0,005), respectively.
Summary and Conclusions: These results show that genetic polymorphisms in oxidative-stress related genes might be related with myeloid malignancies development and may constitute novel genetic markers for MPN, MDS and AML susceptibility.
This work was supported by CIMAGO (Project 22/09).

PB1710

VORINOSTAT IMPAIRS VIABILITY, DIFFERENTIATION AND REDOX HOMEOSTASIS IN BCR-ABL-NEGATIVE MPN PATIENTS

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Background: The classical BCR-ABL-negative myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (PMF). They are characterized by increased proliferation of hematopoietic precursors in the bone marrow resulting in an elevated number of terminally differentiated cells. Despite the recent description of JAK2 activating mutations and other mutations, these do not completely explain the pathophysiology and clinical heterogeneity of MPN. Epigenetic modifications, particularly histone acetylation, play pivotal roles in the pathogenesis of several hematological malignancies, and treatment of such disorders with histone deacetylase inhibitors results cell death and proliferation arrest. Importantly, epigenetic agents have proven to be quite effective in the clinical practice, by producing remissions and increasing the overall survival in several hematological disorders.
Aims: HDAC inhibition has been shown to reduce tumor burden in patients with MPN. In order to investigate the effects of HDAC inhibitors in MPN, we analyzed the impact of Vorinostat in the cellular biology of MPN cell lines and primary bone marrow samples.
Methods: MPN bone marrow samples were collected at diagnosis following informed consent and in course of routine clinical tests. Mononuclear cells from primary MPN samples were isolated by gradient separation and MPN derived cell lines and primary cells were incubated with Vorinostat. At different time points of culture, the cells were harvest, lyzed for RNA extraction and stained with different antibodies, Annexin-V/PI and DCF-DA to analyze cellular differentiation, apoptosis and Reactive Oxygen Species (ROS) respectively.
Results: Incubation of MPN cell lines with Vorinostat induced apoptosis in a time- and dose-dependent manner. Vorinostat induced the expression of genes associated with apoptosis and growth arrest and down-regulated the expression of genes associated with proliferation, growth arrest and also JAK-STAT signaling pathway target genes. Furthermore, incubation of primary MPN bone marrow samples with Vorinostat induced apoptosis, blocked differentiation and reduced ROS levels in a dose dependent manner. These effects were most marked in the monocytic lineage, a population which expresses the highest levels of ROS. Vorinostat also reduced the levels of GPA and CD61, markers of erythroid and megakaryocytic differentiation, respectively.
Summary and Conclusions: We have found that Vorinostat incubation impairs differentiation in MPN and also reduces cellular viability and ROS, possibly through the down-regulation of genes associated with cellular and proliferation, particularly the JAK-STAT target genes, and up-regulation of genes important for apoptosis and growth arrest. These results hold therapeutic promise as Vorinostat reduced differentiation markers associated with Polycythemia Vera and Essential Thrombocytosis. Furthermore, the discovery that Vorinostat is particularly noxious to the monocytic lineage is interesting since it has recently been shown in a mouse model that bone marrow monocytes are crucial for Polycythemia Vera pathogenesis. Our results point towards the potential of Vorinostat (and possibly other HDAC inhibitors) to treat MPN. This potential would require more clinical trials to confirm their clinical efficacy.

PB1711

MUTATIONS OF THE CALRETICULIN GENE IN MYELOPROLIFERATIVE NEOPLASMS IN JAPANESE PATIENTS

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Background: Frequent somatic mutations of the *calreticulin* (*CALR*) gene in myeloproliferative neoplasms (MPN) patients were recently reported. These mutations were located in exon 9 and included several types of insertions or deletions, which cause a frameshift to the same alternative reading frame and generate an altered C-terminal peptide. Klampfl et al reported that the Ba/F3 cells expressing the 52-bp deletion showed cytokine independent cell growth and STAT5 auto-phosphorylation. Detecting specific mutations are useful for diagnostic tool, classification, and prognostic estimation. Elucidating the incidence of the mutations and clinical characterization in different race and country was important for establishing the disease classification system.
Aims: To determine the relevance of the somatic mutations in the *CALR* gene and its association with type of MPN in Japanese patients, we analyzed *JAK2* V617F, *MPL* W515L and the *CALR* gene mutations.
Methods: A total of 132 patients with MPN (68 essential thrombocytosis (ET), 42 polycythemia vera (PV), 7 primary myelofibrosis (PMF), and 15 other MPN) were analyzed (Table 1). Somatic mutations in the *CALR* gene were analyzed in MPN patients without *JAK2* V617F or *MPL* W515L mutations. Granulocytes were separated from peripheral blood after obtaining written informed consent. *JAK2* V617F and *MPL* W515L mutations were detected by allele-specific polymerase chain reaction (PCR) as previously reported (Yoshinaga K *et al.* 2008). Somatic mutations in the *CALR* gene were analyzed by bidirectional direct sequencing of purified PCR products covering the exon 9 of the *CALR* gene. The primer sequences were previously published (Nangalia J *et al.*, 2013). Current study was conducted within the guidelines and with the approval of the institutional ethical committee.
Results: *JAK2* V617F mutation was found in 33 of the 68 patients with ET and 40 of the 42 patients with PV. *MPL* W515L mutation was found in two of 22 patients with ET. *CALR* gene mutations were analyzed in 34 patients (30 patients with ET, 2 patients with PMF, one patient with AML from ET, and one patient with MF from ET). *CALR* gene mutations were found in 14 of the 30

patients with ET and one of the two patients with PMF. The identified *CARL* gene mutations were the followings; 52-bp deletion (p.L367fs*46) in 8 patients; 5-bp insertion (p.K385fs*47) in 6 patients; and 34-bp deletion in one patient (Table 1). We were unable to identify an apparent point mutation.

Table 1. Type of MPN and mutation status.

Summary and Conclusions: Sequencing analysis revealed that 46.7% of ET patients without *JAK2* V617F or *MPL* W515L mutations carried a somatic mutation of the *CALR* gene. This incidence of the *CALR* mutation may be slightly lower than those reported by Nanglia et al (71%) and Klampfl et al (67%). The mutations consisted of two common variants of 52-bp deletion and 5-bp- TTGTC insertion which accounted for 14 of 15 (93.3%) of the *CALR* mutations. This percentage is slightly higher than the previous reports. These discrepancies may reflect the differences between race or patient selection bias.

PB1712

JAK-2 V617F ALLELE BURDEN INCREASES OVER TIME IN HETEROZYGOUS PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA RECEIVING OR NOT TREATMENT WITH HYDROXYUREA

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Background: JAK-2 V617F mutation is a cornerstone in the diagnosis of Essential Thrombocythemia (ET); however, it is still unclear if the JAK-2 V617F allele burden could be modified by the natural evolution of disease over time or by the cytoreductive treatment.
Aims: To evaluate allele burden variations over time in subjects receiving or not treatment with hydroxyurea (HU), 86 patients with ET and a 1st evidence of JAK-2 V617F mutation in a heterozygous state (allele burden<50%) were prospectively studied.
Methods: There were 30 males and 56 females, with a median age at diagnosis of 56.6 years [interquartile range (IR) 45.9 – 68.7]. Median time from diagnosis to 1st JAK-2 allele burden evaluation was 4.2 years (IR 0.5 – 9.6); in particular, 54 patients received 1st allele burden evaluation<6 years since diagnosis and 32 patients ≥ 6 years since diagnosis. Median interval between 1st and 2nd JAK-2 allele burden evaluation was 12.1 months (IR 11.1 – 14.1). As to treatment, 29 patients (33.7%) were not receiving any treatment while 57 patients (66.3%) were receiving HU when both JAK-2 allele burden analyses were done.
Results: JAK-2 V617F allele burden median values at 1st and 2nd analysis in the whole population were 17.2% (IR 6.1 – 32.7) and 22.3% (IR 8.9 – 35.3), respectively (p=0.003). In the 29 patients without treatment, 1st and 2nd analysis were 22.7% (IR 14.2 – 31.6) and 25.9% (IR 14.7 – 34.7) (p=0.013); in the 57 patients receiving HU, 1st and 2nd analysis were 15.2% (IR 4.2 – 34.5) and 20.0% (IR 6.8 – 36.3) (p=0.038). Patients with a shorter disease duration (< 6 years since diagnosis) showed a V617F allele burden increase from the 1st median value of 14.3% (IR 3.5 – 28.0) to the 2nd median value of 15.0% (IR 6.7 – 31.8) (p=0.028); patients with a longer previous disease duration (≥ 6 years since diagnosis) had an allele burden increase from the 1st median value of 29.0% (IR 10.4 – 35.4) to the 2nd median value of 31.0% (IR 17.9 – 40.0) (p=0.027). Moreover, 64/86 patients had a 3rd JAK-2 V617F allele burden evaluation after a median interval of 11.9 months (IR 11.2 – 13.4) from the 2nd sample: in these 64 patients, JAK-2 V617F allele burden median values at 1st, 2nd and 3rd analysis were 16.9% (IR 4.7 – 32.7), 21.6 (IR 10.1 – 36.4) and 28.2% (IR 11.6 – 41.7), respectively (p<0.001).
Summary and Conclusions: JAK-2 V617F allele burden seems to increase over the time in patients with ET; this increase seems to be significant during all disease course. In addition, HU does not seem to reduce the allele burden rise. However, a larger cohort of patients is warranted to confirm these data.

PB1713

THE FREQUENCY AND PROGNOSTIC OF EZH2 MUTATIONS IN PATIENTS WITH BCR-ABL-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Recent studies have revealed a number of epigenetic alterations that contribute to myeloproliferative neoplasms (MPNs) pathogenesis and determine the clinical outcome. Mutations involving the *EZH2* gene which encodes the catalytic component of the histone methyltransferase have been described in MPN patients. These mutations are thought to result in loss of methyltransferase activity suggesting a potential role of tumor suppressor gene silencing as a mechanism in disease progression. According to the published data, mutations in *EZH2* are founded in 6% cases of primary myelofibrosis (PMF), 1% of polycythemia vera (PV) and 1-3% of essential thrombocythemia (ET) regardless of the presence of mutations in *JAK2* or *MPL*. *EZH2* mutations are thought to be of prognostic value in MPN's at the time of transformation to the blastic phase but data are inconsistent and require further verification.
Aims: The goal of our research was to determine the frequency of mutations in *EZH2* in two groups of patients with different chromosomal aberrations.
Methods: We have examined 48 patients with *BCR-ABL*-negative MPNs (24 pts with PV, 16 pts with ET and 8 pts with PMF). According to results of the cytogenetic studies 32/48 patients have a favorable karyotype, 4/48 have abnormalities with intermediate risk and 12/48 have chromosomal aberrations with unfavorable prognosis. Patients were divided into 2 groups. The first group included 20 patients with normal karyotype, and 16 patients with the isolated chromosomal aberrations del(13)(q22), del(20)(q12), -Y associated with favorable prognosis, and add(22)(q13), del(1)(p32), del(6)(q15), t(10;12)(q22;p13) that are referred to as an intermediate risk. The second group included 12 patients with complex abnormalities and the other chromosomal aberrations of unfavorable prognosis: +8, +7,-7, inv(7)(p11;q21). Mutations in 8, 10, 17, 18, 19 exons of *EZH2* were defined by sequence analysis.
Results: 2 mutations of *EZH2* gene have been found in 2 individuals with PMF (2/8) belonging to different groups of prognosis. Both mutations are located in the 19 exon. The Ile713Thr mutation was detected in the patient with a del(6)(q15) karyotype which is associated with an intermediate risk. This patient subsequently underwent transformation from PMF to myelodisplastic syndrome in 9 months after the disease onset. Another case of mutation harboring (Thr731Asp) was detected in a patient with PMF and poor prognosis karyotype (chromosome 7 monosomy). This patient had transformation PMF to acute myeloid leukemia and was died after 20 months.
Summary and Conclusions: Mutations in the *EZH2* gene could be preliminarily assessed as additional prognostic markers of unfavorable prognosis in patients with *BCR-ABL*-negative MPNs and with different chromosomal aberrations. Our results confirm its prognostic significance for PMF patients but further studies are needed to consider patients with other types of MPNs.

PB1714

TGF-B SIGNALING PATHWAY MOLECULAR REGULATION BY SMADS IN MYELOFIBROSIS PATIENTS

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Background: Myelofibrosis (MF) is a complex clonal hematopoietic stem cell malignancy classified as a myeloproliferative neoplasm. Besides the known TGF-β functions in MF fibrosis progression, detailed mechanism of TGF-β signaling in the pathophysiology of this disease remains unclear. Bone morphogenetic proteins (BMPs) are multifunctional growth factors, members of the TGF-β superfamily. Smads are a group of proteins critical for transmitting signals from TGF-β superfamily of the cell surface to the nucleus. They are classified as receptor-regulated (R-Smads: 1, 2, 3. 5 and 8/9), common-mediator (Co-Smad: Smad4), and antagonistic/ inhibitory (I-Smads: 6 and 7) Smads. As they regulate TGF-β signal transduction, they may have an important role in TGF-β modulation in MF process.
Aims: To investigate the molecular regulation of TGF-β signaling pathway by SMADs in MF patients, quantifying their mRNA expression and comparing it with controls.
Methods: Forty-two MF patients diagnosed according to the World Health

Organization criteria (2008) were selected from the Hematology Department of the Universidade Federal de São Paulo (UNIFESP) for this study. Twenty-three controls were also included and matched by age and gender with patients. Plasmatic TGF-β1 was measured by Luminex technology, and quantification of TGFB1 and SMADs 1 to 8 mRNA expressions was performed by real time qPCR, using Taqman assays. The statistical analyses were carried out with data of 42 MF patients when this group was analyzed separately. However, when the comparison was performed among MF group and matched controls, the number of subjects in each group was 23.
Results: TGF-β plasmatic concentrations of MF patients were similar to the matched controls (P> 0.05). Regarding to mRNA expression, SMAD6 was higher in MF patients in relation to controls (P=0.01), while SMAD7 was lower (P=0.01). When each group (MF or control) was analyzed separately, according to the median of TGFB1 mRNA (median of values in 42 MF patients = 0.138841; 23 controls = 0.08563) and classified as low and high expressions, it was observed that MF patients with high TGFB1 mRNA expression presented higher SMADs 1 to 7 mRNA expressions than those with low expression (p< 0.05). The controls with high TGFB1 mRNA expression had higher SMADs 2, 3, 5, 6 and 7 mRNA expressions than those with low expression (P< 0.005).
Summary and Conclusions: It seems that TGF-β molecular regulation by SMADs is different among MF patients and controls. Our findings suggest that MF patients have a lower negative feedback of TGF-β signaling by SMAD7 than controls. Furthermore, the fibrotic process involved in the disease may be modulated by SMAD6 overexpression, which inhibits BMPs signaling and its anti-fibrotic activity. (FAPESP 2012/12957-5).

PB1715

DETERMINING THE NUMBER OF CIRCULATING CD34+ CELLS FOR THE DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS IN DAILY PRACTICE
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Background: A high number of circulating CD34+ cells has been advocated to distinguish primary myelofibrosis (PMF) from other Philadelphia-negative myeloproliferative neoplasms (MPN). However, this test is not included in the current WHO diagnosis criteria and literature remains sparse on the subject.
Aims: In this oligocentric study, we reevaluate the diagnostic interest of measuring circulating CD34+ cells for the diagnosis of MPNs in daily practice.
Methods: Between 2010 and 2012, the number of circulating CD34+ cells was determined in 26 healthy volunteers and 286 consecutive patients at diagnosis in whom the diagnosis of PMF was suspected (suggestive blood count, splenomegaly or investigation of a splanchnic thrombosis). The determination of circulating CD34+ cells was obtained through flow cytometry with a standardized simple platform technique (BD™ Stem Cell Enumeration Kit, BD Bioscience ; Stem-Kit™ CD34 HPC Enumeration kit, Beckman Coulter). Results for growth of progenitors on a collagen gel without and with growth factors (Stem Alpha ou StemCell Technologies) were available for 82 patients. Results were correlated with the entire clinical and biological data and the final diagnosis in regard to the 2008 WHO criteria for each patient.
Results: 31 patients were excluded from the analysis because they were diagnosed with acute leukemia, lymphoid neoplasm or thrombosis without MPN. The normal number of CD34+ determined in healthy volunteers is <5.4/µl (median number of 2.6/µl + 2 standard derivations of 1.32). The median number of CD34+ is significantly higher in MPNs than in healthy volunteers, but at levels somewhat different, in the following diseases (Mann et Whitney test): PMF (n=34 pts ; 51/µl [2.5–717]), essential thrombocytemia (ET) (n=93 pts ; 3.5/µl [1–32]), polycythemia vera (PV) (n=42 pts ; 4/µl [0.5–18]), chronic myeloid leukemia (CML) (n=10 pts ; 144/µl [3.4–1930]), chronic myelomonocytic leukemia (CMML) (n=8 pts ; 8.6/µl [1–899]) while the number of CD34+ was similar to healthy volunteers in patients with myelodysplatic syndrome (MDS) (n=11 pts ; 2.4/µl [0,5–378]) or with non-malignant transitory polyglobulia or thrombocytosis (n=43 pts ; 2.5/µl [0.5-18], n=12 pts ; 2.5µl [1-14], respectively). No correlation was found between the JAK2 V617F or MPL W515 molecular status and the number of CD34+ cells. The determination of the CALR molecular status in the 134 JAK2 V617F negative cases is underway. The ROC curve analysis shows that a number of CD34+ <10/µl excludes the diagnosis of PMF with a negative predictive value of 99.5% (ROC curve parameters: area under the curve: 0.93 [0.89-0.98] ; p<0.001 ; sensibility : 97%, specificity : 90%, positive predictive value : 60%). Endogenous growth in cell culture distinguishes MPNs (number of colonies: PV 10 [3-34], ET 9 [0-49] and PMF 8 [0-53]) from other diseases such as atypical cases of MDS (no growth) with a specificity of 92% and a positive predictive value of 96%.
Summary and Conclusions: Determining the number of circulating CD34+ cells is a fast, simple, and standardized test when a diagnosis of MPN is suspected: its excellent negative predictive value excludes the diagnosis of PMF when <10/µl. It is a tool of particular interest when a bone marrow biopsy is not informative or difficult to realize. Cell culture can differentiate PMF from other diseases with a high number of circulating CD34+ cells.

PB1716

HYPOXIC AND NON-HYPOXIC PROANGIOGENIC STIMULI IN MYELOPROLIFERATIVE NEOPLASMS
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Background: The role of nitric oxide (NO) has not been entirely clarified in tumor angiogenesis. NO may induce synthesis of vascular endothelial growth factor (VEGF) via PI3K/Akt/HIF1α pathway, while VEGF enhances NO production by endothelial NO synthase (eNOS). Additionally, hipoxia seems to induce NO production. In contrast, high NO concentration leads to HIF1α down-regulation, thus decreasing VEGF production.
Aims: A target of this study is to correlate the expression of key angiogenic molecules VEGF, eNOS and HIF-1α in myeloproliferative neoplasms (MPN): polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) according to JAK2V617F mutation status.
Methods: The angiogenic factors VEGF, eNOS and HIF-1α mRNA levels are measured by real time PCR, while their protein expression is examined in granulocytes and CD34$^+$ hematopoietic progenitor cells of MPN using immunocytochemistry and Western blot. ELISA-based assay is used for the quantitative determination of eNOS concentrations in granulocytes of MPN.
Results: Using DNA sequencing, JAK2V617F mutation is detected in all PV and per 60% of both ET and PMF patients, out of total 198 MPN patients. eNOS mRNA levels are generally increased in granulocytes in comparison to CD34$^+$ hematopoietic progenitor cells of MPN patients. eNOS and HIF-1α mRNA levels are increased in MPNs *vs.* controls both in granulocytes and hematopoietic progenitors. Presence of JAK2V617F mutation exaggerated eNOS gene expression in hematopoietic progenitors, but not in granulocytes. JAK2V617F mutant allele burden increases HIF1α gene expression in hematopoietic progenitors of MPN patients and granulocytes of ET patients (3-5 fold). VEGF gene expression has the highest levels in hematopoietic progenitors of PMF patients, further on augmented by JAK2V617F allele burden. eNOS protein levels are increased in granulocytes of MPN patients, especially in ET heterozygous for JAK2V617F mutation. Analyses of protein expression of VEGF, eNOS and HIF1α in granulocytes show a significant increase in MPN patients in comparison to healthy controls. The activated HIF1α is significantly increased in nuclear fractions of PV and ET granulocytes (2 fold). In addition, there is a positive correlation between VEGF and eNOS as well as eNOS and HIF1α protein in MPN patients. Further on, the immunocytochemical analyses of granulocytes confirm that the percentage of VEGF- and eNOS-positive cells is generally increased in PV, ET and PMF JAK2 homozygous patients with a positive correlation among them. Moreover, in CD34$^+$ cells, VEGF- and HIF1α-positive cells are also increased in MPN patients with a positive correlation.
Summary and Conclusions: The presented triangle of angiogenic factors exhibit mostly elevated gene and protein expression in hematopoietic primitive and mature cells of MPN patients, confirming its importance in pathogenesis of disorders. The expression demonstrates tendency, but is not generally augmented by JAK2V617F mutation.

PB1717

JAK-STAT PATHWAY INHIBITOR DO NOT MODULATE DRUG TRANSPORTERS GENE EXPRESSIONS IN JAK2V617F POSITIVE CELL LINE
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Background: Discover of JAK2V617F mutation and the role of JAK-STAT pathway disregulation on myeloproliferative neoplasms (MPNs) pathogenesis lead to development of drugs targeted to inhibition of this pathway. The recent introduction of JAK-STAT pathway inhibitors in clinical practice represented a great advance in MPNs therapy. Little is known about the drug transporters expression effect on JAK inhibitors pharmacokinetics in MPN patients. The drug transporters play a role in drugs efflux (ABCB1 and ABCG2 molecules) or uptake (SLC22A1 and SLCO1A2 molecules) and could impact in patient response to therapy, causing variability of response and resistance to treatment.

Aims: To evaluate the effect of JAK inhibitors on ABCB1, ABCG2, SLC22A1 and SLCO1A2 mRNA expression in Caco-2, HepG2 and SET-2 cell lines.
Methods: Caco-2, HepG2 and SET-2 (JAK2V617F positive) cell lines were treated with 0 to 4 μM of *JAK Inhibitor I* (Merck/Calbiochem®, Darmstadt, Germany), a commercial ATP-competitive JAKs inhibitor, for 24 hours. After treatment, Caco-2 and HepG2 cells were submitted to viability and DNA fragmentation tests. JAK-STAT pathway inhibition was verified by STAT-5 phosphorylation inhibition (western blot) in SET-2 cells. The ABCB1, ABCG2, SLC22A1 and SLCO1A2 mRNA expressions were evaluated in Caco-2, HepG2 and SET-2 treated with *JAK Inhibitor I* by real time PCR, using Taqman® Gene Expression Assays (Applied Biosystems, Foster City/CA, USA).
Results: *JAK Inhibitor I* did not affect HepG2 cell line viability or DNA fragmentation when used in concentrations of 0 to 4 μM after 24h of cells treatment (P>0.05). In Caco-2 cells, the treatment with *JAK Inhibitor I* in a concentration of 2 μM and 4 μM by 24 hours leads to cell viability loss (P<0.001), although the DNA fragmentation were similar to control (P>0.05). The gene expression was analyzed in cell lines treated with *JAK Inhibitor I* in a concentration range of 0 to 1 μM. In SET-2, the treatment with 1 μM for 24 hours leads to JAK-STAT pathway inhibition. In HepG2, treatment with *JAK Inhibitor I* did not change ABCB1 and ABCG2 mRNA expression (P>0.05). Likewise, *ABCB1*, *ABCG2*, *SLC22A1* and *SLCO1A2* expressions were similar before and after *JAK Inhibitor I* treatment in Caco-2 and SET-2 cells lines (P>0.05).
Financing: FAPESP, process no. 2012/01081-1
Summary and Conclusions: Taken together, the data suggest that *JAK Inhibitor I* did not change the *ABCB1*, *ABCG2*, *SLC22A1* and *SLCO1A2 mRNA* expression in HepG2, Caco-2 and SET-2 cell lines. These findings indicate that JAK-STAT pathway inhibitors might not offer resistance potential by upregulation/downregulation of these drug transporters genes.

PB1718

GENETIC AND EPIGENETIC ABNORMALITIES IN MYELOPROLIFERATIVE NEOPLASMS
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Background: Recent studies have revealed a number of genetic and epigenetic alterations that likely contribute to disease pathogenesis and determine clinical outcome. In addition to the *JAK2 V617F* mutation, pathogenesis of myeloproliferative neoplasm (MPN) has evolved from a simple to a complex model, including a number of novel mutations. However, little is known about the frequencies and distribution patterns of genetic and epigenetic mutations in Korean population.
Aims: We investigated the frequencies and distributions of genetic and epigenetic mutations in Korean patients with MPN.
Methods: We investigated 75 patients with *BCL-ABL1* negative MPN. The *BCR-ABL1* rearrangement was assessed by reverse transcription polymerase chain reaction (RT-PCR). The *JAK2* V617F mutation was assessed by allele-specific PCR or direct DNA sequencing. We searched for mutational hot spots in *IDH1* (R132), *IDH2* (R140 and R172), *DNMT3A* (R882), *CBL* (exons 8 and 9), *EZH2* (exon 17~19), *WT1* (exons 7 and 9), *JAK2* exon 12, *MPL* (W515L), *ASXL1* (exon 13), *SH2B3* (exons 2, 7 and 8) and *SRSF2* (P95) using direct sequencing.
Results: In this study, *JAK2* V671F mutation was detected in 64% of *BCR-ABL1*-negative MPN. We found two *SH2B3* mutations in exon 8 which is recently reported mutational region in the Korean population study. Compared to the previous reports, the mutational hot spots in this study are not found except *SH2B3* (2.7%). However, we found 88% of *WT1* rs16754 and analyzed genotype-specific risks by comparing the genotype distribution. Especially, the individuals carrying mutant G alleles of *WT1* rs16754 found low prevalence of MPN for *WT1* rs16754 (Hazard ratio 0.103~0.648, p<0.05), therefore, G allele for *WT1* rs16754 might be risk reducing alleles for developing a MPN. There was no significant difference in overall survival between genotypes.
Summary and Conclusions: This study identified a very low prevalence of genetic and epigenetic mutations in the Korean patients with MPN except *SH2B3* .We observed a significant difference in allele and genotype frequencies of *WT1* rs16754 in an Asian population compared to a western population and wild allele G of *WT1* rs16754 might be a risk-reducing association for developing MPN in Korean population.

PB1719

OXIDATIVE STRESS IN MYELOPROLIFERATIVE NEOPLASMS
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Background: Oxidative stress is an invasive condition with increased reactive oxygen species, now recognized as an important characteristic of malignant disorders as well as their progression.
Aims: The aim of this study was to evaluate the role of oxidative stress induced genes and antioxidative enzymes in myeloproliferative neoplasms (MPN): polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).
Methods: Using microarray analysis we studied oxidative stress induced gene expression in $CD34^+$ hematopoietic progenitors of MPN patients. An assay for superoxide dismutases (SOD) was based on the ability of SOD to inhibit the autoxidation of epinephrine at alkaline pH. The activity of glutathione reductase (GR) was based on the capacity of GR to catalyze the reduction of oxidized to reduced glutathione using NADPH as a substrate. Glutathione peroxidase activity was assayed following the oxidation of NADPH with t-butyl-hydroperoxide as a substrate. The antioxidative enzymes activities were determined in red blood cells lyzate.
Results: Oxidative stress induced FBJ murine osteosarcoma viral oncogene homolog (FOS) gene expression was highly elevated in ET (3.1 fold) and PV (3.7 fold) comparing to healthy controls. FOS gene expression was higher in JAK2V617F heterozygous PV patients (4.1 fold). Less prominent expression was observed for kelch-like ECH-associated protein 1 (KEAP1) gene in PV (1.6 fold) and PMF (1.8 fold). Regarding ET patients, heme oxygenase 1 (HMOX1) gene was preferentially expressed in JAK2V617F positive ET (2.4 fold), significantly higher than in healthy controls (p<0.05). Also, HMOX1 was significantly more expressed in JAK2V617F homozygous PV patients (2.5 fold), than in healthy controls (p<0.05). In contrary, v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (MAFF) gene was significantly less expressed in JAK2 homozygous PV and PMF than in healthy controls (p<0.01). The levels of superoxide dismutase and glutathione peroxidase were the most abundant in PMF of MPN. The level of glutathione reductase was the highest in PV, not influenced by JAK2V617F mutant allele burden. However in PMF, the level of glutathione reductase was the most increased in JAK2 homozygous PMF and reduced in JAK2 negative patients, in opposite to glutathione peroxidase levels.
Summary and Conclusions: Presented oxidative stress induced gene expression demonstrated JAK2 dependence in MPN. The antioxidative enzymes activities were the most prominent in PMF. So, oxidative stress effects both at gene and enzyme levels revealed a variation specific for certain type of MPN.

PB1720

GENE EXPRESSION AND PROTEIN LEVELS OF MMPS, TIMPS AND SPARC AND THEIR RELATIONSHIP WITH PLASMA MARKERS OF ANGIOGENESIS IN MYELOFIBROSIS AND ESSENTIAL THROMBOCYTHEMIA PATIENTS
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Background: Myelofibrosis (MF) and essential thrombocythemia (ET) are myeloproliferative neoplasms (MPNs) resulting from acquired hematopoietic stem cell mutations. The molecular mechanisms involved in MPNs pathogenesis are not completely elucidated. It is known that angiogenesis plays an important role in hematological neoplasias progression and prognosis. Matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and secreted protein acidic and rich in cysteine (SPARC) are involved in tumor neoangiogenesis. In this context, evaluation of genes related with angiogenesis could be useful to better understand MPN pathobiology.
Aims: To investigate the mRNA and protein expressions of MMPs, TIMPs and SPARC and their relationship with plasma markers of angiogenesis (VEGFA and bFGF) in MF and ET patients.
Methods: Forty-three MPNs patients (MF=29 and ET=14) and 36 control were studied. The controls were matched with patients according age and gender. The MPN diagnosis was defined according World Health Organization criteria (2008). The MMP2, MMP9, TIMP1, TIMP2 and SPARC gene expression were evaluated in peripheral leukocytes and VEGFA and bFGF concentration were measured in plasma from MPN patients. mRNA gene expression and proteins levels were measured by real time PCR and Luminex technology, respectively.
Results: High MMP2, MMP9 and TIMP1 mRNA expression were observed just in MF patients compared to controls (P<0.05). High SPARC mRNA levels was detected in MF and ET patients when compared to controls (P<0.05). In MF group, SPARC mRNA expression was inversely associated with MMP9 expression (β=-0.436 ,R^2=0.3672 and P=0.002) and directly associated with TIMP1 and TIMP2 expression (β=0.625, R^2= 0.4984 and P<0.001; β=0.346, R^2=0.2510 and P=0.015). MF patients showed higher levels of VEGFA and bFGF then controls. Two models of multivariate linear regression were performed in MF

group. In model 1, it was observed that MMP9 levels were directly associated to dependent variable VEGFA levels (β=0.828, R^2=0.4556 and P=0.001). In model 2, MMP9 and TIMP1 levels were directly associated to dependent variable bFGF levels (β=0.866, R^2=0.4802 and P<0.001; β= 0.625, R^2=0.2641 and P=0.014, respectively). A third model of multivariate linear regression was performed with ET group and it was found that TIMP2 levels were directly associated to dependent variable bFGF levels (β=0.940, R^2=0.2641 and P=0.014). In controls, the independent variables MMPs, TIMPs and SPARC serum levels were not associated with each dependent variable (VEGFA or bFGF).
Summary and Conclusions: These preliminary results showed high SPARC mRNA expression in MF and ET. MMP9 and TIMP1 serum levels are associated with plasmatic VEGFA and bFGF in MF, whereas TIMP2 is associated with plasmatic bFGF in ET. These findings might be useful on understanding MF and ET pathobiology due to their role in angiogenesis. (FAPESP 2012/12957-5).

PB1721

WHOLE BLOOD PLATELET AGGREGATION IN PATIENTS WITH V617F JAK2 AND CALR MUTATIONS

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Background: Platelet activity changes could cause the development of clinical symptoms of chronic myeloproliferative neoplasms (CMN). As we previously found, the mutation in the JAK2 gene leads to increase all parameters of whole blood aggregation (Olkhovskiy, Stolyar; 2013) and had weak or non significant correlation with platelet counts. In december 2013 two research groups independently demonstrated more than half of all cases of JAK2 negative, associated with a novel somatic mutations in the gene of CALR (Nangalia J. *et al.* and Klampfl T. *et al.*). CALR mutations associated with more marked thrombocytosis, but lower risk of thrombosis.
Aims: To measure platelet aggregation in whole blood in patients with V617F JAK2 and CALR mutations and evaluate the relationship between platelets number and aggregation in patients with CMN.
Methods: 120 patients: 51 women 55.6 (31-74) age and 69 men 55.5 (27-87) age with suspicion a CMN were examined. CALR gene deletion was determined by PCR and followed by electrophoresis. CALR insertion and mutations in JAK2 and MPL genes was detected by RT-PCR (IQ5, BioRad). Platelet aggregation in whole blood was measured in 67 healthy subjects and 91 patients with CMN by impedance method at Chronolog -700, with ADP inducer (5 μM).
Results: Total of 15 patients was found with a deletion in CALR gene and 5 with insertion in it. Including CALR mutation was found in 43% of all 42 patients without mutations V617F JAK2 and MPL but with thrombocytosis more than 500 000 per μl of blood. The parameters of platelet aggregation was increased in all patients with thrombocytosis and independent of the availability of mutations in JAK2 or CALR (Table 1). There is no correlation between aggregation and platelet counts both in general for the entire group of patients with CMN, and separately in subgroups with different mutations.

Table 1. Parameters impedance platelet aggregation (Me; Q25-Q75) in whole blood of healthy donors and patients with chronic myeloproliferative neoplasms (CMN) with thrombocytosis more than 500 000 per μl.

Summary and Conclusions: Increasing the platelet counts corresponds to the degree of increase of it aggregation in whole blood samples in patients with CMN. However, differences in the degree of thrombocytosis depend on JAK2 or CAL mutations to a greater extent than platelet aggregation. Functional activity of platelets in whole blood CMN rather determined by their quality than quantity.

PB1722

OXIDATIVE STATUS IN ESSENTIAL THROMBOCYTHEMIA

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Background: Oxidative stress (OS) is due to an excess of pro-oxidant species, like a Reactive Oxygen Species (ROS) and not counterbalanced by an adequate endogenous antioxidant molecules such as Reduced Glutathione (GSH). It has been demonstrated that it is involved in pathogenesis of atherothrombotic disease. Essential Thrombocythemia (ET), a BCR-ABL1-negative MPN, is characterised by increased platelet production and sustained thrombocytosis which can lead to thrombotic or hemorrhagic complications.
Aims: To investigate oxidative status in ET.
Methods: Thirty ET patients (13M/17F, age range 29-66 years) were recruited at our Hospital and compared to 26 healthy volunteers matched by age and gender (12M/14F, age range 28-69). All participants gave their informed consent. Serum ROS and whole blood GSH levels were measured by using a spectrophotometric method (dROMs test, Diacron International, Grosseto, Italy) and the HPLC method (Chromsystems Instruments & Chemicals, Munich, Germany) respectively. Reduced/oxidised glutathione ratio (GSH/GSSG) was also calculated. JAK2 mutational analysis was confirmed by direct sequencing (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Warrington, UK); for quantitative analysis of the allele burden of the JAK2 V617F mutation, we had to perform RQ-PCR using JAK2 MutaQuant™ (Ipsogen Inc., New Haven, CT). We compared levels of markers of OS between ET patients and healthy subjects using multiple linear regression models adjusted for gender, age (continuous), smoking (never, former, current), dyslipidemia (yes, no) and treatment with acetylsalicylc acid (yes, no).
Results: Oxidative stress results of our study group are reported in Table 1. No significant differences of oxidative status parameters were found between JAK2 positive and JAK2 negative groups.

Table 1. Results are expressed as mean (standard deviation).

*From multiple regression model.

Summary and Conclusions: Even if GSH levels were unusually augmented in ET, patients showed significantly increased levels of GSSG as well as a reduced GSH/GSSG ratio compared to the controls in place, therefore underlying that ET patients showed an altered oxidative status. Further studies are necessary to investigate if oxidative stress plays a role in ET thrombotic complications.

PB1723

8P11 MYELOPROLIFERATIVE SYNDROME: DIAGNOSTIC CHALLENGES AND PITFALLS

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Background: 8p11 myeloproliferative syndrome (EMS) is a very rare clinicopathological entity which is characterized by the appearance of a myeloproliferative neoplasm (MPN) in the bone marrow, peripheral lymphadenopathy, usually caused by a T or B lymphoblastic lymphoma/leukemia, a peripheral blood eosinophilia in the absence of basophilia, and a reciprocal translocation involving chromosome 8p11. Disruption of the fibroblast growth factor receptor 1 (*FGFR1*) gene at the 8p11-12 chromosomal locus, and creation of novel fusion genes and chimeric proteins result in constitutive activation of FGFR1 tyrosine kinase which, consequently, promotes activation of downstream signaling pathway resulting in oncogenesis.
Aims: We want to present a patient with a very rare disease such as EMS, and underline an importance of comprehensive diagnostics in patients presenting with eosinophilia in peripheral blood, lymphadenopathy and pattern of MPN in bone marrow biopsy specimens.
Methods: We analyzed medical records of patient with EMS.
Results: We described 22 year old male patient with unusual clinical presentation of EMS. Namely, initially he presented with prolonged epistaxis.

Complete blood count showed elevated hemoglobin (17,7g/dl), thrombocytopenia (98x10^9/l) and a leukocytosis (57x10^9/l). The white cell differential revealed an eosinophilia of 9%, with a leukoerythroblastic picture and a left shift (neutrophils 51%, bands 16%, metamyelocytes 3%, myelocytes 1%, promyelocytes 3%, lymphocytes 7%, monocytes 9%). Bone marrow aspirate and biopsy findings corresponded with the presence of a myeloproliferative neoplasm while cytogenetic analysis revealed t(8;13)(p11q12). After that ZMYM2-FGFR1 in-frame fusion was confirmed at the molecular level. Immediately after establishing diagnosis of MPN generalized lymphadenopathy was developed. Histopathologic examination of lymph node sample confirmed the diagnosis of a T cell lymphoblastic lymphoma without bone marrow involvement. Four cycles of Hyper CVAD chemotherapy was applied with effect of complete morphological and cytogenetic remission and resolution of lymphadenopathy. During the preparation for allogeneic (allo) stem cell transplant (SCT) from his HLA matched sister, 4 weeks after completion of chemotherapy, he developed a peripheral blood monocytosis and eosinophilia, along with a left shift, but without blasts. A bone marrow aspiration was performed confirming the recurrence of the myeloproliferative neoplasm. The repeat cytogenetic analysis on this marrow sample showed t(8;13) accompanied by complex numerical and structural aberrations. Because of generalized lymphadenopathy recurrence, lymph node biopsy was repeated with confirming previous diagnosis of T cell lymphoblastic lymphoma. The patient underwent an allo SCT and subsequently achieved a complete remission.

Summary and Conclusions: Patients with MPN and translocations involving chromosome 8 need to be carefully evaluated for EMS. However, having in mind very aggressive clinical course of EMS allogeneic SCT is the only potential curative option.

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PB1724

THE MPN FATIGUE PROJECT STAGE 2: UNDERSTANDING THE ROLE OF COMORBID CONDITIONS AND FATIGUE-ALLEVIATING STRATEGIES IN MPN FATIGUE

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Background: The Philadelphia chromosome negative chronic myeloproliferative neoplasms (MPNs) are a group of diseases characterized by myeloid clonal lineage with hypersensitivity to or independence from cytokine regulation. These disorders include essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis. Patients with these disorders suffer from a unique constellation of symptoms which includes severe, prevalent, and debilitating fatigue. Although the cause of this fatigue is not entirely clear, it is thought to arise from significant cytokine dysregulation and impaired hematopoiesis. Overall, it is a predominant cause of reduced quality of life and impaired functioning among MPN patients. Given the unique constellation of disease-related fatigue, this symptom has become known as MPN-Related fatigue (MRF). We have recently published the results of the initial phase I project initiated and hosted by *MPN Forum* evaluating fatigue-relieving strategies among MPN patients (Blood 2013;a1595). Among the 879 online respondents, patients most often employed exercise, diet, social interaction, nutrition, rest, and relaxation techniques to combat fatigue. Use of non-prescription energy-enhancing supplementation was common. Pharmacologic interventions included MPN-specific treatments, steroids, noradrenergic stimulants, prescription vitamin supplements, and erythropoietin analogues. Despite our previous efforts, little is known to date about the degree of relief from specific fatigue-alleviating strategies and the contribution of comorbid conditions to MRF.

Aims: The aim of this study is to evaluate the role of comorbidities and fatigue-alleviating strategies in MRF.

Methods: Our survey was developed using a compilation of validated questionnaires along with items developed by a team of investigators for specific use in this study. In total, a 70-item internet-based survey was developed and hosted by the Mayo Clinic Survey Research Center. The survey was promoted online via multiple MPN-related webpages including the *MPN Forum*, *MPN Net*, *MPN Research Foundation*, and *MPN Voice* during late February to March of 2014. Surveyed data included disease demographics such as splenomegaly, thrombosis, hemorrhage transfusions, medications, and phlebotomies. The MPN-SAF including the 10-item brief fatigue inventory was used to assess disease burden (Blood. 2011 Jul 14;118(2):401-8). Comorbid conditions were assessed, including hypothyroidism, anemias, sleep disturbances, obesity, and heart and lung disorders. Mental health was assessed using the POMS-short form (J Nerv Ment Dis. 1979;167(10):612-4), PHQ-2 (Ann Fam Med. 2010 . 8(4): 348–353) and MHI-5 (J Consult Clin Psychol. 1983. 51;730-742). Participant's attainable metabolic equivalents were also assessed as a measure of functional status. Fatigue was characterized using a set of questions to evaluate timing, frequency, duration, triggers, and impact on daily activities. Interventional strategies to cope with fatigue were complied based on responses from our previous study. Patients were queried as to whether they had utilized individual interventions and were asked to rate the success of each intervention on a 1 (not at all successful) to 5 (very successful) scale. All survey and study protocols were approved though the Mayo Clinic IRB prior to survey implementation. We anticipate a sample size of 850 participants.

Results: This trial began open online enrollment in late February 2014 and remains in the recruitment phase. Our updated preliminary data will be presented at the EHA 2014 meeting.

Summary and Conclusions: Overall patients with MPN experience an exclusive pattern of disabling fatigue. Further study of MRF will 1) help to better characterize this fatigue, 2) improve our knowledge on comorbid conditions that may be contributing to fatigue severity and prevalence, and 3) glean knowledge on non-pharmacologic interventions can be utilized to alleviate fatigue. We plan to use the results of this intervention to inform an interventional trial which pairs standard MPN therapies with integrational non-pharmacologic fatigue reduction techniques.

PB1725

Abstract withdrawn

PB1726

ORAL IRON-CHELATION WITH DEFERASIROX IN PRIMARY MYELOFIBROSIS: A SINGLE CENTER EXPERIENCE.

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Background: Transfusion-induced iron overload is a frequent problem that clinicians have to face in the treatment of patients affected by myelodysplastic syndrome (MDS), resulting in multiple organ failures, with significant hepatic and cardiac involvement. Deferasirox (DSX) is the principal option currently available for chelation therapy, but the expertise in the management of iron overload in patients with primary myelofibrosis (MF) is limited.
Aims: We wanted to analyze our experience regarding iron-chelation therapy (ICT) with DSX in MF patients in order to evaluate safety, efficacy and hematological responses.
Methods: We identified in our MPN Ph- database, 154 patients affected by primary MF referred to our Division from 1990 to 2012: of them, 47 patients (30,5%) presented TD anemia at onset or during follow-up of disease; we analyzed our initial experience in 10 patients with MF treated with oral DSX from September 2010 to May 2013, starting from dose of 10 mg/kg/day, up to the maximum tolerated dose. Criteria for initiating ICT were an estimated life expectancy of at least 1 year and at least one of: ferritin level > 1000 microg/L, transfusion of > 20 RBC units, or organ dysfunction from iron overload, refractoriness and/or absence of concomitant therapy with stimulant erithropoietic agents.
Results: The median values of ferritin pre-treatment was 2280 microg/l and the median red blood cells (RBC) transfused was 2 units/month. The median dose tolerated of the DSX was 750 mg/day (10 mg/kg/day), with 3 transient interruption of treatment for grade 2 of drug-related adverse events (AEs), in particular 1 rash, 1 diarrhea and 1 transaminitis; 3 patients experienced a definitive discontinuation of the drug for grade 3 AEs (1 hepatitis, 2 renal failure). 2 patients interrupted DSX for leukemic evolution of disease. Overall, only 4/10 patients (40%) continued permanently the oral ICT. According to IWG criteria 2006, erythroid responses with DSX, comprising reductions in transfusion requirements or increases in hemoglobin levels, were observed in 4/10 patients (40%), with 2 patients (20%) who obtained transfusion independence. Median changes in serum ferritin levels were greater in hematologic responders (HR) compared with non-responders (NR) at any time from start of ICT, in particular already at 6 months, as showed in Figure 1. A trend of a better overall survival was present in HR respect to NR (90.7 *versus* 35 months, respectively), probably due to a longer time exposure to DSX (14.5 *versus* 6.5 months) and a lower incidence of AEs in HR. Moreover, the incidence of definitive discontinuation of ICT for AEs was higher in NR patients (66% *vs* 25%).

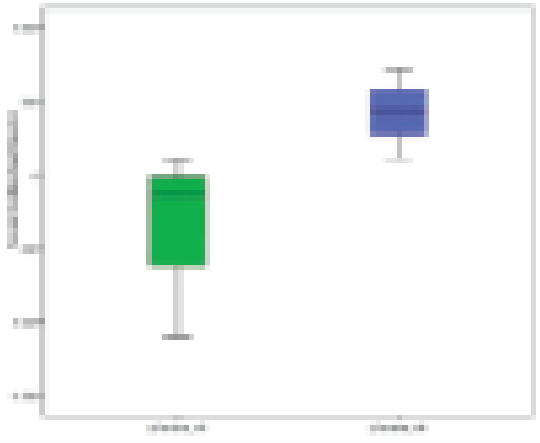

Figure 1.

Summary and Conclusions: Our preliminary data open new insights regarding the benefit of ICT not only in MDS, but also in MF patients with the possibility to obtain a partial or complete erythroid response, overall in 40% of patients. Moreover, there seems to be a potential positive impact of the ICT on survival in responding patients. However, the tolerability of the drug seems to be lower compared to MDS patients, both in terms of lower median tolerated dose (10 mg/kg/day) that for higher frequency of discontinuance for drug related AE (40%). The biological mechanism of action of DSX in this specific myeloproliferative setting, through an independent NF-κB inhibition and not based on the reactive oxygen species scavenging properties of the drug, could explain a direct action on the malignant clone during *in vivo* therapy, inducing a hematopoietic improvement, but further investigations are required.

PB1727

CALR MUTATIONS ARE RARE IN CHILDREN WITH ESSENTIAL THROMBOCYTHEMIA

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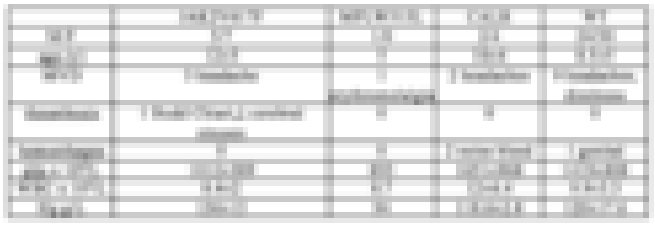
Background: Sporadic essential thrombocythemia (ET) is rare in children; they display rarely than adults a clonal pattern. *JAK2*V617F mutation occurs in 20-25% of cases and *MPL*W515L has been described only in 1 child. Therefore, diagnosis of ET in children is based on exclusion criteria in most cases. Somatic mutations in calreticuline gene (CALR) have been recently described in 80% of adult ET wild-type for *JAK2*V617F, thus reducing the rate of non-clonal cases in adults to 10-15%.
Aims: Research of CALR mutations in a large cohort of children with ET.
Methods: CALR mutations were searched in 72 children with a clinical diagnosis of ET, who were consecutively referred to our laboratory from 11 Italian Hematological Pediatric Centers. Platelet count >450x10^9/L was observed for at least 6 months; reactive thrombocytosis,familial patterns and BCR/ABL rearrangements were excluded in all cases. Bone marrow aspirates were performed in all cases; 40 cases had a histological picture consistent with ET. None of the children received any cytoreductive treatment before the study. The study was approved by the Ethic Committee of the Padua Hospital and informed consent was provided according to the Helsinki Declaration. *JAK2*V617F mutation was searched by quantitative real time-polymerase chain reaction assay. *MPL* mutations and CALR mutations were searched by direct sequencing.Comparison between means was made with ANOVA followed by Bonferroni post hoc test.
Results: 11 children (15.2%) harbour *JAK2*V617F and 1 (1.4%) *MPL*W515L mutations. Within the remaining 60 cases, 6 had one CALR abnormality (8.3% of total population & 10% of *JAK2/MPL* negative cases) and 54 (75%) were wild type (WT). In summary, 25% of patients had a clonal mutation as identified by current molecular studies: their clinical features are summarized in the Table 1.

Table 1.



No significant statistical differences were found among the different groups.

Summary and Conclusions: We present the first 6 cases of children with ET carrying a *CALR* mutations,albeit in a smaller proportion of cases than in adults. Other Authors did not found any mutations within 6 children with ET. In our large cohort of children,75% were WT for all known mutations: the proportion of clonal cases is smaller in children with clinical diagnosis of ET, than in adults. The absence of significant clinical or laboratory differences among the children with different molecular pattern confirms that ET in children is, at least in part, a different entity, may be not deserving the term myeloproliferative neoplasm. The children might suffer from an undefined thrombocytosis and their diagnostic process and follow-up need to be further established. In any case, the use of cytoreductive drugs in children with an increased platelet count has to be cautiously evaluated and deferred as much as possible.

PB1728

LEUKOCYTOSIS DURING DISEASE COURSE FOR PREDICTING THROMBOTIC/HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background: Recent clinical and preclinical findings suggest that leukocytosis seems to be related to thrombotic and hemorrhagic complications in polycythemia vera (PV) and essential thrombocythemia (ET).
Aims: The purpose of this study was to evaluate whether white blood cell (WBC) burden during the disease course affects the incidence of thrombotic or hemorrhagic events in PV and ET.
Methods: We have retrospectively analyzed PV and ET patients diagnosed and treated at Seoul National University Bundang Hospital between Jan. 2004 and Aug. 2012. To estimate the WBC burden per patient during the disease course, we calculated time-weighted averages of complete blood cell count (CBC) values of whole follow-up period per each patient. The calculated values were compared between groups with events and without events. Also the time-weighted average values of the 3 months period before the events were compared with those of the whole follow-up period in each individual patient with events.
Results: A total of 102 patients with PV (33 patients, 32.4%) and ET (69 patients, 67.6%) were analyzed. The median follow-up period was 54 months (range, 2-163). Median age at diagnosis was 64 years (range, 24-87); 52.9% were male. Thirty-five events (16 thrombotic, 19 hemorrhagic) occurred in 29 patients. The time-weighted averages of WBC were significantly higher in patients with events (12,015x10^3μL) compared to patients without any events (9,567x10^3μL)(*P*=0.003). There were no statistically significant differences in hemoglobin or platelet counts. The difference in time-weighted averages of WBCs between groups with or without events was more prominent in the subgroup of patients with ET (8,577x10^3μL *vs.* 11,674x10^3μL, *P*=0.007) than with PV, and with hemorrhage (9,567x10^3μL *vs.* 12,823x10^3μL, *P*=0.003) than with thrombosis. In the patients with events, the time-weighted average of WBCs in the 3 months period before the event (16,767x10^3μL) was significantly higher than that of the whole follow-up period (12,015x10^3μL)(*P*=0.002). The time-weighted average of platelets was also significantly higher in the 3 months period, but no significant difference in the average of Hb was observed.
Summary and Conclusions: WBC burden during the disease course seems to be related to higher occurrence of thrombotic and hemorrhagic events in PV and ET patients.

PB1729

BONE MANIFESTATIONS IN PATIENTS WITH MYELOFIBROSIS - A CROSS-SECTIONAL STUDY USING DXA AND HR-PQCT

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Background: In patients with myelofibrosis, osteosclerotic changes appear in the bone tissue as the result of growing and thickening of the bone trabeculae. Despite this bone formation a recent nationwide population-based cohort study showed that patients with chronic myeloproliferative neoplasms (CMPN) in general have a higher rate and risk of hip fractures.
Aims: We conducted a cross-sectional study to evaluate bone structure MF patients using conventional DXA and HR-pQCT.
Methods: Patients who meet the diagnostic criteria of primary myelofibrosis or myelofibrosis secondary to another CMPN according to WHO 2010-criteria with *International Classification of Diseases,* 10th revision, from the department of haematology, Odense University Hospital, Denmark were included. Areal Bone mineral density was assessed by DXA (duel energy X-ray absorption), and 3D assessment of Bone geometry, volumetric BMD, and micro-architecture were measured using XtremeCT (High-resolution peripheral quantitative CT). Data are compared with healthy volunteers matched on age, sex, and height, in a 1:1 ratio. Blood samples were analyzes for markers of bone formation including PINP1 (procollagen type I N-terminal pro-peptide) and measurements of haemoglobin, leucocytes, thrombocytes, lactate dehydrogenase, potassium, sodium, creatinine, alkaline phosphatase, alanine transaminase, ionized calcium, thyroid-stimulating hormone, parathyroid-stimulating hormone (PTH) and 25-hydroxyvitamin (25(OH)D). Data are presented as mean-values including 95% confidence intervals (CI). Results from patients and controls are compared using t-test and the statistical significance level set at p<0.05. All diagnostic bone marrow biopsies (BMB) were reviewed by a hematopathologist, confirming diagnosis, grading of fibrosis and presence of osteosclerotic changes. p<0.05. Informed consent was obtained.
Results: 20 MF patients were included, mean age 69.8 (95% CI: 66.3-73.4). 11 patients had grade MF 1-2 and 9 patients had grade MF-3 fibrosis in their bone marrow biopsy, while osteosclerosis including focal ostesclerosis was observed in 12 patients. The patients had higher BMD in spine 1.10 (0.93-1.08) *vs.* 0.93 (0.87-1.00), but not of the hip 0.93 (0.85-1.02) *vs.* 0.90 (0.82-0.98). HR-pQCT showed consistently increased bone mass, particular trabecular volumetric BMD, trabecular BV/TV, and trabecular number, although statistical significance was not reached. Haemoglobin was below normal range (F: 11.6 (10.2-12.9) g/dL, ref: 11.8-15.3 g/dL; M: 10.8 (9.5-12.2) g/dL, ref: 13.4-16.9 g/dL) and lactate dehydrogenase is above normal range (374.11(269.89 - 478.34) U/L, ref: 155-255 U/L) as expected for a patient with myelofibrosis. 10 patients were below reference level of 25(OH)D, and respectively 7 patients had increased levels of PTH. Remaining biochemical measurements were within normal range. PINP was increased in patients (Figure 1).

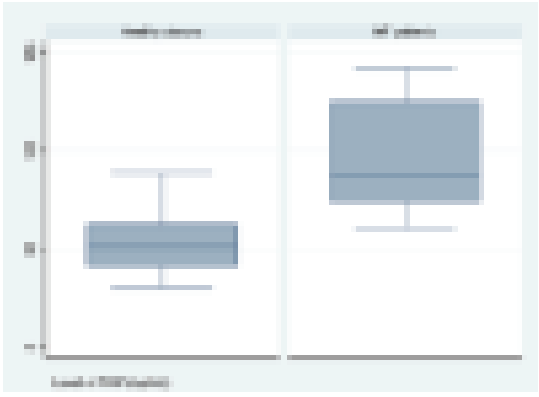

Figure 1.

Summary and Conclusions: This study demonstrated elevated level of PINP in MF patients indicating increased formation rate of collagen. Deficient level of 25(OH)D is common in the Nordic countries among the elder population. The DXA and HR-pQCT results indicate that these patients have increased trabecular bone mass, but did not reach statistical significance. Myelofibrosis is a rare disease and the number of patients included was small.

PB1730

RETROSPECTIVE ANALYSIS OF 260 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: IWG-MRT (IPSET MODEL; PASSAMONTI ET AL, BLOOD 2012) PROGNOSTIC SCORE IS A GOOD PREDICTOR OF SURVIVAL

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Background: Life expectancy in Essential Thrombocythemia (ET) patients is generally considered similar to that of normal population of the same age, at least during the first decade from diagnosis. The main causes of death are thrombotic events and evolution into myelofibrotic phase or secondary myelodisplasia/acute leukemia. Recently, in a large study published by IWG-MRT (Passamonti et al, Blood 2012), authors developed a widely applicable prognostic model, delineating three different prognostic risk groups by age, leukocyte count and history of thrombosis (IPSET model).
Aims: In this study we tested the IPSET model in a retrospective cohort of 260 ET patients defined according to WHO criteria, diagnosed from 1974 to 2013 in a single Italian haematological centre (Turin).
Methods: We analysed 260 ET patients. According to the IPSET model, patients were divided into three groups: adverse points are assigned to age ≥60 years (2 points), leukocyte count ≥11 x109/l (1 point) and venous thrombosis (1 point). In this way patients were divided in low-risk(0 points), intermediate-risk (1 or 2 points) or high-risk (≥3 points) groups. We evaluated overall survival (OS) from diagnosis to death from any causes by Kaplan Meyer method; Hazard Ratio were estimated with Cox Models.
Results: Characteristics at diagnosis were as follows: median age was 59 years, 144 patients (55%) were female, cardiovascular risk factors and palpable splenomegaly were observed in 158 (61%) and 42 (16%) patients, respectively. Forty-one (16%) patients showed leukocytosis (≥11 x 109/L) whereas elevated HCT (≥45%) was present in 51 out of 125 evaluated patients (41%). Forty-seven (18%) patients had previous thrombotic events. As expected, approximately 119 patients (46%) resulted positive for JAK2 mutations (V617F). With a median follow-up of 73 months we observed 14 deaths (5.3%), 27 myelofibrotic transformations (10%) and 4 leukemic evolution (2%). The incidence of post diagnosis arterial and/or venous thrombosis were 8.8%, in the sub-group of 125 patients evaluated for HCT. The incidence was higher but not significantly (p=0.742) in patients with HTC>45 (9.8% *vs* 8.1%) than in patients with HTC<45%. OS at 6 years is 97.5% (95%CI: 93.5-99.1). The IPSET prognostic model displayed discrimination between high-risk (n= 95; 6-year survival 93.3%; *vs* low-risk HR 5.7; 95%CI: 0.9-34.7), intermediate-risk (n= 124; 6-year survival 96.0%;

vs low-risk HR 4.7; 95%CI: 1.3-17.6) and low-risk (n=91; 6-year survival 100%) groups. The C-statistic was 0.6814. (Figure 1).
Summary and Conclusions: In our retrospective cohort of ET patients, IWG-MRT (IPSET model) prognostic score was confirmed as a good predictor of survival. Elevated HCT seems to represent an additional thrombotic risk factor, besides in Polycythemia Vera, also in ET patients. This promising results need to be confirmed in larger prospective studies.

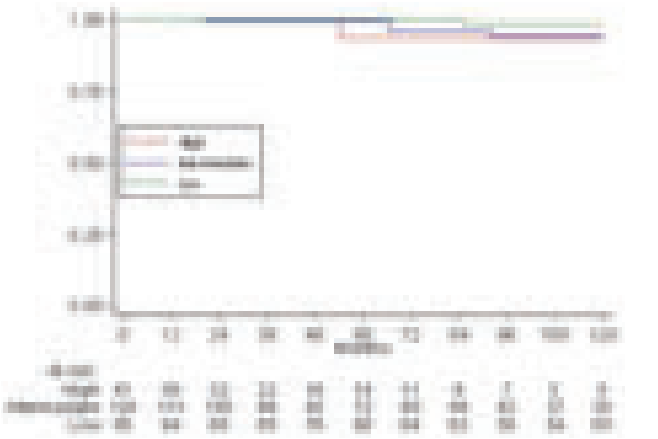

Figure 1.

PB1731

SECOND MALIGNANT TUMORS ASSOCIATED WITH HEMATOLOGICAL MALIGNANCIES - FREQUENCY AND SIGNIFICANCE
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Background: Malignant diseases, regardless of the affected organs, have a common etiopathogenic background.
Aims: If malignancies involve a genetic susceptibility or if malignant diseases pathogenesis has common links or if a cancer therapy promotes the development of second malignancies, it is expected that the development of a second malignancy in patients with malignant disease to be more common as in the general population.
Methods: We've analyzed (retrospective and prospective) the frequency of the association of two different primary malignancies in patients with malignant hemopathies diagnosed in our clinic over the past five years. 486 patients have been reviewed: 261 (53.7%) females and 226 (46.3%) males. The average age was 59.9 years (patients between 18-98 years). Of these patients 301 (62%) had lymphoproliferative disorders whereas 185 (38%) had myeloproliferative disorders.
Results: A second malignancy has been identified in 68 patients: 32 males and 36 females, with an average age of 67.6 years. The overall frequency of the second neoplasm was 13.99%, with no significant difference between males and females (14.2% and 13.8% respectively). The frequencies of the second neoplasm in patients with lymphoproliferative disorders and myeloproliferative disorders were 13.82% and 15.13% respectively. We emphasize that in subjects with RAEB - 1 MDS and RAEB - 2 MDS, 17.5% had a history of neoplasms that required proapoptotic chemotherapy and radiation. The identified second primary malignancies associated with malignant hemopathies and their frequencies were: cutaneous cancers (melanoma, squamous-epidermoid carcinoma, basal cell carcinoma) - 30%, neoplasms of genital / breast area (carcinoma of prostate, carcinoma of the cervix , malignant ovarian tumors, breast carcinoma) – 27%, gastrointestinal carcinomas - 15%, neuroendocrine neoplasia – 7%, pancreas carcinoma – 7.5%, renal cell and bladder carcinoma 5%, connective tissue sarcomas - 4%, bronchogenic carcinoma – 3%, a second different hematological neoplasia - 1.5%.
Summary and Conclusions: A significant part (13.99%) of our patients with malignant hemopathies developed a second primary neoplasia. There was no significant difference between males and females regarding the frequency of the second neoplasm. Radiation / radiotherapy, chemotherapy and anti CD20 antibody therapy seem to be associated with the induction / unmasking of a second neoplasia. Regarding the genetic predisposition we note down that none of the eight patients (4 with CLL, 2 with NHML, and one with HD) diagnosed in our clinic with familial malignant hemopathies developed a second primary neoplasm. These data sustain the theory of the existence of a common ethiopathogeny of neoplastic afflictions.

PB1732

IMPACT OF MOLECULAR REMISSION ON CLINICAL OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN 14 PATIENTS WITH MYELOFIBROSIS
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Background: Myelofibrosis (MF) may occur as a primary disease (PMF) or as evolution of previous essential thrombocythemia (post-ET MF) or polycythemia vera (post-PV MF). Allogeneic hemopoietic stem cell transplantation (allo-SCT) is currently the only curative option for these patients, although associated with high rate of transplant-related mortality. Patients with primary or secondary MF may carry the *JAK2* V617F mutation, mutations in *CALR* gene or, more rarely, mutations within the *MPL* gene; few patients do not carry any mutation (triple-negative patients).
Aims: To evaluate the impact of mutational status (*JAK2*, *MPL*, *CALR*) and molecular remission on clinical outcome in patients with MF undergoing allo-SCT.
Methods: Diagnosis of PMF, post-ET MF and post-PV MF was made according to WHO 2008 criteria and International Working Group on Myelofibrosis Research and Treatment (IWG-MRT) criteria. Complete remission (CR) was defined according to the revised IWG-MRT and European Leukemia Net (ELN) response criteria. The absence of minimal residual disease was assessed by chimerism analysis (through microsatellites or FISH evaluation). Mutations of *JAK2*, *MPL* and *CALR* were assessed by allele specific qPCR, high resolution melting and Sanger sequencing, respectively.
Results: We analyzed 14 patients with MF (7 PMF, 4 post-ET MF, 3 post-PV MF) who received allo-SCT. In detail, 2 patients were *CALR* mutated, 9 patients were *JAK2* V617F mutated, 1 patient carried *MPL* W515A mutation, whereas 2 patients were triple-negative. The median age was 48.7 years (20.6-58.3). The median time from MF diagnosis to transplant was 53 months (range 8-118). The dynamic score (DIPSS) at time of allo-SCT was intermediate-1 in 2 patients, intermediate-2 in 10 patients and high in 2 patients. At the time of analysis 4 of 14 (28.5%) patients were dead. We did not find an impact of mutational status (*JAK2*, *MPL*, *CALR*) on overall survival (*P* 0.568) and achievement of CR (*P* 0.491), perhaps due to the low number of patients. The assessment of molecular remission after allo-SCT was feasible in 11 of 14 patients as one patient lacked a molecular marker (triple-negative) and two patients died early after allo-SCT for transplant related complications. Of the 11 evaluable patients, 9 patients achieved molecular remission: 7 were in CR according to the revised IWG-MRT and ELN criteria whereas 2 showed an incomplete blood counts recovery, perhaps due to the short time elapsed from allo-SCT. The remaining 2 patients who still carried the molecular marker after allo-SCT showed persistence of disease with a 100% pre-allo-SCT chimerism. Molecular response after allo-SCT was significantly correlated with the achievement of a clinical CR (*P* 0.028).
Summary and Conclusions: Driver founding mutations in myelofibrosis may be used to evaluate minimal residual disease after allo-SCT and their eradication appears to be predictive of clinical complete remission.

PB1733

Abstract withdrawn

PB1734

SYMPTOMS TRIAL UPDATE: SYMPTOMS YIELDED IN MYELOFIBROSIS PATIENTS AFTER TRANSPLANT AS OBJECTIFIED BY THE MPN-SAF TSS
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Background: Patients with myelofibrosis (MF) including post-polycythemia vera (PV) and post-essential thrombocythemia (ET) MF, suffer from debilitating symptoms profiles, splenomegaly, cytopenias, reduced quality of life and shortened life expectancies. With standard medical therapy primarily focused on symptom management, allogeneic stem cell transplant (ASCT) remains the only available curative option for intermediate to high-risk disease. The

procedure, however, is associated with significant risks, symptomatology, financial burdens and impact on quality of life. To date, no study has investigated the MF symptom profile and its evolution in patients who undergo stem cell transplant.

Aims: This prospective clinical trial aims to asses the symptomatic burden in primary and post-PV/ET MF patients undergoing ASCT using the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS), FACT-BMT, Global Impression of Change and financial assessment scoring tools prior to and post-allogeneic stem cell transplant.

Methods: This international study was approved by the Mayo Clinic Institutional Review Board and remains in active accrual. A total of 110 participants with PMF or post-ET/PV MF planning to undergo allogeneic ASCT will be prospectively enrolled. Patients will be randomized to the ASCT arm *vs.* the Standard Medical Therapy (SMT) arm based on matched or mismatched related/unrelated status. Patients undergoing ASCT will complete the MPN-SAF TSS, Global Impression of Change, FACT-BMT and financial questionnaire items pre-transplant (day 0) and post-transplant on days 30, 100 and 365. Patients randomized to SMT will complete the same packets at similar time points with day 0 representing completion of the first packet. Paired t-tests will be utilized to compare pre- and post-transplant results between MPN-SAF TSS and other scoring tools at each post-baseline time point. Global Impression of Change items will be used to assess responsiveness of the MPN-SAF TSS in each study. Descriptive and graphical techniques will include mean plots and stream plots from each continuous or ordinal scale/subscale item.

Results: Since trial initiation (June, 2012), seven institutions have been enlisted to participate (Mayo Clinic, AZ USA; MD Anderson, Texas USA; University Medical Center, Hamburg Germany; Memorial Sloan Kettering, NY USA; Medical College of Wisconsin, Wisconsin USA; Princess Margaret Cancer Center, Toronto, Ontario Canada). A total of 9 patients have been enrolled with data available on 7 patients. Two patients have been randomized to HSCT and 5 patients have been randomized to SMT. Baseline patient demographic and clinical data is listed in Table 1. Additional patient information will be presented at the conference.

Summary and Conclusions: The symptomatic burden associated with allogeneic stem cell transplant in myeloproliferative neoplasm patients remains uninvestigated. The MPN-QOL Study Group aims to evaluate improvements in MPN symptoms and quality of life via the use of MPN-specific, validated survey instruments in this unique population. The study remains in active recruitment with updated results to be presented at the conference.

Table 1. SYMPTOMS patient demographics and clinical data.

PB1735

HYDROXYCARBAMIDE AS A RISK FACTOR FOR TRANSFORMATION TO ACUTE MYELOID LEUKEMIA IN ESSENTIAL THROMBOCYTHEMIA: SINGLE CENTER LONG TERM FOLLOW UP STUDY

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Background: Essential thrombocythemia (ET) is a classical myeloproliferative neoplasm characterized by sustained clonal thrombocythosis and bone marrow megakaryocytic hyperplasia with a prolonged clinical course and a near-normal life expectancy. Evolution to myelofibrosis is observed in 4%>8% of pts at 10 years, whereas leukemic transformation is rare but can increase with the use of certain cytoreductive drugs. Ideal treatment of ET is towards preventing vascular complications but without increasing the risk of transformation of the disease. Hydroxycarbamide (HU) is a well established effective first-line therapy. However there are controversial data regarding the possible leukemogenic effects of HU. Several new studies indicates dose dependent leukemogenic effect of HU, while other denied any association. In order to evaluate further those observation we analyzed in a retrospective study all patients diagnosed and followed in a period longer than 10 years at the University Clinic of Hematology-Skopje.

Aims: In order to evaluate further those observation we analyzed in a retrospective study all patients diagnosed and followed in a period longer than 10 years at the University Clinic of Hematology-Skopje.

Methods: Our study group consisted of f 164 pts diagnosed at the University Clinic of Hematology in Skopje, Macedonia, before 2006, was made according to the WHO diagnostic criteria for ET. We analyze their clinical characteristics at diagnosis, therapies received, and development of myelofibrosis or acute leukemia (MF/AL).

Results: Of 164 pts, 49 were men and 115 women (median age 55), all of them had a sustained platelet count of >450x10^9/L (median platelet count 1122x10^9/L).; JAK2V617F mutation was demonstrated in 105 pts (64%). Twelve patients presented with splenomegaly (7,3%); Fifteen patients (9,1%) presented with history of previous thrombotic events at the moment of diagnosis. Pts with low-risk disease received low dose Aspirin; 123 high-risk pts were treated with Hydroxyurea 2gr/per day, 10 pts were treated with Anagrelide and 12 with Interpheron alpha. During the follow up period, 22 pts (13,4%) experienced thrombotic and hemorrhagic complications. Progression to myelofibrosis occurred in 6 pts (4,8%) treated with Hydroxyurea after 9 yrs of diagnosis and only one HU treated patient experienced a leukemic transformation (0,9%), 10 yrs after diagnosis; among the group treated with anagrelide, one of the patient transformed to myelofibrosis.Statistical analysis did not show any association between clinical characteristics at diagnosis, or HU therapy, and development of MF/AML. In our study group 6,5% of the pts treated with Hydroxyurea, experienced disease transformation.

Summary and Conclusions: Result from our follow up study showed that Hydroxyurea is effective and safe therapy of choice for ET patients and does not increase leukaemogenicity or disease transformation risk . It is in our opinion that major role in disease transformation in ET patients have nontreatment-related factors. However, larger, prospective studies are needed to study this issue further.

PB1736

MUCOCUTANEOUS TOXICITY IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS TREATED WITH HYDROXYUREA: A RETROSPECTIVE MONOCENTER COHORT STUDY

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Background: Hydroxyurea (HU) is the mainstay treatment in high-risk patients with myeloproliferative neoplasms (MPN). Mucocutaneous (MC) toxicity HU-related was 4.8% and 8.3% in two retrospective studies on MPN patients, respectively (Antonioli E et al, Am J Hematol 2012; Latagliata R et al, Cancer 2012) and 11.1% in a prospective trial in patients with essential thrombocythemia (ET) (Harrison C et al, N Engl J Med 2005).

Aims: To assess the probability over time of MC toxicity HU-related in a retrospective monocenter cohort of MPN patients.

Methods: The cohort included 250 patients (males 105, 42%) with MPN (polycythemia vera [PV]=89, ET=153, myelofibrosis [MF]= 8) who started HU treatment from 1994 to 2014. Patients were followed by an unique medical team and 92% of them started HU from 2000. All cutaneous or oral lesions of clinical significance, *i.e.* requiring consultation with dermatologists and/or discontinuation of treatment, were recorded. The interval between the start of HU and discontinuation due to unacceptable MC toxicity (uncensored observations) or discontinuation due to other causes or the last visit to the center (censored observations) was analysed. Moreover, the total HU dose intake was calculated in patients having had MC toxicity.

Results: The median time of exposure to HU was 37 months (range 1-217). Overall, 52 patients (20.8%) had MC toxicity (leg ulcers=21, oral aphtosis=12, keratosis=6, basalioma=6, dermatitis=4, squamous skin cancer=2, melanosis=1). In 15 of them (12 with aphtosis) toxicity was mild and allowed continuation of HU; 37 patients (14.8%) discontinued treatment. The median interval between start of HU and discontinuation for severe toxicity was 40 months (range 7-169). The cumulative probability of toxicity requiring discontinuation was 1.4% at 1 yr, 3.9% at 3 yrs, 18.7% at 5 yrs, and 30.5% at 10 yrs. Out of the 37 patients with severe

toxicity 17 were males (46%), 20 had PV (54%), 16 ET (43%), 1 MF (3%); 34 patients carried JAK2V617F (92%), at variance with those without MC complications (150/198, 76%, p=0.02). Among the 52 patients with MC toxicity the median total HU dose intake was 821 gr (range 209-4536) in those with leg ulcers, 852 gr (range 226-6166) in those with skin cancer, and 648 gr (range 69-12805) in those with other MC toxicity (p=0.5).
Summary and Conclusions: In this cohort the probability of MC HU-related toxicity was higher than previously reported and seems time-dependent and associated with JAK2V617F; interruption of treatment was required in 15% of the cohort.

PB1737

MOLECULAR MONITORING OF PCM1-JAK2 FUSION TRANSCRIPT IN A CASE OF ATYPICAL CHRONIC MYELOID LEUKEMIA WITH T(8;9)(P22;24)
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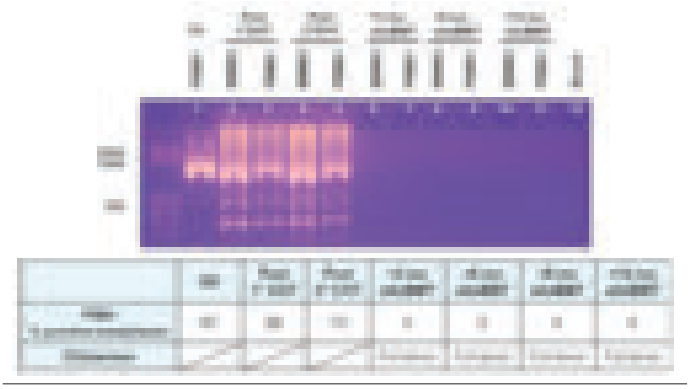
Background: Janus activated kinase 2 *(JAK2)* translocations have been described in hematologic malignancies involving both lymphoid and myeloid lineages. Better characterized translocation partners are *ETV6/TEL* on chromosome 12, *BCR* on chromosome 22 and the autoantigen Pericentriolar Material-1 *(PCM1)* on chromosome 8.
t(8;9) leading to *PCM1-JAK2* fusion are extremely rare and, to our best knowledge, less than 30 clinical cases have been reported so far in the literature. Although the clinical onset of these disorders is extremely heterogeneous, several patients present with a myelodysplastic/myeloproliferative neoplasm with a rapidly progressive disease and poor prognosis.
Aims: In this study we sought to assess the feasibility of a molecular monitoring of the *PCM1-JAK2* fusion transcript in a patient affected by atypical chronic myeloid leukemia (aCML) associated with t(8;9)(p22;p24)/*PCM1-JAK2* at time of diagnosis (Dx) and after: induction chemotherapy (1° CHT); consolidation chemotherapy (2° CHT); allogenic bone marrow transplant (alloBMT).
Molecular results are compared to the conventional monitoring tools for this peculiar disease such as FISH analysis and chimerism after alloBMT.
Methods: Break-apart FISH assays for *JAK2*/9p24, RP11-125K10 (5'*JAK2*) and RP11-39K24 (3'*JAK2*) and for *PCM1*/8p22, RP11-156K13 (5'*PCM1*) and RP11-484L21 (3'*PCM1*) was perfomed at time of diagnosis, after 1° and 2° CHT and at 3, 6, 9 and 12 months after alloBMT from a HLA-match sibling donor. Chimerism was assessed at same time points after transplant. Nested RT-PCR were performed using primers derived from *PCM1* Exon 25 and *JAK2* Exon 9 (as described by Reiter *et al. Cancer Res* 2005) on peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) at time of diagnosis, after 1° and 2° CHT and at 3, 6 and 12 months after alloBMT.
Results: As shown in the Figure 1, the sole CHT wasn't able to reduce the number of metaphases carrying the t(8;9) and, consistently, nested RT-PCR documented persistence of the fusion transcript both in BMMC and PBMC (lane 2-5). Only the alloBMT was able to restore a normal karyotype and to clear the pathological transcript, both from PBMC and BMMC (lane 6-11). Molecular data correspond to chimerism results, revealing full donor chimerism at the time points indicated.



Fgure 1.

Summary and Conclusions: In this report we provide the first evidence that the detection of the fusion transcript by RT-PCR is a reliable tool to monitor the residual disease also in t(8;9)(p22;p24)/*PCM1-JAK2*-related disorders, similarly to what routinley used in the setting of *BCR-ABL* positive CML. Since we know that RT-PCR is very sensitive technique capable to predict an early disease relapse, our study suggests that this type of molecular monitoring may be extended also in this peculiar setting of hematologic neoplasms.

PB1738

ADHERENCE SUPPORT PROGRAM FOR PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) TREATED WITH ANAGRELIDE
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Background: Increasing patient medication adherence should be considered for chronic diseases such as ET, where treatment is prescribed to avoid thrombotic and hemorrhagic events. Patient support programs may substantially improve non-adherence by providing patients with information about pathology, treatment adherence, dose reminders, and cardiovascular risk factor management. One of the treatments used in ET is anagrelide.
Aims: To assess how an adherence patient support program (APSP) can improve treatment engagement and satisfaction in patients with ET treated with anagrelide and to evaluate APSP physicians' acceptance.
Methods: Canonical action research methodology was used (Principles of Canonical Action Research. Info System J 2004;14:65–86). A committee of experts (four hematologists, one cardiologist) designed the APSP. Trained nurses were responsible for keeping phone contact with patients and for providing educational materials. Patients were offered participation in the APSP according to their hematologists' criteria. 158 patients with ET from 149 sites gave written consent to enter this program. Two types of programs were offered: a) the basic program (BP) to help patients to learn more about their pathology and increase awareness of the need for treatment compliance, including the importance of taking prescribed doses, and b) the extended program to support patients in the management of cardiovascular risk factors (RFP). In order to assess and improve the implementation of the program, two surveys were performed among patients and doctors.
Results: In total, 190 patients were invited to enroll in the programs and 158 participated for 18 months: 138 patients in the BP and 20 in the RFP. Results from both programs show an adherence to treatment by 78% of patients in the first year. Overall, 130 patients participated in the survey, which showed an average satisfaction rating of 9 out of 10. 44% of the respondents thought that the program had helped them to better understand and treat their disease, and the majority of patients (85%) confirmed that it had been useful for them in some way. Physicians' survey outcomes from 106 hematologists showed that 15% frequently prescribed the APSP, 57% occasionally, and 8% never prescribed it. The main reasons for not using the program were: not having time, heavy workload, and forgetting about the program. The level of global satisfaction was rated 3.9 out of 5.
Summary and Conclusions: Adherence level achieved in the first year with the APSP was 78%. Most patients (85%) confirmed that it has been useful for them in some way. Overall satisfaction from patients and physicians was high. Other complementary interventions would probably be required to encourage greater participation in patient programs.

PB1739

AN OBSERVATIONAL, HYPOTHESIS-GENERATING, MULTICENTER STUDY INVESTIGATING THE IMPACT OF JAK2(V617F) MUTATIONS ON RESPONSE TO ANAGRELIDE IN ESSENTIAL THROMBOCYTHEMIA
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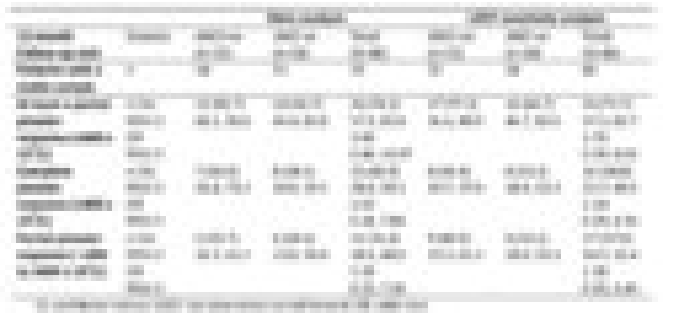
Background: A *JAK2(V617F)* mutation is found in approximately 55% of patients with essential thrombocythemia (ET), and represents a key World Health Organization (WHO) diagnostic criterion.
Aims: To explore the impact of *JAK2(V617F)* mutation status on treatment response to anagrelide in patients with ET.
Methods: Patients with WHO-diagnosed ET, who were intolerant/refractory to their current cytoreductive therapy (CRT) and had started or were due to start anagrelide up to 7 days before study entry, were enrolled. Informed consent was obtained from all patients. Blood samples were collected to determine platelet response, hematologic values, and allele burden at baseline, 6, and 12 months. The primary objective was to compare the proportion of *JAK2*+ve patients vs *JAK2*-ve patients with ET who achieved at least a partial platelet response (≤600 x 10^9/L) after anagrelide therapy. Secondary objectives included comparing the proportion of patients in each group who achieved a complete platelet response (≤400 x 10^9/L), and evaluating the relationship between platelet response and allele burden in *JAK2*+ve patients. This study (NCT01352585), sponsored by Shire Pharmaceutical Development Ltd, was not statistically powered.

Table 1. Summary of patients with a platelet response at 12 months (full analysis set).



Results: Of the 47 patients enrolled, 46 were included in the full analysis set (*JAK2*+ve, n=22; *JAK2*-ve, n=24). Baseline characteristics were well balanced except for median time since ET diagnosis, which was greater in *JAK2*+ve patients than in *JAK2*-ve patients (8.2 *vs* 3.5 years, respectively). At 12 months, 35 patients (*JAK2*+ve n=14; *JAK2*-ve n=21) had a suitable platelet sample, of which 74.3% (n=26/35) achieved at least a partial response. The response rate was higher in *JAK2*+ve patients (85.7%, n=12) *vs JAK2*-ve patients (66.7%, n=14); odds ratio [OR] 3.00, 95% confidence interval [CI] 0.44, 33.97 (Table 1). A last observation carried forward sensitivity analysis was performed to account for the imbalance in patients with suitable samples between the groups due to a slightly higher withdrawal rate in the *JAK2*+ve group. The total response rate was 71.7% (n=33/46), with 77.3% (n=17/22) of *JAK2*+ve patients and 66.7% (n=16/24) of *JAK2*-ve patients achieving at least a partial response (OR 1.70, CI 0.39, 8.02). The median allele burden in the 12 patients who achieved at least partial platelet response at 12 months and had a suitable sample was 41.0% (17–47) in granulocyte DNA and 49.5% (31–58) in platelet RNA. There was no significant allele burden change over the 12 months. 31.9% of patients (n=15) experienced an adverse drug reaction (ADR), most commonly headache (12.8%, n=6). More *JAK2*-ve patients had an ADR than *JAK2*+ve patients (37.5%, n=9 *vs* 26.1%, n=6). No severe, serious, or fatal ADRs were reported.
Summary and Conclusions: The overall platelet response rate was high in both *JAK2*+ve and *JAK2*-ve patients with ET, but the odds of achieving at least a partial response were slightly higher if patients had a *JAK2(V617F)* mutation than if they did not. These results were not statistically significant and should be interpreted with caution due to the study size and imbalance in suitable samples. No clear relationship was found between allele burden and platelet response rate. Anagrelide was well tolerated and the safety profile was consistent with current literature. These data, presented on behalf of the EMIX investigators, are hypothesis generating and a controlled study would be required to further pursue any of these hypotheses.

PB1740

CLINICAL ASSESSMENT OF AQUAGENIC PRURITUS IN 70 PATIENTS WITH MYELOPROLIFERATIVE NEOPLASM

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Background: Aquagenic pruritus (AP) is an intense prickling and stinging sensation induced after contact with water. It is a frequent symptom of polycythemia vera (PV) and described by patients as one of the most troublesome aspect of their disease. Cytoreductive treatments can sometimes relief AP but classical symptomatic treatments are mostly inefficient. Pruritus has also been reported in other myeloproliferative neoplasms (MPN) such as essential thrombocythemia (ET) or myelofibrosis (MF) without specify if it was AP or not.
Aims: We conducted a prospective study on MPN patients who reported suffering from AP at the onset or later during the course of their MPN. The main objective of this study was to describe circumstances of occurrence, duration and characteristics of the pruritus, and the influence of received cytoreductive treatments. Secondary objective was to determine whether there is a single symptom whatever the MPN or if there is several types of AP depending on MPN.
Methods: This study was in the form of a questionnaire with 26 open and multiple choice questions. 70 MPN patients (44 PV, 14 ET or 12 MF, all JAK2V617F positive) were included.
Results: Our results revealed that AP occurred earlier prior to diagnosis in PV ($p<0.05$) and appeared to be more sensitive to any contact with water ($p<0.05$). A significant influence of the water temperature on pruritus was revealed only in PV and MF but not in ET. Furthermore, these patients experienced longer and more disabling crisis of AP with scratching till excoriation ($p<0.05$). In contrast, we didn't found any statistical difference between MPNs concerning the inducer of AP, the type of pruritus, the affected body areas, the intensity and the overall impact on quality of life. At the time of questionnaire, 42 of 70 patients (8 ET, 6 MF, 28 PV) still suffered from AP despite a complete hematological response (CHR) for 20 of these patients. Cytoreductive treatments appeared to be significantly more efficient to resolve AP in ET and MF.
Summary and Conclusions: In summary, this study shows that AP is not an exclusive symptom of PV and precisely describes some clinical differences between MPN subtypes that could help to better understand pruritus physiopathology and find appropriate therapies.

PB1741

ANAGRELIDE AFTER FAILURE AFTER HYDROXYUREA TREATMENT IN ESSENTIAL THROMBOCYTHEMIA: EXPERIENCES OF A SINGLE HEMATOLOGICAL INSTITUTE

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Background: Essential thrombocythemia(ET) is a chronic myeloproliferative neoplasm with risk of bleeding and thromboembolic complications during course of illness.Cytoreductive treatments, like non-selective hydroxyurea (HU) or interferon as first-line and specific,megakaryocyte-thrombocyte reductive anagrelide chosen as second-line therapy in cases of adverse,intolerable effects of HU can lower incidence of bleeding-thrombotic episodes in patienst(pts)with ET.
Aims: In this observational survey we investigated the effect of anagrelide in pts of ET with high risk,who were getting former HU and failed to have clinicopathological response (HU-resistant)or became HU-intolerant (adverse effects). Doses of HU and anagrelide were adjusted achieving clinicopathological response according to the criteria of European Leukemia NET, as well. Effect of anagrelide as monotherapy (first-line or second-line after HU)or in combination with hydroxyurea was followed during a teratment period in their pts with ET.
Methods: Between 2000 and 2013 in our hematological outpatient department 97 pts with ET were diagnosed (according to the updated WHO-ET classification)and treated primarily with HU(median weekly dose of 7500mg),anagrelide was given after intolerance and/or resistance to HU(median weekly dose of 7.5mg).Combination medication was used when response to monotherapy was insufficient.Bleeding and thrombotic events were also registered.Statistical analysis was made by the Windows Statistical package Software Program.
Results: From a total of 97 pts with ET (JAK2V617F mutation positive 55,negative 42 ones) HU as initial therapy was given to 86 pts, four were treated continuously with interferon and seven ones were getting only aspirin.Because of HU-intolerance (5pts) and -resistance (21 pts) second-line anagrelide was introduced as monotherapy in 16,combined again with HU in 10 pts, moreover 5 pts were getting as fisrt-line monotherapy(mostly younger ones),total of 31/97(31.9%). All pts of this latter group achieved complete remission(CR), from the second-line anagrelide monotherapy group CR and partial response in 16, and in the combined HU+anagrelide group nine pts responsed completely. During anagrelide medication maior bleeding and thrombotic events(myocardial infarction)in two pts were observed.Serious adverse events due to anagrelide were not detected.
Summary and Conclusions: Anagrelide as first- or second- line monotherapy or in partial response combined with hydroxyurea is a useful approach in treating patients of essential thrombocythemia.

PB1742

OUTCOMES FOR MYELOFIBROSIS PATIENTS WHO SWITCHED FROM FEDRATINIB TO RUXOLITINIB THERAPY

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Background: Fedratinib (FED), a JAK2-selective inhibitor, has recently been evaluated for treatment of myelofibrosis (MF) with demonstrated benefits of statistically significant reductions in splenomegaly and constitutional symptoms. Other studies suggest benefit of FED for patients who had previously been

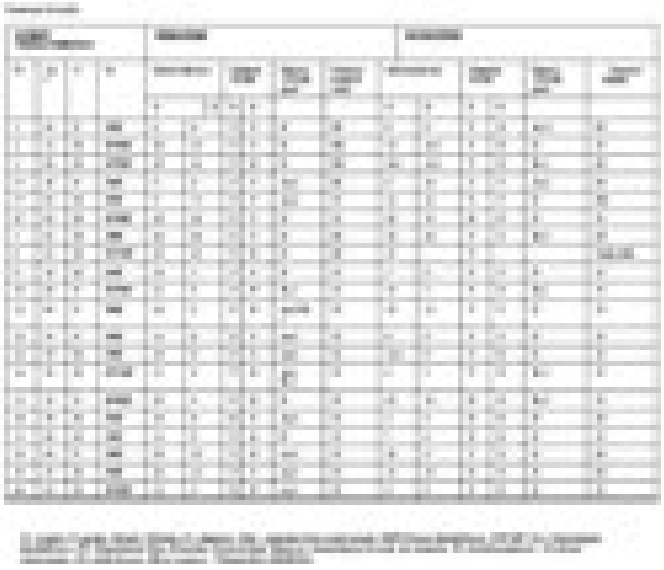
exposed to ruxolitinib (RUX) in the JAKARTA-2 study. However in 2013 development of FED was put on permanent clinical hold after reports of neurological adverse events (Wernicke's encephalopathy). As a result treatment for some patients with MF was switched from FED to RUX. Outcomes for such switches of therapy have not previously been reported.

Aims: We describe our experience in a cohort of these patients.

Methods: We recorded 20 MF patients who received treatment with FED in the JAKARTA trials whose treatment was switched to RUX therapy either following toxicity from FED (n =5), or after clinical hold of this drug (n = 15); 3 of these 20 patients had already switched from RUX to FED in the JAKARTA 2 trial. Responses are defined using IWG-MRT criteria and toxicity with CTCAE grading.

Results: See Table 1 below. Median age was 64 years, there were 13 male, 7 female, 12 PMF, 8 PPV-MF. At FED baseline, median spleen size by palpation was 17cm, and 18 had symptoms; at completion of FED median spleen size was 8cm, with a > 50% reduction in palpable spleen length achieved in 9, and resolution of symptoms in 8. After stopping FED splenomegaly and symptoms recurred or worsened in the majority of patients. No SAEs occurred on FED withdrawal. At initiation of RUX therapy median spleen size was 13cm and 15 patients reported symptoms; currently the median spleen size is 9cm, with > 50% reduction achieved in 6, and symptom resolution in 8. Responses with RUX following FED therapy were as good as those with FED and for symptom response were perhaps better. CI was achieved following FED in 13 patients for spleen and 4 for symptoms, and was generally maintained after RUX. One patient was transfusion dependent on FED and became transfusion independent on RUX, but later progressed to AML and died. Concerning patients (no. 4, 5, 6 in Table 1) who were switched to FED after previous RUX intolerance: re-exposure to RUX delivered a response similar to initial RUX therapy and intolerance has not yet recurred. Regarding hematological toxicity following switch to RUX there were 5 patients with thrombocytopenia – 3 CTCAE grade1, 2 grade3; of these 2 had experienced thrombocytopenia with FED (same grade). For anemia, on RUX there were 2 grade2 (1 experienced with FED); on FED there were 10 anemia-related toxicities (2 grade1, 2 grade2, 6 grade3). Thus far these patients have not experienced the same degree of anemia on RUX therapy although the duration of follow up is shorter.

Table 1.



Summary and Conclusions: Patients who switched JAK inhibitor therapy from FED to RUX experienced rebound of splenomegaly and symptoms after stopping FED. Symptom and spleen responses for MF patients after RUX were similar to those obtained with FED therapy. There was no unexpected toxicity, in this cohort of pre-treated patients. RUX therapy was possibly associated with less anemia, 9 patients experienced this with FED but not RUX, in contrast 3 patients experienced new onset thrombocytopenia on RUX. These data suggest that RUX following FED therapy is well tolerated and efficacious.

COI:T.Somervaille and M.Drummond have research funding and Ad visory boards for Novartis and Ad visory board for Sanofi. M.Drummond has also Speaker Fees from Novartis.A.Mead has consultancies for Sanofi and Novartis and research funding from Novartis.C Harrison has research funding from Novartis, speaker fees and advisory boards from Novartis and Sanofi.

PB1743

JAK2 V617F ALLELE BURDEN LESS THAN 20% HAS NO INFLUENCE ON OUTCOME OF PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: JAK2V617F mutation has been described in 40-60% of patients diagnosed with essential thrombocythemia (ET).

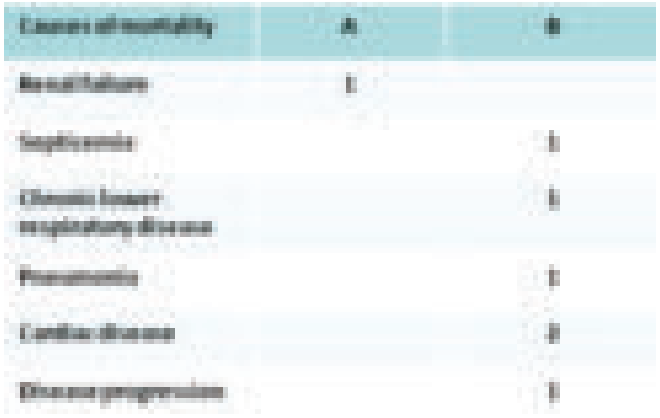
The role of this mutation and its allele burden in terms of thrombotic risk and clonal progression in this subgroup of patients is not well established. The early age of onset less than 40 years up to 15% of patients, makes it important to have tools to predict clinical behavior and new therapeutic approaches.

Aims: To analyze risk of thrombosis, risk of transformation to myelofibrosis (MF) and acute leukemia (AL) and survival in patients in patients diagnosed with ET and to correlate these parameters with mutational status and allele burden.

Methods: A total of 100 patients with ET were retrospectively analized from our center from 1996 to 2013. All patients received anti-platelet aggregation and/or cytoreductive treatment . JAK2 V617F mutation was detected by allele specific real-time quantitative in peripheral blood samples. Homozygous patients for the mutation occurred when the load allelic was > 50%, being the rest considered heterozygous.

Results: Median age at diagnosis was 63 years. A total of 73% presented JAK2 V617F mutation, 7% being homozygous. A lower overall survival was observed in mutated *versus* unmutated patients (88% *vs* 100%) without significant differences between both groups (p=0.07). Upon analyzing patients with JAK2 V617F mutation, displaying an allele burden above or below 20% the latter group had an overall survival similar to that observed for unmutated patients. Furthermore, unmutated and low allele burden patients (group A) were significantly younger as compared to those with ≥ 20% allele burden (group B) (59 *vs* 74 years, respectively; p=0.005), had a lower platelet (739x10e9 / L *vs* 896x10e9 / L p=0.024) and leukocyte counts (10.05x10e9 / L *vs* 11.82x109 / L p=0.010). Interestingly, patients in group A had a significantly better survival as compared to those in group B (98% *vs* 79% at 7 years, p=0.006). Nevertheless, no differences in terms of disease related mortality: progression to myelofibrosis (6.6% *vs* 13.9%, p=0.285);thrombotic events (8.2% *vs* 14.3%, p=0,489) was observed between groups A and B. None progressed to acute leukemia. Causes of mortality are shown in Table 1 and are related to age-related comorbidities in 1.58% *vs* 13.8% of patients from groups A and B, respectively.

Table 1.



Summary and Conclusions: Patients diagnosed with ET with unmutated JAK2 V617F or with a low allelic burden have a significantly better survival than those with a higher allelic burden. Nevertheless, according to the current study, these differences can not be attributed to disease related complications but to a different pattern of comorbidities among patients from both subgroups.

PB1744

COMBINATION THERAPY WITH RUXOLITINIB PLUS 5-AZACYTIDINE OR CONTINUOUS INFUSIONAL LOW DOSE CYTARABINE (LDAC) IS FEASIBLE IN PATIENTS WITH BLAST-PHASE MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Leukaemic transformation of 'Philadelphia-negative MPN' or 'MPN/MDS overlap' syndromes occurs infrequently, but poses significant therapeutic challenges. Conventional AML treatment has limited efficacy and high toxicity, with poor outcome. Most patients are unsuitable for allogeneic stem cell transplant, the only definitive treatment. Estimated survival of blast phase (BP) is less than 6 months. Novel therapeutic approaches are required. Single agent Ruxolitinib displayed only modest anti-leukaemic activity in refractory/relapsed acute leukaemias.
Aims: We present three cases of BP-MPN treated with combination Ruxolitinib and 5-azacytidine or low dose cytarabine (LDAC) and demonstrate safety and efficacy in controlling disease progression and improving symptoms and quality of life.
Methods: Case 1: 71year old with *JAK2* V617F positive 'MPN/MDS', rapid progression to accelerated phase (16% blasts). Failed to respond to single agent 5-azacytidine or decitabine and developed BP. Ruxolitinib introduced, with marked improvement in symptoms, spleen size and transfusion requirements with stable disease (SD) for 4 months. LDAC then introduced as a single agent but failed. Ruxolitinib was re-introduced with infusional LDAC (3 weeks 20 mg/m2/day). After 17 weeks, has good leukaemic cytoreduction, complete resolution of splenomegaly and symptoms and has stable transfusion requirements. Case 2: 65 year old with PV. Ruxolitinib responder but progressed to post-PV MF 12 months later and then frank AML (37% blasts; complex karyotype). Standard dose 5-azacytidine was introduced and he continues on dual therapy. After 34 weeks, he remains asymptomatic, transfusion independent and has SD. Case 3: 69 year old with progressive *JAK2*V617F negative MF and worsening portal hypertension. 18 months after Ruxolitinib therapy progressed to BP. LDAC was ineffective. Switched to combination therapy with 5-azacytidine and Ruxolitinib, with marked objective improvements in portal hypertension, constitutional symptoms and reduced transfusion requirements. Stable blood counts sustained for 20 weeks, after which she succumbed to sudden overwhelming sepsis.
Results: These cases demonstrate the feasibility and tolerability of Ruxolitinib in combination with either LDAC or 5'aza in BP-MPN.
Summary and Conclusions: Conclusions are limited due to cohort size but these patients demonstrated good responses as regards attenuation of symptoms, splenomegaly and disease stability with minimal haematological toxicity. No adverse events occurred. A future UK trial is planned.
Conflict of interest: CHarrison declares research funding, speaker fees and consultancy for Novartis.

PB1745

USE OF ANAGRELIDE IN A COHORT OF PATIENTS FROM THE LAZIO REGION: COMPARISON WITH RECOMMENDATIONS FROM THE ITALIAN GUIDELINES

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Background: The aim of treatment of Essential Thrombocythemia (ET) is to prevent thrombotic/hemorrhagic complications, or leukemic transformation, due either to the natural history of the disease or possibly to the use of chemotherapeutic agents. The Italian guidelines for ET therapy were developed from an Expert Panel and an Advisory Committee, and published in 2004. Anagrelide (ANA) was recommended as 1st line therapy in patients with no child-bearing potential, either <40 years or ≥40<60 years but without a history of major thrombotic events.
Aims: To describe the use of ANA in a population of ET patients from the Lazio region and to evaluate the adherence between the Italian Guidelines recommendations on the use of this drug in the clinical practice.
Methods: Between 1981-2013, 153 ET patients (103F;50M) were treated with ANA for a median period of 4.6 years (0.1-23.2). Median age at diagnosis: 42.6 years (20.9-87.7); median age at the start of ANA: 46.3 years (22.7-87.8); median follow-up: 11.1 years (0.6-31.9).
Results: ANA was used as 1st line treatment in 52/153 patients (34%), as 2nd line treatment in 81/153 (53%) and as 3rd line therapy in 20/153 (13%). Responses (plts <600x10^9/L) were obtained in 125/153 patients (81.7%). While on ANA, 11 thrombotic (3 venous, 8 arterial) and 48 hemorrhagic events (8 major, 40 minor) were observed. One hundred and seven patients (76 F; 31 M) started ANA after the publication of the Italian guidelines: in 26/107 (24.3%) ANA was used as 1st line treatment, in 68/107 (63.5%) as 2nd line and in 13/107 (12.2%) as 3rd line therapy. In the table, age, platelet number at the start of therapy and previous thrombosis are described for patients undergoing ANA as 1st line medication.

Table 1.

In 81/107 (75.7%) patients treated with ANA as 2nd/3rd therapy line, 16 were <40 years and 29 between 40-60 years (45/81, 55.5%).
Summary and Conclusions: These data show that although ANA was indicated as 1st line treatment in patients <40 and ≥40<60 years with no thrombotic events, it has been more widely used as 2nd/3rd line therapy. In our series, when prescribed as 1st line, in 5/26 cases the recommendations were not followed: 3 patients were ≥40<60 years with previous thrombotic events and 2 patients were >60 years. We conclude that the use of ANA has not been very adherent to the Italian recommendations, but it depended more on the experience of the single center.

PB1746

PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS - ROLE OF INTERFERON ALPHA 2-A

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Background: Role of interferon in MPD has been documented over 25 years. Peginterferon alfa-2a (Pegasys) is a covalent conjugate of IFN alfa produced by recombinant DNA technology in Escherichia coli with polyethylene glycol. Starting dose of IFN is usually 45 mcg sc once weekly as 2nd line or 1st line option (< 40 years). It is considered safe in pregnancy. The most Common side effects are anorexia, nausea, flu like symptoms and myelosupression. Rarely severe depression and suicidal behaviour has been documented with interferon treatment. It should be used withcaution in IHD.Pegylated interferon-α2 (peg-IFN) hasemerged as treatment option for patients with myeloproliferative disorders (MPD). In particular, it may be suitable for younger patients and those who are unable to tolerate standard agents. Furthermore, several recent studies have shown that peg-IFN is able to induce molecular remission with undetectable JAK2V617F in a subset of patients with essential thrombocythemia (ET) and polycythemia vera (PV).
Aims: We performed a retrospective review of 15 patients with MPDs who received treatment with peg-IFN with a focus on efficacy and tolerability.
Methods: Of 15 patients treated with peg-IFN, 11 were diagnosed with ET, 3 with PV and one patient had chronic neutrophilic leukaemia (CNL). JAK2V617F mutation was detected in 5 patients (PV 3, ET 2) with 8 patients being negative for this mutation. JAK2 mutation remains unknown in 2 patients who were diagnosed more than 10 years ago. 10 patients were younger than 60 years at the time of treatment.
Results: All patients with PV and 10 patients with ET had excellent response with either reduction in venesections or in platelet counts. In one patient therapy was discontinued due to worsening depressive symptoms. In all other patients IFN-α was well tolerated. The single patient with CNL had stable disease during treatment with no significant reduction in neutrophil count.
In one patient with essential thrombocythemia pregnancy was successfully contemplated with pegylated interferon alpha.
Summary and Conclusions: Peg-IFN is well tolerated and effective in patients with MPD. However, randomised studies and much longer term follow-up will be required to determine the overall tolerability of this agent and whether patients can enter durable molecular remissions.

PB1747

EFFICACY OF PEGYLATED INTERFERON IN ESSENTIAL THROMBOCYTHEMIA: A MONOCENTRIC EXPERIENCE

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Background: Essential Thrombocythemia (ET) is a clonal myeloproliferative chronic neoplasm: in Europe, its incidence is 0.38-0.6/100000 per year, with a prevalence in the old women subgroup. The molecular markers of disease are

represented by the JAK2V617F mutation, accounting about 50% of patients, by the less frequently detected MPLW515L7K mutation, and by the most recently identified calreticulin gene, that is mutated in about the half of the remaining cases. In young people affected by ET, the Interferon-alpha has showed good tolerance and efficacy, because of its antiapoptotic, anticytokine and anti-inflammatory effects. Recently, also the pegylated formulation showed a good toxicity profile and efficacy, with more than 60% complete responses (CR).
Aims: To assess the safety profile and efficacy of the pegylated interferon in the Essential Thrombocythemia
Methods: Twenty patients with ET received subcutaneous recombinant pegylated-interferon alpha2a (peg-IFN, PEG-INTRON®) at the Hematology Division of Pisa (Italy), from May 2004 to December 2013. The median age at diagnosis was 37 years (range 21-54 years), with an equal gender distribution. The drug was dispensed as off label use, after obtaining the informed written consent. The median weekly dose was 50 ug/week. All patients were also assessed at diagnosis for the JAK2V617F mutation.
Results: Five patients presented a thrombotic event at the time of diagnosis, and only one developed thrombosis during the peg-IFN treatment. Half of cases showed splenomegaly at the first visit; the median value of platelets was 917x 10^3/mmc (range 700-1800x10^3/mmc). With a median follow-up of 58 months, the CR rate was 88%, and in the majority of cases the response had been achieved during the first year of therapy. Early discontinuation of the drug was documented in 13.6% of patients, after a median of five months, due to primary intolerance (itch, skin atrophy in the side of injection, thyroid disfunction). Other four patients have stopped therapy (two female patients for procreation, another one for the hematology toxicity, and one for dysphoria), after a median of 2 years of treatment. A total of 31% of patients definitively discontinued the peg-IFN at the last observation. The efficacy of the peg-IFN is also sustained by the fact that all patients previously received the conventional IFN2b (INTRON A®) for almost one month; for all responders, at the start time of peg-IFN, the median value of platelets was still above the normal level. JAK2 V617V mutation was found at diagnosis in 72% of patients. Data were checked at least one year later during peg-IFN therapy in 12 patients. We observed a reduction in the mutated allele burden in 8/12 cases (66%), and the molecular remission was documented in another patient.
Summary and Conclusions: Recombinant IFN therapy has been shown to suppress megakaryopoiesis, induce cytogenetic remission and also reduce the JAK2V617F allele burden, sometimes completely. Efficacy has been demonstrated in myeloproliferative disease, but toxicities are still frequent and the adverse events often restrict the use of the drug, also in young patients. Changing it with peg-IFN, because of less toxicity, may be a valid alternative. In our patients, peg-IFN were well tolerated and allowed to reach good results in terms of hematological and molecular responses in the large majority. Finally, we observed a significant improvement in the quality of life, consequent to the less iatrogenic side effects and the reduction of the injection frequency for the pegylated formulation.

PB1748

LATE ONSET NON-SYNDROMIC JUVENILE MYELOMONOCYTIC LEUKEMIA WITH PTPN11 MUTATION

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Background: JMML is a rare hematological malignancy of early childhood. Somatic mutations in PTPN11 are the most frequent molecular lesions in patients with JMML.
Aims: Clinical and laboratory features of these patients are rarely reported. Here we report a non-syndromic JMML in a nine-year old patient.
Methods: A nine year-old girl referred to our hematology outpatient unit for mild thrombocytopenia and neutropenia. Initial physical examination was normal and there was no hepatosplenomegaly. After 11 months, she presented with malaise, fever and abdominal enlargement. Liver and spleen were 10 and 11 cm palpable below the costal margins respectively. Laboratory workup revealed anemia, leukocytosis and thrombocytopenia (Hb 9.6 g/dl, WBC 37,900/mm^3, ANC 21800/mm^3, monocyte 1895/ mm^3, PLT 29100/mm^3 and MCV 74 fl). Total bilirubine was 3.3 mg/dL, direct bilirubine 1 mg/dL, haptoglobin 8 mg/dL and HbF was 18%. Peripheral blood smear, showed basophylic stippling, cabot rings, polychromasia, anisocytosis and megaloblastic changes in red blood cells. There were large platelets, some lobulation defects in neutrophyl nuclei, and large basophylic granules in some lymphocytes. Nucleated cells showed 22% band, 22% lymphocyte, 21% neutrophyl, 9% normoblast, 9% erythroblast, 7% myeloblast, 5% monocyte, 1% metamyelocyte, 3% eosinophyl, 3% basophyl, and 1% promyelocyte. Repeated bone marrow smear showed hypercellularity, no megakaryocytes. Erythroid/myeloid ratio was 40/1. Almost all cells were erythroblast and normoblasts. Very few promyelocytes, lymphocytes and neutrophyls were seen. There was 85% celularity in marrow biopsy, megacaryocytes were normal in morphology and number. There was erythroid hyperplasia with increased glycophorin positive young cells. Granulocytic series were only seen in patchy areas and showed maturation and eosinophylic predominance. Monocytes were increased, erythroid/myeloid ratio was 2/1, 3/1 and there were no collagen fibers and hemosiderin. Cytogenetic analysis in 30 methaphases showed 46,XX [8]/46,XX,del (8q)[6]/45,XX,-8[16]. In FISH analysis 7.5% of interphase cells showed monosomy/deletion with 8q22 probe. After only one week, she had fever, tachypnea, tachycardia and edema in her legs. Hb was 7.2 g/dL, WBC 6360/mm^3, ANC1440/mm^3 and PLT 34,900/mm^3. Reticulocyte count was 49,000/mm^3. No chemotherapy was administered due to uncertainty in diagnosis. Piperasillin/tazobactam and supportive therapy with packed red cell and platelets and nasal oxygen were administered. She died with respiratory failure. Genetic study was completed after her death.
Results: DNA analysis performed from bone marrow sample, revealed a heterozygous mutation in the PTPN11 gene, exon 13 c.1508 G>T, aminoacid p.503 G>V. In her past medical history she was hospitalized when she was five-year-old due to multiple liver abcess and pulmonary empyema. *S aureus* was isolated from liver abcess and pleural effusion samples. No immunodeficiency could be defined by laboratory investigations.
Summary and Conclusions: Non-syndromic JMML with PTPN11 mutation can be seen in patients older than 5 years. A cytopenic phase may precede JMML. Leucocytosis may be transient and there may be erythroid predominance in the bone marrow. PTPN11 mutation may cause immune disregulation.
Acknowledgement: Thanks Prof.C.M.Niemeyer and EWOG-MDS for genetic study.

PB1749

CYCLIC THROMBOCYTOSIS IN A PATIENT WITH PV RESOLVED BY RUXOLITINIB TREATMENT

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Background: Cyclic thrombocytosis is a seldomly described and poorly understood phenomenon that occurs in conjunction with some congenital or acquired disorders.
Aims: We report two female patients with a long history of polycythemia vera (PV). A cyclic undulation of thrombocytes was observed in these patients following several years of treatment with hydroxyurea (HU).
Methods: Both patients belong to our single center register, with includes 191 MPN patients with 1163 patient-years of follow up treated in the medical practice Lüdenscheid. Of these, 138 patients were treated with HU, for which 558 years of follow-up are available.
Patient 1 is a 74 year old female, diagnosed with PV in 1987 at the age of 47. She was initially treated with two doses of ^{32}P in the year of her diagnosis. Following a wait and watch period, HU therapy was initiated in 1997 because of a global rise in blood cell counts. In 2013 clinical progression was noted, consisting of elevated LDH and progressive splenomegaly. A bone marrow biopsy revelaed fibrosis and ruxolitinib treatment was initiated in January 2014.
Patient 2 is a 70-year old female diagnosed with PV in 2006, at the age of 62. HU therapy was begun immediately upon diagnosis. Because of clinical progression, again manifested by elevated LDH, splenomegaly and progressive fibrosis in the bone marrow ruxolitinib treatment was started in the fall of 2013.
Results: Undulating platelet counts were noted in patient 1 in 2009, 22 years after diagnosis and in patient 2 in 2013, 7 years after diagnosis. The period of undulation was approximately 28 days in both patients, with thrombocyte counts ranging from 50 to 1350 x 10^9/l. Leukocyte counts varied concordantly but the undulation was less pronounced. No complications (thrombosis, bleeding) were observed. Both patients carry a $JAK2^{V617F}$ as well as a TET-2-mutation. Ruxolitinib promptly abolished platelet undulation and resolved the aquagenic pruritus in patient 2.
Summary and Conclusions: This is the first report of a resolution of undulating platelets following ruxolitinib treatment. Platelet undulation has previously been reported as a rare, often benign observation in some patients with PV, predominantly females. As HU treatment is common in PV and the co-occurrence of $JAK2^{V671F}$ and a TET2 mutation are far more frequent than undulating platelets, it is unlikely that either fact contribute to this poorly understood phenomenon, which appears amenable to treatment with ruxolitinib.

PB1750

IN PATIENTS WITH POLYCYTHEMIA VERA OLDER AGE IS THE PROGNOSTIC FACTOR AT HIGHEST IMPACT ON SURVIVAL

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Background: Life expectancy of Polycythemia Vera (PV) patients is reduced compared with that of the general population mainly due to thrombotic events and evolution into myelofibrotic phase or secondary myelodisplasia/acute leukemia. Recently, in a large study published by IWG-MRT (Tefferi et al, Leukemia 2013) authors developed a widely applicable prognostic model, delineating three different prognostic risk groups by age, leukocyte count and venous thrombosis.
Aims: In this study we tested the IWG-MRT prognostic score in a retrospective group of 204 World Health Organization-defined PV patients, diagnosed from 1974 to 2013 by a single Italian haematological centre (Turin).
Methods: We analysed 204 PV patients. According to the IWG-MRT score, patients were divided into three groups: adverse points are assigned to age ≥67 years (5 points), age 57–66 years (2 points), leukocyte count ≥15 x109/l (1 point) and venous thrombosis (1 point). In this way patients were divided in low-risk (0 points), intermediate-risk (1 or 2 points) and high-risk (≥3 points) groups. We evaluated overall survival (OS) from diagnosis to death by the Kaplan Meyer method and Hazard Ratio were estimated with the Cox Models. The cumulative incidence of death due to PD was estimated by the method proposed by Gooley et al, accounting for competing events.
Results: Characteristics at diagnosis were as follows: median age was 65 years (31% were below age 57 years), 104 (51%) were male, constitutional symptoms and palpable splenomegaly were observed in 42 (21%) and 36 (18%) patients, respectively. Twenty-one (10%) patients showed leukocytosis (≥15 x 109/L) whereas thrombocytosis (≥450 x 109/L) was present in 77 (38%) patients. Fifty-three patients had prevoius thrombotic events (40 arterious and 13 venous). As expected, approximately 58% of the patients were positive for JAK2 mutations (V617F or exon12). At a median follow-up of 93 months we observed 27 (13%) deaths, 26 (13%) myelofibrotic transformations and 7 (3%) leukemic evolution. The incidence of post diagnosis thrombosis arterial *vs* venous and major hemorrage were 15%, 11% and 9% respectively The overall survival at 10 years was 85.3% (95%CI:77.6-90.5) and the cumulative incidence of death due to PD (leukemic transformation or evolution in myelofibrosis) adjusted for competitive risk event was 9.8% (95%IC: 4.5-15.0). The prognostic model displayed discrimination between high-risk (n= 91; 10-year survival 68.3%; *vs* low-risk HR 8.48; 95%CI: 1.94-37.16), intermediate-risk (n= 49; 10-year survival 94.7%; *vs* low-risk HR 3.23; 95%CI: 0.62-16.68) and low-risk (n=42; 10-year survival 100%) patient groups. The C-statistic was 0.751. Age was the prognostic factor at highest impact on OS (fig.1); when we consider the older age as a only prognostic factor the discrimination between risk group was similar to those defined by IWG-MRT. The C-statistic was 0.748.

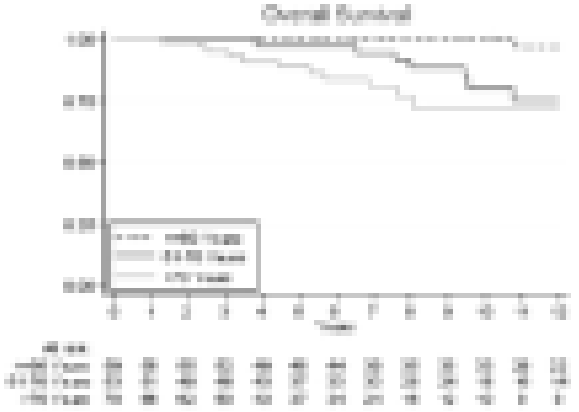

Figure 1.

Summary and Conclusions: In a retrospective cohort of PV patients, IWG-MRT prognostic score was confirmed as a good predictor of survival and prognosis. Older age was described with the prognostic factor at highest impact on OS.

PB1751

CLINICAL STUDY OF SRSF2 MUTATION IN CHINESE WITH CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Recently, spliceosome mutations have been identified in myeloid malignance. SRSF2, as one of components of the splicing machinery, has a high mutation frequency in chronic myelomonocytic leukemia (CMML) according to previous reports. However, little is known of it in Chinese people.
Aims: In our study, we enrolled 38 Chinese patients with CMML and analyzed the status of SRSF2 mutations and their clinical features.
Methods: 38 patients with CMML were recruited from department of hematology in Jiangsu province hospital and Wuxi people hospital bwtween 2012-01 and 2013-12. The diagnoses of all of them and acute myeloid leukemia(AML) transformation were according to the criteria of WHO 2008 classification. We analyze the state of SRSF2 and clinical features(age, sex, karyotype,peripheral blood count) by polymerase chain reaction (PCR) followed by direct sequencing.
Results: 8 of 38 patients(21%) existed SRSF2 mutations, including 4 P95R, 295H and 2P95L point mutations. No significantly statistical differences were observed with regard to their clinical characteristics such as sex, preipheral blood count and karyotype between mutant and wildtype group except age. 1 case with mutation had a early evolution to AML.
Summary and Conclusions: We conclude that SRSF2 mutation probably has a low frequency in Chinese patients with CMML and might be related to old age and unfavorable prognosis. It might be a potentially valuable diagnostic maker and therapeutic target in CMML.

PB1752

NOVEL QUANTITATIVE BEAD-BASED SUSPENSION ASSAY FOR DETECTION OF DNMT3A R882 MUTATIONS USING BNA(NC)-MODIFIED PROBES

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Background: Somatic mutations in the human DNA methyl transferase 3A (*DNMT3A*) gene are found in a number of myeloid malignancies such as Philadelphia chromosome-negative myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS). and acute myeloid leukemia (AML). They have been shown to confer worse prognosis in most of these entities. Notably, about 2/3 of these mutations are missense mutations in codon R882 of the gene. The identification of these mutations is gaining a wider recognition in the field of clinical hematology as part of the integrated molecular profiling of myeloid malignancies.
Aims: We aimed at the development and validation of a novel easily applicable in routine practice method for quantitation of the *DNMT3A* R882C/H/R/S mutant alleles using a bead-based suspension assay.
Results: We initially tested the performance of two sets of beads carrying specific hybridization probes. One set of the beads carried LNA-modified probes while the other set carried BNA(NC)-modified hybridization probes. Initial testing on plasmid constructs showed a better performance of the BNA(NC)-modified probes with an optimal hybridization temperature of 66°C. The method was further validated on the plasmid standards at different ratios between the wild type and mutant alleles and clinical samples from 120 patients with known or suspected myeloid malignancies. The method appeared to be quantitative and showed sensitivity of 2.5% for all mutant alleles, making it significantly superior to direct sequencing.
Summary and Conclusions: This is the first report on the detection of DNMT3A R882 mutations using bead-based suspension assay with LNA/BNA-modified probes. Our data showed that it could be successfully implemented in the diagnostic work-up for patients with myeloid malignancies, as it is rapid, easy and reliable in terms of specificity and sensitivity. Besides, coupling of the assay with techniques for mutations enrichment (*e.g.* COLD-PCR) or fluorescent signal enhancement can make it useful even for quantitative assessment of MRD.
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PB1753

DIABETES MELLITUS TYPE 2 AT DIAGNOSIS OF PRIMARY MYELOFIBROSIS AFFECTS THE PATIENT OVERALL SURVIVAL

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Background: Many evidences suggest that diabetes mellitus 2 (DM2) increases the risk and the mortality of several type of solid cancers. These findings are still debated in hematologic tumors, including Ph-negative myeloproliferative neoplasms (MPNs).

Aims: In the present study we aimed to evaluate whether DM2 affected the clinical outcome of 230 patients with primary myelofibrosis (PMF).
Methods: All the 230 PMF patients were diagnosed and followed in the Latium region (Italy) between 1981 and 2010. Diagnosis of PMF was made according to the criteria accepted at the time the patients were seen. Patients with a PMF following polycythemia vera or essential thrombocythemia were excluded. DM2 was defined as a fasting glucose level >126 mg/dL or a postprandial glucose level >200 mg/dL. Therapy for PMF was variable and reflected the disease individual characteristics and the therapeutic strategies adopted by single Institutions at the time of PMF diagnosis. During the patient follow-up we recorded the occurrence of thrombotic events and leukemic transformations (LT), defined as the appearance of 20% blast cells in peripheral blood and/or in bone marrow.
Results: At diagnosis of PMF, 44 of the 230 patients (19.1%) had DM2. Except for a platelet count that was significantly lower in the diabetic than in non-diabetic patients (333.53 x10^9/L *vs* 443.25 x10^9/L, P=.047), no other considered clinic-biologic features differed between the two studied groups. However, the 10 year overall survival rates and the median survival time in non–diabetic and diabetic patients were 37.1% *vs* 12.6%, and 101 (95% CI: 81-121) *vs* 52 (95% CI: 33-70) months, respectively (p=.0009). At the multivariate analysis DM2, older age, anemia and leukocytosis resulted the only independent prognostic factors affecting patient survival. Thrombosis and leukemia free survival rates were similar in either patient groups (Figure 1).

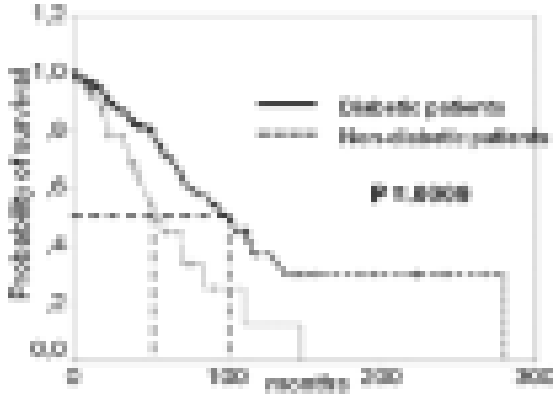

Figure 1. Probability of overall survival in PMF diabetic and non-diabetic patients.

Summary and Conclusions: The retrospective nature of our study did not allow to clarify whether DM2 would affect the probability of survival by acting on the progression of the disease or by promoting fatal events independently by PMF. However, our data still recommend to explore the possible pathogenic link between DM2, inflammation and Ph-MPNs and to be extremely careful to the diagnosis and the treatment of DM2, even increasing in this patient setting the use of metformina, an insulin sensitizers with proven anticancer activities.

Hodgkin lymphoma - Clinical

PB1754

BRENTUXIMAB VEDOTIN FOR RELAPSED OR REFRACTORY HODGKIN LYMPHOMA: EXPERIENCE IN TURKEY

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Background: Current treatment modalities can cure up to 70-80% of patients with classical Hodgkin lymphoma (cHL) and 20-30% of patients require further treatment options. Brentuximab vedotin (BV) is an antibody-drug conjugate showing an impressive activity against relapsed/refractory cHL.
Aims: In this study we aimed to assess the efficacy and safety of BV and help determination of its best role and timing.
Methods: Eleven Turkish institutions participated in this multicenter, retrospective study. Eligible patients were required to be treated with at least two courses of BV without any limitations regarding performance status and organ function. The primary endpoint of the study was the objective response rate (ORR), secondary endpoints were safety, overall survival (OS) and progression free survival (PFS). Response was assessed by PET/CT or CT early during disease course after 2-5 cycles and after ≥ 6 cycles. Analysis of the data was carried out using STATA 11.1 SE software. All patients provided written informed consent.
Results: 58 patients treated from March 2011 to July 2013 were included. Demographics and baseline disease characteristics are summarized in Table 1. In total a median of 7 (range, 2-18) courses of BV were given as a single agent. Relative dose intensity was calculated as 81.56%. ORR of 63.5% with 26.5% complete remission (CR) was achieved at early assessment. Response assessment at ≥6 cycles showed an ORR of 32.4%. PFS at 12 months was 32.83% (95% CI, 19.74%>46.56%) and the median PFS was 7 months (95% CI, 4.81%>11.27%). Forty-four patients were alive at the time of data analysis. OS at 12 months was 70.58% (95% CI, 54.37%>81.94%) and the median OS has not been reached yet. Twelve patients had been treated with 3 or less previous chemotherapy regimens before BV and half of the patients achieved CR. CR rates in patients treated with >3 chemotherapy regimens before BV are significantly lower (p=0.016). Fourteen patients proceeded to transplantation (7 allogeneic, 5 haploidentical and 2 autologous transplantation) among whom 85% were alive. In general the treatment was well tolerated with dose reduction necessary in 5 patients (3 due to cytopenias, 1 peripheral neuropathy, 1 unknown). The most common (≥ 10%) adverse events were fatigue, nausea, neuropathy, neutropenia, vomiting, myalgia, alopecia and extremity pain. Two patients from different centers suffered from generalized tonic convulsions one of whom was on renal replacement therapy. Convulsions occurred after 5 and 2 cycles of BV treatment. Since drug induced neurotoxicity could not be excluded, BV was stopped in both patients.

Table 1. Patient demographics and disease characteristics.

Summary and Conclusions: In general treatment was well tolerated and toxicities were generally grade 1 and 2 in severity. As might be expected patients with less prior chemotherapy regimens had better CR rates compared with more heavily pretreated patients (p=0.016). Our study indicates that best responses to BV are observed early during the treatment course. Since best results are obtained with chemosensitive disease and minimal tumor burden both in autologous and allogeneic stem cell transplantation, transplantation should be considered earlier during BV treatment when best responses are achieved.

PB1755

AN ADDITIONAL POINT TO CONFIRM A REMISSION IN HODGKIN'S LYMPHOMA

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Background: In recent years in the treatment of Hodgkin's lymphoma was achieved a considerable success. Nevertheless, in patients with large tumor mass in the onset of the disease, mediastinum involvement, a residual tissue after the end of therapy makes it necessary to clarify remission. We believe that determining an expression profile of cancer-testis genes (CTG) could help with it. These genes are expressed in germinal cells and tumor cells only.
Aims: To analyze CTG expression profile in biological samples of primary Hodgkin's lymphoma patients and compare this data with expression profile after an achieving of a complete remission (CR).
Methods: Peripheral blood (PB) and lymph nodes (LN) from 27 primary patients with Hodgkin's lymphoma were examined using RQ PCR to evaluate mRNA expression levels of CTG *GAGE1*, *NY-ESO-1*,*MAGEA1*, *PASD1*, *SCP-1*, *SEMG1*, *SPANXA1*,*SSX1* and *PRAME* in comparison with *ABL*expression level. The group of patients consisted of 12 men and 15 women. Median age was 36 y.o. Majority of patients had advanced stage of disease according Ann-Arbor classification, mediastinum involvement (20/27). All patients were treated according to the protocol BEACOPP-14. Also we examined a CTG expression profile in a PB of 7 patients after therapy. We PB samples of 15 healthy donors and 5 reactive lymph nodes were as a negative control.
Results: In PB of primary Hodgkin's lymphoma patients we observed an expression of *GAGE1* (2/27, 7%), *NY-ESO-1* (6/27, 22%), *PASD1* (2/27, 7%), *SEMG1* (12/27, 44%), *SPANXA1* (3/27, 11%), *SSX1* (6/27, 22%) and *PRAME* (4/27, 15%) in different levels. For example, median value for *GAGE1* was 1%; *NY-ESO-1* - 0,2%; *PASD1* - 0,3%; *SEMG1* - 11%; *SPANXA1* - 1,7%; *SSX1* - 0,1% and *PRAME* - 0,06%. We didn't observe an expression of *MAGEA1* and *SCP-1* in PB samples. We revealed an expression of *GAGE1* (2/27, 7%), *NY-ESO-1* (6/27, 22%), *MAGEA1* (2/27, 7%), *PASD1* (4/27, 15%), *SCP-1* (1/27, 4%), *SEMG1* (5/27, 27%), *SPANXA1* (4/27, 15%), *SSX1* (2/27, 7%) and *PRAME* (17/27, 63%) in a LN samples. Neither of analyzed CTG wasn't expressed in a PB of 7 patients after therapy has been finished.There wasn't any CTG expression in a control samples.
Summary and Conclusions: A CTG expression in a PB indicated that the tumor cells could circulate between healthy cells. A lack of these genes expression confirms the success of the therapy and can support a remission achievement.

PB1756

EVALUATION OF BONE MARROW INVOLVEMENT IN PATIENTS WITH LYMPHOMA: F-18 FDG PET/CT VERSUS BONE MARROW BIOPSY

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Background: The evaluation of bone marrow infiltration (BMI) is of crucial importance in the staging of lymphoma. Although bone marrow biopsy (BMB) is the reference method for the evaluation of BMI, it has limitations (e.g taken from a single field, invasiveness).
Aims: The aim of this study was to assess the performance of 2-deoxy-2-[18F]fluoro-D-glucose (18F-FDG) PET/CT against bone marrow biopsy (BMB) in the initial diagnosis of bone marrow infiltration (BMI) in patients with Hodgkin's Lymphoma (HL) and Non-Hodgkin's Lymphoma (NHL), retrospectively. Newly diagnosed 172 consecutive patients (50 female, 122 male; 64 HL, 108 NHL) who were referred to our department between July 2009 and December 2013 were included in this study. All patients were examined by whole-body 18F-FDG PET/CT scan for initial staging. Evaluation of BM with PET/CT scan was done visually and semi-quantitatively. The maximum standard uptake value (SUVmax) was used as quantitative parameters in the evaluation of PET/CT. PET/CT was considered positive for BMI in cases of uni-or multifocal bone marrow (18)F-FDG uptake that could not be explained by benign findings on the underlying CT image or history. A final diagnosis of BMI was considered if the BMB was positive.
Methods: Newly diagnosed 172 consecutive patients (50 female, 122 male; 64 HL, 108 NHL) who were referred to our department between July 2009 and December 2013 were included in this study. All patients were examined by whole-body 18F-FDG PET/CT scan for initial staging. Evaluation of BM with PET/CT scan was done visually and semi-quantitatively. The maximum standard uptake value (SUVmax) was used as quantitative parameters in the evaluation of PET/CT. PET/CT was considered positive for BMI in cases of uni-or multifocal bone marrow (18)F-FDG uptake that could not be explained by benign findings on the underlying CT image or history. A final diagnosis of BMI was considered if the BMB was positive.
Results: For all cases, in visual assessment for BMI with PET/CT, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 31%, 85%, 39% and 79%, respectively. In subgroup analysis sensitivity, specificity, PPV and NPV were; 80%, 78%, 24%, 98% in HL patients and 24%, 90%, 56%, 70% in NHL patients, respectively. In semi-quantitative evaluation, when the SUV max was set as 4 in HL, the sensitivity was 80% and the specificity was 68%. When the SUVmax was set as 3,2 in NHL the sensitivity was 65% and the specificity was 58%.
Summary and Conclusions: Currently, BMB is recommended for the detection of BMI in lymphoma. However PET/CT imaging is more valuable than BMB for the initial staging of the lymphoma. PET/CT detects more BM involvement in HL and NHL compared with BMB. Thus it can be also used as a complementary technique to determine the biopsy site for BMB.

PB1757

TAILORED THERAPY FOR ADVANCED-STAGE HODGKIN LYMPHOMA, GUIDED BY INTERIM 18FDG-PET, WITH BEACOPP-ESCALATED FOLLOWED BY ABVD REGIMEN DECREASES REFRACTORY DISEASE RATES

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Background: Hodgkin lymphoma has become a curable disease with the introduction of ABVD regimen (Doxorubicin, Bleomycin, Vinblastine and Dacarbazine), but some patients (pt) with advanced-stage Hodgkin Lymphoma (adv-HL) go through insufficient results: 15-20% of total behave as primary refractory, 20-30% undergo early and long-term relapses and 60-65% of freedom for treatment failure at 5 years. It means that 30%>40% of adv-HL pt treated with ABVD will experience treatment failure after first-line treatment, and will require salvage autologous stem-cell transplantation (ASCT) in most cases. The German Hodgkin Study Group developed a more intensive regimen for treatment of adv-HL consisting of Bleomycin, Etoposide, Doxorubicin, Cyclophosphamide, Vincristine, Procarbazine, and Prednisone (BEACOPP). An escalated dose variant, BEACOPP escalated (BEACOPP-esc) was studied in the HD9 trial, where it showed 86% of freedom for treatment failure at 10 years. However, BEACOPP-esc was more toxic than the other regimens.
Aims: Developing a tailored treatment BEACOPP-esc and ABVD-based guided by interim ^{18}FDG-PET (PET) in adv-HL, to try decreasing primary refractoriness to first-line treatment with least toxicity.
Methods: We defined adv-HL as stages IIB, III and IV, and International Prognosis Score (IPS) was used to stratify pt into intermediate risk (0-3) and high risk (4-7) of relapse. It was regarded classic treatment as ABVD regimen for 6-8 cycles followed by radiotherapy (RT) over bulky areas (especially mediastinal masses). Since July 2011, we changed therapy for adv-HL to that we named tailored regimen: BEACOPP-esc for two (IPS 0-3) or four cycles (IPS 4-7) followed by early response evaluation with interim PET. Patients with negative-PET, therapy was deescalated to ABVD for 4 cycles and RT was finally indicated over initial bulky areas or residual areas with positive-PET.
We analyzed retrospectively 33 consecutive adult pt with classic adv-HD treated since November 2008 until July 2013. Median age was 38 years [16-62]. Fourteen pt received the classic treatment (group 1) and 19 pt were treated with the tailored regimen (group 2). Pt older than 65 years were excluded because were uniformly treated with COPP (Cyclophosphamide, Vincristine, Procarbazine and Prednisone).
Results: After a median follow-up time of 24 months, we observed the results that are expressed in the Table 1. Twenty-one percent (3/14) of primary refractoriness and 21% of relapses was observed in Group 1, while only 1 refractory patient (1/19, 5%) was present in Group 2 with no relapses; there were not statistically significant differences because of the small number of patients in each group. Fifty-seven percent of relapsed/refractory pt were

underwent to ASCT, being successfully only in 50% of them. Acute toxicity was manageable, but the follow-up is not enough to describe deleterious late effects as secondary malignancies.

Table 1.

Summary and Conclusions: ABVD treatment is insufficient in terms of control of the disease in adv-HL, with a high rate of relapse and primary refractoriness, which only can be rescued with ASCT in half of cases. Initial treatment with BEACOPP-esc, guided by an early response evaluation with PET to deescalate therapy intensity to ABVD in responders allowed us reducing the rate of primary refractoriness to 5%, with no relapse. Based in our results, we propose this therapeutic strategy in pt with adv-HL.

PB1758

BONE MARROW INVOLVEMENT IN HODGKIN LYMPHOMA: CAN 18F-FDG PET/CT REPLACE TRADITIONAL BIOPSY?

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Background: The bone marrow biopsy (BMB) in iliac crest, either unilateral or bilateral, is the standard method for detecting marrow involvement in Hodgkin lymphoma (HL). However, involvement use to be infrequent (5-14%) and mainly focal, reasons why some authors consider this procedure unprofitable. Moreover, ^{18}F-FDG (fluorodeoxyglucose-^{18}Fluor) is very rapidly absorbed by this type of lymphoma.
Aims: Our goal is to compare the BMB and ^{18}F-FDG PET/CT to detect marrow involvement in patients with HL, and to analyze the impact of this new technique in the staging and clinical management of the disease.
Methods: We retrospectively analyzed a total of 65 HL patients in which ^{18}F-FDG PET/CT and unilateral BMB was performed at initial staging or relapse. Clinical and biological parameters were recorded, together with the results of both procedures. We also analyzed those patients in whom the ^{18}F-FDG PET/CT caused a change in stage or a modification in treatment, when compared with the diagnostic combination of BMB plus CT.
Results: The 65 HL patients included 36 men and 29 women, mean age 37 years (range 13-76). Marrow involvement was detected by either of the two techniques in 23% (15/65) of patients: BMB was positive in 3/15 cases, while the ^{18}F-FDG PET/CT showed marrow disease in 15/15 cases. The 3 patients with positive BMB showed diffuse pattern of marrow involvement by ^{18}F-FDG PET/CT adding multifocal involvement in 2 of them. Of the 12 marrow-affected cases by ^{18}F-FDG PET/CT but negative by BMB, 6 had a multifocal pattern, 4 unifocal, one diffuse pattern without focal lesion, and one diffuse and multifocal. None of the cases with focal pattern had involvement in the iliac crest. In a case with unifocal rib lesion, directed biopsy confirmed marrow involvement. Forty of the 65 patients had a concomitant CT and in this group the ^{18}F-FDG PET/CT led to a change in staging in 15 cases (37%), always towards a more advanced stage. Finally, in 8 of these 15 patients, this change in disease stage was also associated to a change in the therapeutic approach, always towards a more aggressive treatment.
Summary and Conclusions: Although these data should be confirmed in larger series of patients, we have not found any diagnostic benefit of BMB over ^{18}F-FDG PET/CT in HL staging. Our results are consistent with those reported in the literature, showing all the series a greater sensitivity for 18F-FDG PET/CT in the diagnosis of bone marrow involvement, when compared with BMB. This increased sensitivity is probably related to the intense avidity of HL to ^{18}F-FDG of LH and to the higher frequency of patchy (instead of diffuse) marrow involvement. Directed biopsy to specific accumulation of ^{18}F-FDG may help to investigate the possibility of false positive cases.

PB1759

TREATMENTS AND CLINICAL BURDEN AMONG HODGKIN LYMPHOMA PATIENTS AT HIGH RISK OF RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANT: A LITERATURE REVIEW

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Background: Hodgkin lymphoma (HL) patients with certain clinical characteristics such as refractory, relapsed, or progressive HL, extranodal involvement, or B symptoms are at high risk of relapse after receiving an autologous stem cell transplant (ASCT).
Aims: To conduct a literature review of the treatments and clinical outcomes among patients with HL at high risk of relapse after ASCT.
Methods: A literature review was conducted (search period: January, 1993 to December, 2013) to identify publications of randomized or observational studies that included HL patients who had received adjuvant therapy after ASCT and before relapse, and reported characteristics and outcomes in this subgroup of patients. Patient demographics, disease characteristics, prognostic factors, adjuvant therapies, and clinical outcomes were extracted from the full text of the identified studies.
Results: Of the 147 studies retrieved, 15 (9 prospective and 6 retrospective) studies met the inclusion criteria. The prognostic factors reported varied across studies. Most studies reported proportions of patients bearing a certain risk factor, but not overall proportions of high-risk patients. No studies included only high-risk patients. Reporting of key trial and patient characteristics was variable, with ranges as follows: sample size, n=13–245; patients' median age, 15–44 years;% male, 39%–69%; history of refractory HL, 15%–61%; relapsed/progressive HL, 13%–69%; extranodal involvement, 34%–54%; and B symptoms, 11%–85%. Adjuvant therapy use varied from 3% to 100%; the most common adjuvant therapies used included radiotherapy, tandem ASCT, and chemotherapy. No universal post-ASCT or pre-relapse standard of care was mentioned in the included studies. Clinical remission rates and survival were reported for overall study populations rather than for the high-risk population. Complete response rates after ASCT ranged from 71% to 92%, while 3-year survival ranged from 55% to 75%.
Summary and Conclusions: While factors prognostic for post-ASCT HL relapse are known, patients at high risk of relapse following ASCT are not a well-defined population in the literature. Although several studies suggest that patients with specific prognostic factors suffer from a higher clinical burden than those patients without the respective risk factors, no established guidelines exist for the treatment of such HL patients at high risk of relapse. Data on the efficacy of adjuvant treatments are sparse and come from subgroup analyses in a small set of non-randomized studies. Randomized studies in this population will be critical to establish effective adjuvant therapies and address this unmet need.

PB1760

SALVAGE TREATMENT WITH SINGLE-AGENT BENDAMUSTINE FOR RELAPSED/REFRACTORY HODGKIN LYMPHOMA: AN ITALIAN MONOCENTRIC EXPERIENCE

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Background: The prognosis of patients (pts) with Hodgkin lymphoma (HL) who had multiple relapses or refractory disease remains poor. In transplant-ineligible pts and post-autologous stem cell transplantation (ASCT) recurrences, a standard treatment doesn't exist and allogeneic SCT can achieve a long-term disease free survival only in selected pts. However, some novel agents with promising single-agent activity, including bendamustine, has lately emerged.
Aims: To evaluate the safety and efficacy of bendamustine in advanced HL.
Methods: We retrospectively evaluate our experience on relapsed/refractory HL pts who received single-agent bendamustine at the dose of 120 mg/m2 every 21-28 days.
Results: From 2/2010 to 8/2013 10 pts affected by HL (8 with refractory disease and 2 in second relapse) received bendamustine at our Institution. Before starting bendamustine treatment, the clinical characteristics of pts were as follows: median age 39 years (range 22-70), 6 pts male and 4 female, median number of prior treatment lines 3 (range 2-3), including ASCT in 3 pts, and median time from initial diagnosis 29 months (range 11-103). The patients received a median of 4 courses of bendamustine (range 2-6). Treatment was well tolerated in all patients, without life-threatening or unexpected extra-haematological adverse events. Particularly, no cases of cutaneous adverse effects were recorded. Haematological toxicity was limited. Grade 3-4 thrombocytopenia occurred in 10% of cycles, grade 3-4 anaemia in 4% and

grade 3-4 granulocytopenia in 2%. The overall response rate was 50%, with 4 complete response (CR), 1 partial response, 1 stable disease and 4 progressive disease. Two of the 4 pts who achieved CR relapsed after 12 and 18 months, respectively. Today, after a median follow-up of 35 months for living pts, overall survival is 35% and median time to progression or relapse 6 months (range 2-24). Overall, 5 pts deceased for HL progression and 5 pts are still alive.
Summary and Conclusions: Our results confirm the safety and the efficacy of bendamustine in pts who had undergone multiple lines of treatment. With this in mind we look at some possible scenarios involving bendamustine. Firstly, because allogeneic SCT represents a chance of cure in a subset of relapsed/refractory pts, bendamustine may be considered a bridge to transplant. Furthermore, combination studies of bendamustine with other anticancer drugs or biological agents aimed to maximize the response rate and response duration are ongoing. In this regard, combination of brentuximab vedotin and bendamustine seems very promising. Currently, a phase I/II multicentre prospective study of bendamustine in combination with lenalidomide (LEBEN; ClinicalTrial.gov identifier NCT 01412307) is ongoing at our Institution. Finally, considering its favourable safety profile, single-agent bendamustine could be a valuable resource for frail patients who are not eligible to intensive salvage regimens for Hodgkin lymphoma.

PB1761

ABVD WITH OR WITHOUT RADIOTHERAPY IS SAFE AND EFFECTIVE FOR THE TREATMENT OF PATIENTS AFFECTED BY CLASSIC HODGKIN LYMPHOMA AGED 18 YEARS OR YOUNGER

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Background: ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) with or without radiotherapy (RT) is the standard of care for the majority of adult patients affected by classical Hodgkin lymphoma (cHL). The treatment of pediatric cHL is based on the experience of adult treatment regimens. Since most pediatric patients with cHL are successfully treated and late toxicities vary according to the treatment modality, an important consideration in children and adolescents is the selection of the treatment regimen, particularly in reference to the anticipated late toxicities associated with anticancer therapy.
Aims: To evaluate safety and efficacy of ABVD alone or combined with RT in young patients affected by cHL.
Methods: We performed a retrospective analysis on cHL pts aged 18 years or younger, treated at our Institution with ABVD with or without RT as well as adults.
Results: From June 1991 to June 2013, 26 consecutive young pts affected by cHL were diagnosed and treated at our Institution. The clinical characteristics of pts at diagnosis were as follows: median age 16 years (range 6-18), 11 pts male and 15 female, clinical stage I-II 19 pts (73%) and III-IV 7 (27%), B symptoms 9 (35%), Bulky mass 9 (35%). Treatment strategy varied according to the extent of the disease: the pts with clinical stage I-II received ABVD plus involved field RT (IFRT) and the pts with stage III-IV received ABVD alone. According to the different periods of treatment, the number of cycles ABVD varied from 3 to 4 for initial stages, and from 6 to 8 for advanced stages. The dose of IFRT varied from 20 to 36 Gy. Overall response rate was 92%. In detail, 24 pts achieved complete remission (CR), while 2 pts failed to respond to ABVD. One of them died after 13 months for progressive disease, while the second one was rescued with IGEV salvage chemotherapy plus autologous PBSCT. After a median follow-up of 94 months (range 7-278) overall survival rate is 96% and freedom from treatment failure 92%. Among the 24 CR pts no relapses were recorded until now. With regard to the late toxicities, all 15 female pts present normal menses today, while 2 pts developed a mild hypothyroidism. Finally, there aren't serious cardiac and pulmonary complications so far.
Summary and Conclusions: Our results obtained in a small group of children and adolescents with cHL who received ABVD alone or in combination with IFRT seem very encouraging. The major concerns for children with HL treated with ABVD plus RT regard the risks of late toxicities, especially in the case of mediastinal RT. Modern pediatric treatment strategies have been focused on reducing late effects of therapy with the development of risk-adapted chemotherapy alone or response-adjusted combined-modality regimens. We think that the low burden of late effects that occurred in our experience may be in part due to the short follow-up of some pts. However, irrespective of this point of view, we believe that achieving CR is always the primary goal of a treatment regimen, because the prognosis of relapsed/refractory HL pts remains still poor. Therefore, outside of clinical trials, we think that ABVD alone or combined with modern RT, in agreement with clinical stages, may still constitute the standard of care for the majority of both pediatric and adult pts affected by cHL. In this context, the use of reduced volumes and doses of RT and the availability of liposomal doxorubicin could contribute to further reduce the risk of late toxicities.

PB1762

RESPONSE- ADAPTED THERAPY AFTER INITIAL TOW ESCALATED BEACOPP IN ADVANCED ADULT HODGKIN LYMPHOMA: TUNISIAN PROSPECTIVE TRIAL (MDH2008) RESULTS

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Background: Advanced Hodgkin lymphoma (HL) still present many therapeutic problems of primary failure and relapses (20-30%) specially in developing countries. To improve the outcome of this unfavorable disease, Escalated(Esc) BEACOPP was used since 2008 to treat all advanced HL in Tunisia (Tunisian Prospective adult Hodgkin lymphoma trial: MDH2008 Group3).
Aims: We propose to report and analyze therapeutic results and prognostic factors in Tunisian advanced Hodgkin lymphoma patients(pts)
Methods: 85 newly advanced HL pts (Stage II with Bulky mediastinal disease, III and IV) were enrolled in group3 (Gr3) of the Tunisian prospective adult Hodgkin lymphoma trial (MDH2008) and treated in the clinical hematology department of Aziza Othmana hospital-Tunis from July 2008 to December2011. Pts were initially received two Esc BEACOPP followed by reevaluation with CT scans. When response≥75% was achieved, de-escalation of treatment to 6 ABVD was allowed to minimize long-term toxicity.Pts who failed to obtain this response received two other Esc BEACOPP with a second reevaluation:When response≥75% was achieved, pts received four Standard doses BEACOPP and Pts who failed to have this response received salvage therapy with autologous stem cell transplantation.
Results: Median age at diagnosis was 31 years. 22% of pts were classified as stage II with mediastinal Bulky and 45% as stage IV. International prognostic score≥3 was observed in 41% of pts. 77.5% of pts were in complete response after the first line therapy. At the univariate study, the mediastinal bulky (69% *vs* 93%, p:0.009) and response<75% to 2 Esc BEACOPP (70% *vs* 98%, p:0.002) were the adverse prognostic factors concerning response status at the end of therapy. 15% were refractory pts and the mediastinal bulky and response<75% to 2 Esc BEACOPP have consistently been demonstrated to be predictors of poor outcome. Three year event free survival (EFS), relapse free survival (RFS) and overall survival(OS) were respectively 68%, 87% and 86%. In multivariate study, the unfavorable prognostic factors emerging were mediastinal bulky for OS (82% *vs* 92%, p:0.05), lymphopenia <600/mm3 for EFS(20% *vs* 70%, p:0.000) and OS(40% *vs* 90%, p:0.000) and the response<75% to 2 Esc BEACOPP for EFS(57% *vs* 94, p:0.001). Acute hematologic toxicity was observed in about 71% of patients. Death related to acute toxicity was observed in 3 patients.
Summary and Conclusions: In our country, Esc BEACOPP improve the OS and the RFS but the EFS remains lower than in published studies . Bulky mediastinal disease and response <75% to 2 Esc BEACOPP have been demonstrated to be predictors of poor outcome, requiring treatment intensification.

PB1763

SECOND NEOPLASMS IN PATIENTS WITH HODGKIN LYMPHOMA

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Background: As the majority of patients with Hodgkin lymphoma (HL) are able to achieve long-term survival free of HL, late complications of treatment have emerged as a competing cause of death and morbidity. An important complication of the successful treatment strategies and the long-term survival is the occurrence of second malignant neoplasms.
Aims: Evaluate the occurrence of second neoplasms in long-term survivals after HL treatment.
Methods: It was analysed clinical datas of patients diagnosed with HL between January 1990 and December 2012.
Statistic analysis were performed with the SPSS (V20).
Results: We included 314 patients and have identified second neoplasms in sixteen of these patients (5,1%). There were 12 males (75%) and 4 females (25%). Ten patients received combined treatment with chemotherapy and radiotherapy and six patients were treated with chemotherapy alone. The median time to diagnosis of secondary neoplasms was 7,6 years (range, 0,74 to 23.34 years). Median age at diagnosis of second neoplasm was 41.5 years (32-71 years). The median follow-up time of the 314 patients was 5,49 years (range, 2 days – 21 years). Second neoplasms were: 3 non-hodgkin lymphoma, 3 acute myeloid leukemia, 2 bladder carcinoma, 2 gastric adenocarcinoma, 1 breast carcinoma, 1 Leydig cell testicular tumor, 1 cerebral sarcoma, 1 thyroid carcinoma, 1 neuroendocrine tumor, 1 chronic myelomonocytic leukemia, 1 prostatic adenocarcinoma and 1 pancreatic adenocarcinoma. Two patients developed 2 second neoplasms. Only two patients didn't receive treatment for the secondary malignancy. Eight patients are alive, 1 missed follow-up and seven patients died (3 with acute myeloid leukemia, 1 with chronic

myelomonocytic leukemia, 1 with resistant non-hodgkin lymphoma, 1 with gastric adenocarcinoma and 1 with pancreatic adenocarcinoma).
Summary and Conclusions: Patients treated with chemotherapy and/or radiotherapy for HL have an increased risk of developing acute myeloid leukemia, non-Hodgkin lymphoma and solid tumors, according to the literature. Long-term follow-up is mandatory for survivors of HL to early recognition of second neoplasms and initiation of adequate treatment for better results.

PB1764

HODGKIN LYMPHOMA IN THE WEST OF ALGERIA: PANORAMA OF EPIDEMIOLOGICAL AND CLINICAL FEATURES, INITIAL WORK-UP, SURVIVAL AND RISK FACTORS DISTRIBUTION

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Background: In Algeria, the incidence of hematologic malignancies has been difficult to estimate for many years. Today, many hematological centers, including 14 university hospitals, have been developped in the entire north and have useful epidemiological data pertinent to Hodgkin'sLymphoma (HL).
Aims: The principal object of this study is to represent - in maximum accuracy- the real status of Hodgkin's lymphoma in west of Algeria, we think also it serves as an adequate indicator for the epidemiological characteristics of HL patients and as an overview of theses very patients treatment and survival in Algeria in general.
Methods: From January 2008 to December 2012 , 668 patients were included in this study, from the age of 15 to 88 years, and both genders. 8 hematology centers participated covering the west of Algeria. An including criteria which respects the representation of the socio-economic status distribution was adapted for this study. Also we included patients of most professions that present in reality with in the west Algerian community. All diagnostic procedures and treatment policies were unified between the involved center, the pilot center (Oran1) adapted a strict system for data collection, control and confirmation, this system included: standard follow-up and standard software that been installed in all other centers to serve as data collection and organization program which send full and unified tables to the study data manager to be reviewed and confirmed by control committee for the final processing. The therapeutic approach consisted of chemotherapy combined or not with radiotherapy according to clinical stage followed when appropriate with intensification protocol and autologous stem cell transplantation.
Results: Of our 668 patients: 53% were males, 47% females, global median age (36 years) and mean age (33 years). 65% of all patients had nodular sclerosis subtype, localized disease was presented in 22% of our study patients, while 78% presented advanced stages, rates of peripheral bulky disease, mediastinal bulky and non-bulky disease were 8%, 45% and 47% respectively. 9% of patients had bone marrow involved by HL, 18% of patients had imaging of affected spleen, while 9% had features of affected liver. 74% of patients had one or more of B symptoms, 68% of males and 74% of females were anemic, 28% of patients had leukocytosis. Cumulative Overall Survival (OS) was estimated in 352 evaluable patients: 59% (at 60 months) , 69% (at 48 months), while 79% of patients were alive after 5 years of follow-up. Cumulative OS male was 64% (at 48 months), and Cumulative OS female: 75% (48 months) P-value>0.05. For patients aged more or less than 45 years, cumulative OS were 43 and 49 months respectively for the means of OS (p=0.016). Of the 352 evaluable patients, 129 patients had reached CR and been qualified for DFS statistical analysis. Relapse rate was 16%, cumulative DFS was 69% (at 47 months), no statistically significant difference had been detected between males and females (p=0.811).
Summary and Conclusions: Differences in survival rates between this study and publications may be explained partially by the very high frequency of negative prognostic factors in our cohort like bulky and advanced disease, B-symptoms, and anemia positivity rates, lack of PET-CT and radiotherapy centers number may had also affected this rate, even though we found ourselves very interested in deepening further more in diagnosis, treatment and follow – up modalities standardization and development in west of Algeria in order to raise the level of health services offered to HL patients to meet eventually developed countries survival rates.

PB1765

EFFICACY AND TOXICITY OF BRENTUXIMAB VEDOTIN MONOTHERAPY IN RELAPSED OR REFRACTORY CD30+ CLASSICAL HODGKIN LYMPHOMA PATIENTS OUTSIDE CLINICAL TRIALS

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Background: Brentuximab Vedotin (BV) is a drug-conjugate antibody composed of an anti-CD30 antibody linked to a microtubule-disrupting agent monomethyl auristatin E (MMAE) with proven efficacy in various CD30 positive lymphoprolipherative disorders. In Italy BV is approved since October 2012 for the treatment of classical Hodgkin Lymphoma (cHL) patients (pts) relapsed after autologous stem cell transplantation (ASCT) or relapsed/refractory after at least two prior multiagent chemotherapy regimens if not eligible to ASCT.
Aims: We retrospectively collected safety and efficacy data of 9 consecutive CD30+ cHL pts treated in our institution with BV, with the purpose of confirming tolerability profile of this drug in the daily practice, outside clinical trials.
Methods: Nine pts with refractory and relapsed cHL received BV therapy at the standard dose of 1.8 mg/kg every 3 weeks by IV infusion. BV premedication consisted in acetaminophen 500 mg po and chlorphenamin 10 mg iv. No antimicrobial prophylaxis was scheduled. All patients had histologically confimed CD30+ disease. Toxicitiy was assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Response was defined according to the Revised Response Criteria for Malignant Lymphoma. In 8/9 pts BV therapy was started as bridge to allogeneic stem cell transplantation; in these pts BV was continued untill the best response was achieved. One pt refused allogeneic transplant and a full course of BV was planned, up to 16 cycles. BV was stopped in case of disease progression or unacceptable toxicity.
Results: Median age was 38 years (range: 22-46). Nodular sclerosis was the most frequent histological subtype (6/9 pts), while mixed cellularity and not otherwise specified accounted for 2 and 1 pts, respectively. Six pts were refractory to the previous treatment received, while 3 pts had relapsed disease. Median number of prior therapy regimens was 7 (range: 3-10). All pts had failed a prior ASCT and seven pts had received radiotherapy. Median number of BV cycles administered was 7 (range: 1-16). Response to BV was evaluated in all but one pts with FDG-PET after four cycles: 2/8 (25%) pts achieved a complete response (CR), 4/8 (50%) pts achieved a partial response (PR), while 2/8 (25%) pts had progressive disease. Between responding pts, one pt refused to proceed to allogeneic transplant; 2 pts proceeded to MUD allogeneic transplantation with reduced intensity conditioning, 1 pt proceeded to haploidentical allogeneic transplantation. BV therapy is ongoing in two responding pts, waiting for unrelated donor availability. As regard toxicity we registered: grade 3 reactivation of Varicella Zoster Virus followed by grade 2 pneumonia in one patient and grade 3 herpetic ocular infection in one patient. One case of grade 3 necrotic hemorrhagic acute pancreatitis occured in a 22 years old female, with no additional risk for pancreatitis, 10 days after the first BV administration. The event required admission to Intensive Care Unit and completely resolved without surgical intervention. This pt withdrew BV therapy definitely. Neither significat infusional reactions nor peripheral neuropathy occurence were recorded.mHematological toxicity consisted in grade 3 neutropenia in 5/9 pts, with no case of grade 3 and 4 anemia and thrombocytopenia.
Summary and Conclusions: BV confirmed to be effective as single agent therapy in heavily pretreated relapsed/refractory cHL pts and allowed the majority of pts to proceed to allogeneic transplantation. Acute pancreatitis is a threatening, potentially fatal and unpredictable adverse event that should be kept in mind in daily management of BV exposed pts. Viral reactivations in our serie were unexpectedly severe; this finding suggest the opportunity to better investigate the role of routinely antiviral prophylaxis in association with BV therapy.

PB1766

PREVENTION OF REACTIVATION OF CHRONIC HEPATITIS B IN PATIENTS WITH HODGKIN'S LYMPHOMA DURING CHEMOTHERAPY.

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Background: In Russia, chronic hepatitis B (HBV) is a relatively frequent viral infection in patients with Hodgkin's lymphoma (HL). There is no consensus about the advisability prophylactic antiviral therapy during cytostatic therapy.
Aims: The aim of our study is the evaluation of the effectiveness of prophylactic antiviral therapy in patients with Hodgkin's lymphoma with markers of viral hepatitis B.
Methods: We analyzed the clinical data and results of therapy in 38 patients with, which were observed in the Russian Research Institute of Oncology from 2002 to 2012. Stages of HL were II in 21 cases, III-IV in 17 cases. B symptoms were in 17 patients. At diagnosis of LH increase the level enzymes was 21 patients: ALT ranged from 1.25 to 3 upper limit of norm in 21 cases; AST ranged from 1.5 to 2 upper limit of norm in 15 cases; GGTP ranged from 2 to 6 upper limit of norm in 11 patients, alkaline phosphatase was 2 upper limit of norm in 6 patients. HBV DNA was determined in 21 patients. Viral load was from $1.3x10^3$/ml to $6.5x10^7$/ml (median $1.4x10^4$/ml). HBsAg was revealed only in 17 patients. HL treatment was carried out in according to standards of treatment: 21 patients with II stage have received 6 cycles of ABVD, 17 patients with III-IV stages have received 6-8 cycles of BEACOPP. Additionally, high-dose

chemotherapy carried out in 6 patients due to inadequate effect of standard chemotherapy. All 38 patients were underwent antiviral therapy. Therapy with Lamivudine 100 mg/day was started 1-4 weeks before the chemotherapy and continued at the same dose during chemotherapy.
Results: Complete remission (CR) was achieved in 76% (16 of 21) of patients with II stage after ABVD and 59% of patients (10 of 17) with III-IV stages after BEACOPP. CR was achieved in patients after high-dose chemotherapy. The duration of therapy with Lamivudine was from 14 to 24 months. Therapy was effective. HBV DNA was identified only in 1 patient during the chemotherapy. Viral load was $1.5x10^4$. Enzyme levels did not increase. Relapse of HBV infection was in 11 patients (29%) after the end of Lamivudine preventive therapy.
Summary and Conclusions: Therapy with Lamivudine is effective and affordable for the prevention of HBV reactivation in patients with HL lymphoma receiving combination chemotherapy ABVD and BEACOPP. However, HBV infection recurrence rate was 29% after the end of therapy with Lamivudine. Nevertheless, we can conclude that at the time of chemotherapy LH should take preventive therapy with Lamivudine in patients with markers of HBV who previously untreated with antiviral therapy.

PB1767

THE PROGNOSTIC SIGNIFICANCE OF EXTRANODAL DISEASE IN HODGKIN'S LYMPHOMA

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Background: Hodgkin's lymphoma (HL) is a highly curable disease. Usually confined to the lymph nodes, extranodal involvement can occur and has an independent prognosis value.
Aims: To analyze the presenting features and the prognostic significance of extranodal disease in HL.
Methods: We performed a retrospective single institution study of 155 HL cases; from January 1992 to December 2010.
The median age was 27 years (range, 15-80 years), Stages III-IV were present in 85 (54.8%) patients. Combined radio-chemotherapy was administered to 95 (61, 2%) patients and chemotherapy alone to 60 (38.8%).
We analyzed the prognostic relevance of extranodal involvements and their significance was tested according to response rate an overall survival (OS).
Results: Extranodal disease was documented in 52 patients (33.5%) and 49 (44%) had bulky disease. Extranodal sites included the liver in 21 (13.5%), bone marrow in 11 (7%), pleura in 5 (3%), pharynx in 5 (3%), bone in 10 (6%), lung in 4 (1, 7%) and eye in 1 (0, 6%). The 52 patients with extranodal disease had poor prognosis compared with the nodal group (5 year OS, 51% *versus* 77%; p<0.001). Compared with the nodal subset, the extranodal patients presented more frequently with advanced stage disease (92% *vs* 08%; p<0.0001), B symptoms (90% *vs* 10%; p=0.002), a significantly low serum albumin (63.4% *vs* 24%; p<0.001) and a higher ESR (77% *vs* 23% p=0.005). Complete remission rates in the extranodal and the nodal subsets of patients were 46% *vs* 81% (p<0.001), respectively.
Summary and Conclusions: In our study, extranodal disease in patients with HL is frequent, especially in advanced stages,and associated with a poor outcome. This subset of patients is eligible for more effective treatment .

PB1768

A CASE OF BLEOMYCIN TOXICITY AND IMATINIB

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Background: Pulmonary fibrosis secondary to bleomycin for treatment of Hodgkin lymphoma can be potentially fatal, and monitoring of lung function often varies between instituitions. Animal studies had showed tyrosine kinase eg Nilotinib was effective in reversal of bleomycin induced interstitial disease, but this drug or similar TKI is not approved for this indication. There is one case report of Imatinib use in interstial fibrosis published in the literature.
Aims: To report our experience of a case of patient with stage II, early unfavourable Hodgkin lymphoma who developed bleomycin induced pulmonary fibrosis with progession of this on steroid; but subsequent treatment with Imatinib (off-lable use) reveresed the fibrosis. Imatinib was effective in our patient where 300mg daily was used for a total of 6 months successfully.
Results: A 53 year old female, non-smoker, previously well was diagnosed of nodular sclerosing Hodgkin lymphoma. She presented with bilateral cervical lymphadenathy, further staging confirmed Arr Born stage II early unfavvourable disease and underwent 4 cycles of ABVD with routine pulmonary function tests during treatment. She presented with dysponoea and dry cough a week later, PE was excluded, and was initially treated for infection. Bronchoscopy was performed and did not show PCP. HRCT subsequently showed upper and lower lobe interstitial changes with ground glass opacities, consistent with bleomycin induced interstitial fibrosis. Initial LFT showed higher than normal diffusion capacity, DLCO 87% FEV1, RV and TLC all more than 100% of predicted; and at time of diagnosis DLCO was 38%; this decreased further to 18% (FEV1 1.14l 46% or predicted, FVC 1.4l 48% of predicted, TLC 4.93l 54% of predicted) after prednisone was started at 75mg daily for two weeks. At this point, patient required intubation in ICU for two weeks and Imatinib 300mg daily was commenced. She gradually improved in the next 6 months and was able to walk from 50m to 500m after 3 months of treatment. She completed 4 cycles of chemotherapy and achieved CR after two cycles on interim PET scan. Due to development of pneumonitis, IFRT was abandoned. Plot DLCO and lung function against time of treatment and progress will be provided.
Summary and Conclusions: Although this patient achieved complete remission, she suffered severe bleomycin pneumonitis, which progressed with initial steroid treatment; subsequent tyrosine kinase inhibitor, ie imatinib 300mg daily reversed fibrosis and lumg function and diffusion capacity. She was treated for a total of 6 months with tapeing off steroid during this time, near complete resolution of interstitial fibrosis on CT was achieved, and continued to improve after this. Prognosis with bleomycin toxicity can be dismal and with the novel treatment albeit off lable use, there is a potentially effective therapy to reverse interstial fibrosis with TKI such as imatinib.

PB1769

CARDIAC COMPLICATIONS OF SPECIFIC TREATMENT IN HODGKIN'S DISEASE

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Background: In recent decades, the survival of patients treated for Hodgkin's disease has improved considerably. Unfortunately, improving prognosis of the disease was accompanied by a Hodgkin long-term toxicity, such as high risk for cardiovascular complications. The supradiaphragmatic radiotherapy, next to its beneficial role recognized, may be responsible for potentially serious cardiac complications. This risk is increased in patients with conventional combination chemotherapy with anthracyclines.
Aims: We present our experience in the management of cardiac complications in Hodgkin's disease.
Methods: Over a period of 20 years from January 1988 to December 2012, 11 cases of 286 pts presented a cardiovascular complication following chemotherapy regimens with or without radiotherapy. We will describe the type of complication, their time of onset and evolution.
Results: This is 11 cases (3,84%) who presented a cardiovascular complication. 7 men and 4 women with an average age of 37 years [24-65years].2 patients stage II, 4 stage III, 5 stage IV.First-line chemotherapy: ABVD: 9 pts; MOPP: 1 pts, and radiotherapy combined: 5 pts (3 localized stages, 2 extended). Second-linechemotherapy and the third line (refractory disease) CNOP and DHAP: 1 pts, then BEACOP-ESHAP: 2 pts. 11 cases presented acardiovascular complication after a median time of 22 months with intervals [6 months to 56 months].In 2 cases: thrombosis of vessels of the neck, after stopping immediatly radiotherapy. In 8 cases: Cardiomyopathy, after a mediantime of 25 months [4 and 56 months]. Their evolution was unfavorable in 6 cases (54.5%) died in an array of congestive heart failure.1 case with CMD still alive, with a survival of 7 years and 1 case with CMD POS. These two latter cases were respectively treatedwith ABVD (x8) and MOPP (x3) + RT. In 1 case: Treated exclusively by ABVD (x6), presented a 12-month overhaul aortic valvesand received an aortic valve replacement. The latter is still alive, with a survival of 6 years.This is 3.84% or 11 patients who presented a cardiovascular complication. 7 men and 4 women with an average age of 37 {[24-65 years]. 2 patients stage II, 4 stage III, 5 stage IV. This is 11 cases (3.84%) who presented a cardiovascular complication. 7 men and 4 women with an average age of 37 years [24-65 years]. 2 First-line chemotherapy: ABVD: 9 pts; MOPP: 1 pts, and radiotherapy combined: 5 pts (3 localized stage).
Summary and Conclusions: In our series, 3.84% of cases presented with cardiac complications and are consistent with those described in the literature. In a Mexican study, cardiac complications represented 3.5%.
The treatment of Hodgkin's disease is one of the most successful medical hematology. However, progress remains to be made in the therapeutic escalation to limit these deadly complications and some with heavy for other serious functional impairment, psychological and social. Strategies for appropriate risk reduction should beings associated with specific treatment, such as treatment of hypertension, hypercholesterolemia, and not smoking.

PB1770

ANALYSIS OF PATIENTS DIAGNOSED OF NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN´S LYMPHOMA

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Background: Lymphocyte-predominant Hodgkin Lymphoma (LPHL) is a rare entity with differences in pathology and clinical behaviour from classical Hodgkin lymphoma (cHL).
Aims: The objective of this study is to improve the knowledge of this infrequent disease, patient's characteristics and prognosis.
Methods: We retrospectively analysed all cases of Hodgkin lymphoma diagnosed in "Principado of Asturias" in a 10-year period (between 2002 and 2012). 259 patients were diagnosed with cHL, and we identified 21 cases of LPHL.
Results: Patients with LPHL had the following characteristics: median age 37 years (13-30) and 19 were males (90,5%). With regard to the stage, 15 pts (71.4%) presented with stage I/II disease, while N pts had stage III/IV disease. Only 9% of patients had B-symptoms and none had extranodal involvement or bulky mediastinal mass. 9 pts were treated with surgery and/or radiotherapy alone, while chemotherapy (ABVD) was administered to 12 (57,1%). Relapse occurred in 4 pts (19,1%); 2 patients of them with recurrence presented with histological transformation into aggressive lymphoma.
The median follow-up period was 76,27 months (range 8-130). The 10-year progression free survival (PFS) and 10-year overall survival (OS) were 69% and 100% for early stages, and 60% and 84% for advanced stages, respectively. A high level of beta-2 microglobulin was identified as a prognostic factor in our patients (RR 0,06; 0,005-0,68; p=0,020). In Table 1 we summarize the differences between LPHL and cHL. LPHL is an infrequent disease with a favourable prognosis. It's important a long follow-up and a repeat biopsy at the time of relapse in order to exclude histological transformation. The prognostic value of beta-2 microglobulin must be confirmed in larger series of patients.

Table 1.

Summary and Conclusions: LPHL is an infrequent disease with a favourable prognosis. It's important a long follow-up and a repeat biopsy at the time of relapse in order to exclude histological transformation. The prognostic value of beta-2 microglobulin must be confirmed in larger series of patients.

PB1771

TREATMENT OF RELAPSE OF CHRONIC HEPATITIS B VIRUS IN PATIENTS WITH HODGKIN'S LYMPHOMA

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Background: Treatment of HBV relapses in patients with Hodgkin's lymphoma (HL) is a not enough-studied problem. Preventive therapy with Lamivudine in patients with HL with viral hepatitis B was quite effective during chemotherapy LH, but was not effective in the treatment of relapses of HBV.
Aims: The aim of our work was to analyze the clinical presentation and treatment outcomes in patients with Hodgkin's lymphoma, evaluation of the effectiveness of antiviral therapy with recurrent hepatitis B in these patients.
Methods: We analyzed data from 38 patients with Hodgkin's lymphoma and markers of viral hepatitis B. Median HBV DNA was $1.4x10^4$/ml. All of these patients received polychemotherapy and prophylactic therapy with Lamivudine. Hepatitis B relapse developed in 11 patients. All these 11 patients had III and IV stages of HL. These patients had received from 6 to 8 cycles of chemotherapy BEACOPP. 3 patients had received high-dose chemotherapy due to inadequate effect BEACOPP. Relapse of chronic hepatitis B developed by 3-12 months (median 9.5 months) after preventive therapy with Lamivudine. At the time of relapse HBV infection HBV DNA level ranged from $1.5x10^4$/ml to $8.9x10^6$/ml (median $2.6x10^5$/ml), which was significantly higher than the HBV DNA level before start of antiviral therapy. At the relapse HBV ALT level ranged from 3 to 8 upper limit of norm in 11 patients, AST level ranged from 2.5 to 7 upper limit of norm in 7 patients, GGTP level ranged from 4 to 8 upper limit of norm in 5 patients. Therapy with Lamivudine 100 mg/day has been renewed for patients with relapse hepatitis B. Antiviral effect was not obtained at this group of patients. Therapy with Entekavir 1 mg/day was carried out in 6 patients, 3 patients have reached disappearance of HBV DNA and 3 patients had no effect. Therapy with Tenofovir 300 mg/day was appointed for 11 patients with resistant HBV. Level of HBV DNA ranged from $4.5x10^5$/ml to $1.9x10^8$/ml (median $3.9x10^6$/ml). ALT level ranged from 3 to 15 upper limit of norm in 10 patients, ACT level ranged from 2.5 to 9 upper limit of norm in 9 patients, GGTP level ranged from 4 to 7 upper limit of norm in 7 patients.
Results: Biochemical parameters were normalized after 3-6 months of therapy with Tenofovir (median 5 months).HBV DNA disappeared after 6-8 months (median 7.5 months) in all 11 patients. All patients received antiviral therapy during 6 - 8 months. Only 1 patient had relapse after discontinuation therapy with Tenofovir.
Summary and Conclusions: HBV relapses of HBV in patients with HL after chemotherapy developed only in patients with advanced stage HL. In the case of frequently relapsing hepatitis B, tenofovir therapy was effective after the discontinuation of preventive therapy with Lamivudine for relapse of HBV.

Non-Hodgkin & Hodgkin lymphoma - Biology

PB1772

TUMOR ASSOCIATED MACROPHAGES AND FOXP3 LYMPHOCYTES CORRELATES WITH THE OUTCOME IN HODGKIN LYMPHOMA PATIENTS

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Background: In the past decade the researches with the aim to identify powerful prognostic biomarkers for Hodgkin Lymphoma (HL) were very intensive. The structure of the tumor microenvironment and angiogenesis is found to have important role in tumor biology.
Aims: The aim of this study was to evaluate whether tumor associated macrophages (TAM), FOXP3 lymphocytes, microvessel density and the expression of vascular endothelial growth factor (VEGF) in tumor tissue could be important predictive factors for outcome of patients with HL, separately and combined with clinical and laboratory parameters.
Methods: The study included 84 newly diagnosed ABVD treated HL patients in the period 2000-2008. Immunohistochemical analysis using CD68, FOXP3, CD34 and VEGF monoclonal antibodies was performed on paraffin-embedded lymph node specimens. The examined clinical and laboratory parameters were presence of bulky disease, B symptoms, ESR≥50 mm/h, elevated LDH and high IPS score (3-7). By ROC curves were determined the most appropriate cut off values for number of TAM and FOXP3 lymphocytes, as well the microvessel density, which were used in subsequent survival and multivariate analysis.
Results: Five-year overall survival (OS) was 73,8% and 5-year event free survival (EFS) was 59,5%. In univariate analysis patients with high number of TAM, low number of FOXP3 lymphocytes and VEGF positivity had significantly shorter OS (p=0.017, p=0.003, p=0.046, respectively). Shorter EFS was found in patients with low number of FOXP3 lymphocytes (p=0.001) and VEGF positivity (p=0.026), while there was a trend toward worse EFS in patients with high number of TAM (p=0.068). Between clinical and laboratory parameters, patients with bulky disease, B symptoms, ESR≥50 mm/h and high IPS score had significantly shorter OS (p=0.006, p=0.022, p=0.013, p=0.024, respectively), while EFS was shorter in patients with ESR≥50 mm/h (p=0.026) and high IPS score (p=0.008). Multivariate analysis as the independent risk factors for poor OS identified high number of TAM and low number of FOXP3 lymphocytes in the group of biomarkers (p=0.034, p=0.006, respectively), while between clinical and laboratory parameters bulky disease (p=0.002) and high IPS (p=0.004) were identified. Low number of FOXP3 (p=0.002) and high IPS (p=0.011) were identified in analyzed groups as the independent factors for poor EFS. Afterwards, we performed survival analysis with aggregate scores of identified negative prognostic factors for OS for each patient (p=0.000). By merging groups with 0 or 1 and 3 or 4 negative prognostic factors (no difference in OS in intergroup analysis), we developed prognostic model for identifying patients at low (0-1 factors), intermediate (2 factors) and high risk (3-4 factors) for poor outcome (p=0.000). According to this model, in the examined group 28 (33.3%) patients had low, 24 (28.6%) intermediate and 32 (38.1%) high risk for poor outcome, with 5-year OS of 100%, 75% and 50%, respectively (Figure 1). This model retained its prognostic significance in multivariate analysis for OS (p=0.03; RR=3.271; 95% CI 1.516-7.057).

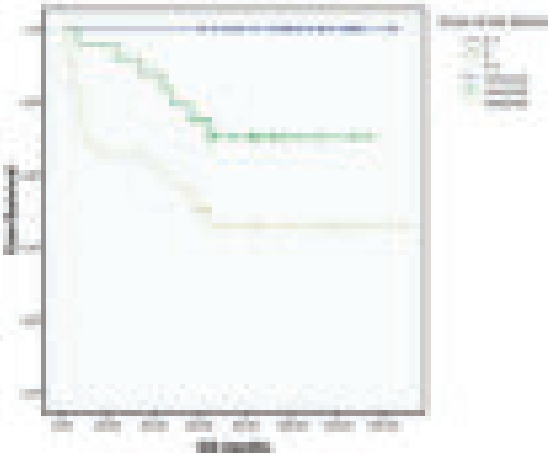

Figure 1.

Summary and Conclusions: Combining of TAM and FOXP3 lymphocytes with established clinical and laboratory prognostic factors could result in better risk stratification of patients with HL.

PB1773

THE PREDICTIVE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) IN LYMPHOMA PATIENTS

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Background: The immune suppression is one the most important mechanisms that contribute to tumor onset by impairing immune surveillance and by promoting tumor escape. Many aspects of lymphoma pathophysiology indicate that dysregulation of mutual interactions between the host immune system and lymphoid cells is involved in lymphoma pathogenesis through the suppression of T cell responses so that supporting escape and survival of lymphoma cells. Furthermore, the treatment outcome and survival were correlated with the presence of immune cells in the tumor microenvironment among which the myeloid-derived suppressor cells (MDSCs) have recently been demonstrated to have a significant ability to suppress innate and adaptive immune response, as resulting a crucial role in tumor progression as supported by their expansion during cancer and other pathological conditions, like inflammation, autoimmunity and infection. MDSCs represent a phenotypically and morphologically heterogeneous population of immature myeloid cells that promotes tumor growth and progression by supporting tumor tolerance as well as by non-immunological functions such as directly inducing tumor angiogenesis and metastasis formation. However, there are few data on their role in the clinical outcome of lymphoma patients as well as their correlation with relapse/progression of disease, especially in the case of Hodgkin's lymphoma patients whose no data are available.
Aims: The aim of this study was to assess the frequency of MDSCs in the Bone Marrow (BM) and Peripheral Blood (PB) of Lymphoma patients and healthy controls, in order to determine if they correlate with tumor burden and so to evaluate their role in clinical outcome and relapse/progression in Lymphoma patients (pts).
Methods: To detect MDSc as prognostic factors,BM and PB samples of 72 Lymphoma pts,with non-Hodgkin's Lymphomas(NHL) and classical Hodgkin Lymphoma(cHL),were collected at diagnosis and prospectively during the follow-up (1-6-12 months after chemo- or immuno-chemotherapy therapy) as well as at the relapse/progression. PB samples of 16 healthy donors were collected as controls. The MDSC population was defined by flow cytometric analysis as lineage: CD33+, HLA-DR$^{low/-}$, CD11b+ , discriminated for CD14+ and CD15+, by following direct antibody staining with: CD33+, CD11b+, CD15+, CD14+, HLA-DR$^{low/-}$. Data were acquired with Navios flow cytometer (Beckman Coulter) and analysed with Kaluza software (Beckman Coulter). All pts provided their informed consent in accordance with the Declaration of Helsinki.
Results: The percentage (mean±SE) of MDSCs in BM (3,770±0,0343) of Lymphoma pts was higher than in PB samples (2,836±0,0147) (p<0,0001) (Figure 1A). As demonstrated in previous studies the frequency of MDSCs in PB of lymphoma pts were increased as compared with healthy donors (p<0,0001) (Figure 1B). More interestingly their number seemed to be modulated both by disease and relapse/progression and correlated with histotypes. Specifically in cHL pts circulating MDSCs were significantly increased as compared with other histotypes (p=0.0003) (Figure 1C).

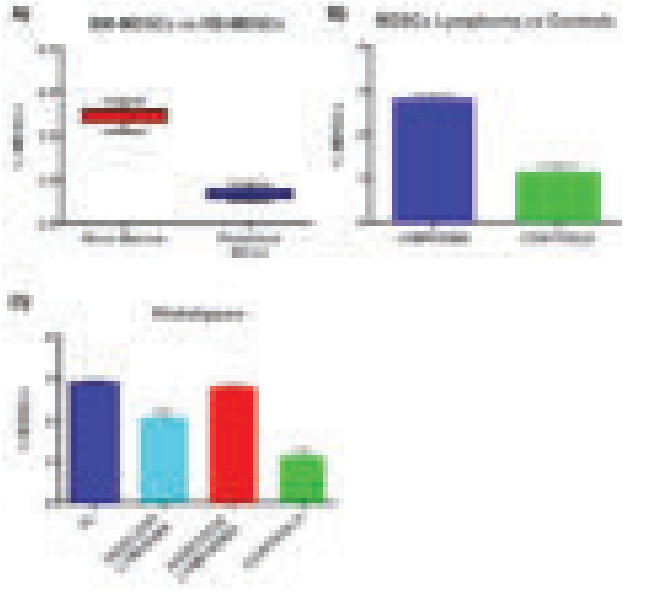

Figure 1.

Summary and Conclusions: Our study has demonstrated that the MDSCs could play an important role in both pathogenesis and progression of lymphomas as well as in treatment outcome. In addition, we demonstrated that circulating MDSCs were significantly increased in cHL as compared with NHL suggesting a characteristic immunosuppressive microenvironment in this histotype.

PB1774

ARGINASE-1 IS AN IMMUNOSUPPRESSOR MEDIATOR INCREASED IN HODGKIN`S LYMPHOMA NEUTROPHILS ASSOCIATED TO POOR OUTCOME

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Background: In Hodgkin Lymphoma (HL) elevated neutrophil (HL-N) count is a well recognized negative prognostic factor but its biological meaning is not elucidated. Our previous work showed that HL-N are dysfunctional and can suppress T-cell activation *in vitro*.
Arginase (Arg-1) in blood cells is mainly stored in N-granules and released as inflammation suppressor.
Aims: In order to investigate immunosuppressive properties of HL-N we evaluated arginase expression and activity in HL-N.
Methods: In N obtained from 15 HL patients we tested phagocytic activity by flow-cytometry, enzymatic activity of Arg-1 by colorimetric method, expression of Arg-1 in RT-PCR, and suppression of healthy T-lymphocytes activation in co-culture experiments. Amount of Arg+ cells was also evaluated in HL lymphonodes by IHC. We prospectively measured soluble Arg-1 (s-Arg-1) in 135 sera obtained from 60 patients with Hodgkin lymphoma, distinguished in a training set (N=25) and a validation set (N=35) and 21 healthy participants. In the training set, blood was taken at three fixed time-points prior, during, and after first-line therapy. Findings were compared with radiological assessment, including 2FDG-positron emission tomography scan after 2 cycle of chemotherapy (PET-2) and clinical variables.
Results: N-HL exhibited a reduced phagocytosis (93.2 ±1.9% *vs* 73.1±3.7, p=0.0008) and an increased arginase activity up to 15 times compared to healthy subjects matched for age and sex. We observed an increase of Arg-1 expression in HL-N up to 100 folds compared to healthy subjects matched for age and sex (p=0.001), independently from tumor load and other well-known prognostic factors, including sex, anemia, stage, bulky disease and IPS. In the lymphonodes, Arg-1 evaluated in immunohistochemistry showed a granular pattern distribution in lack of overlapping with CD68+ staining. s-Arg-1 was increased in HL patients compared to healthy subjects, reduced after therapy in responders and increased in relapsed patients (p<0.0001). s-Arg-1 was positively correlated to the amount of N and Arg-1 in N detected by RT-PCR. A cut-off level of 205 ng/mL for Arg-1 was chosen (equal to 2 times the 95thpercentile in controls and ROC value with sensitivity and specificity of at least 80%) to predict response status at 24 months. In the training set, 32% patients had high s-Arg-1, 24% had positive PET-2 and were addressed to an early salvage therapy accordingly to BEACOPP scheme. A level of 205 ng/mL s-Arg-1 resulted in 83% (95% C.I. 58-96) sensitivity and 81% (95% C.I. 42-96) specificity in predicting response status in the training set (area under curve, AUC, 0.81, p=0.02). In the validation set, baseline levels of s-Arg-1>205 ng/mL resulted in 83% (C.I. 95% 62-95) sensitivity and 87% (C.I. 95% 47-99) specificity in predicting response status. Patients with s-Arg-1 ≥ 205 ng/mL had shorter PFS than patients carrying Arg-1<205 ng/mL (despite both groups did not reach the median, because of the short follow-up, p=0.005).
Summary and Conclusions: Neutrophils in HL are dysfunctional for high amount of Arg-1. S-Arg-1 is a predictor of PFS even in the cohort of advanced-stage patients early addressed to salvage regimen in case of PET-2 positivity.

PB1775

PROGNOSTIC SIGNIFICANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR, OSTEOPONTIN AND MONOCYTE CHEMOTACTIC PROTEIN-1 IN DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH IMMUNO-CHEMOTHERAPY

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Background: Angiogenesis is gaining importance in hematological malignancies; it is regulated by a balance of various enhancing and inhibiting angiogenic factors. However, studies related to the prognostic value of angiogenic factors and aggressive Non-Hodgkin lymphomas are limited compared to solid tumors.
Aims: The aim of this study was to determine pretreatment serum level of vascular endothelial growth factor (VEGF), osteopontin (OPN) and macrophage chemotactic protein-1 (MCP-1), recognized to be involved in the angiogenesis. The expression was analyzed at the protein level in serum in patients with diffuse large B cell lymphoma (DLBCL) and investigated whether these biomarkers provide prognostic information.
Methods: We measured pretreatment serum levels of VEGF, OPN and MCP-1 by Enzyme-Linked Immunosorbent Assay (ELISA) in 76 patients newly diagnosed as diffuse large B-cell lymphoma and in 30 healthy controls. All patients were treated with rituximab-CHOP chemotherapy.
Results: The serum VEGF levels were found elevated in untreated DLBCL patients compared to controls: in patients ranged from 47.4 to 1512.6 pg/ml; median 423.4 pg/ml while VEGF levels of the healthy controls ranged from 41.8 to 289.4 pg/ml; median 192.6 pg/ml (P=0.002). There were significant differences in the serum OPN levels between DLBCL patients and controls (median 81.5 pg/ml *vs.* 29.5 pg/ml, P<0.001). Median serum levels of MCP-1 in patients with DLBCL was 1005 pg/ml and 790 pg/ml in control group (P=0.044). Serum VEGF levels were significantly higher in patients with an more advanced Ann Arbor stage (P=0.038), B symptoms (P=0.011), ECOG ≥2 (P=0.027), International Prognostic Index (IPI) ≥3 (P=0.018). Higher serum OPN levels were correlated with advanced Ann Arbor stage (P=0.006), B symptoms (P<0.001), ECOG ≥2 (P=0.027), IPI ≥3 (P<0.001), elevated serum LDH (P<0.001) and bone marrow infiltration (P=0.008). High MCP-1 was associated with Ann Arbor stage III-IV (P=0.009), IPI ≥3 (P=0.007) and bone marrow infiltration (P=0.016). Patients who achieved complete remission (CR) with therapy had a significantly lower value of VEGF (P=0.004) and OPN (P=0.007) in correlation to patients without CR achieved. The overall survival (OS) in patients with a serum level of VEGF lower than median VEGF was superior to those with VEGF higher than median (P=0.022). Furthermore, better OS was also noted in patients with OPN serum level lower than median (P<0.001). Serum MCP-1 levels did not predict outcome/response to therapy and survival. The univariate analysis for overall survival was identified to have prognostic significance: age, tumor grade, B symptoms, performace status, IPI, LDH, bone marrow infiltration, response to therapy, elevated OPN, VEGF and simultaneous elevation of 3 elevated biomarkers. Nevertheless, multivariate Cox proportional hazard regression analysis showed that no response to therapy (P<0.001), elevated LDH (P=0.006), elevated OPN (P=0.009) and VEGF (P=0.017) were considered independent prognostic factors for overall survival.
Summary and Conclusions: Our results showed that pretreatment serum level of VEGF and OPN are predictors of response to therapy and survival after immunochemotherapy and may help to further stratify DLBCL patients into risk groups.

PB1776

RITUXIMAB-INDUCED HYPOGAMMAGLOBULINEMIA AND SUPPORTIVE INTRAVENOUS IG (IVIG) TREATMENT IN PATIENTS WITH NON HODGKIN LYMPHOMA

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Background: Rituximab is a chimeric murine/human-engineered monoclonal antibody which can selectively deplete CD20-expressing B cells in peripheral blood and lymphoid tissues. It is demonstrated efficacy in patients with various lymphoid malignancies, including indolent and aggressive forms of B-cell non Hodgkin's lymphoma (NHL) and the favorable toxicity profile have led to its broad application in induction and maintenance regimens for B-cell malignancies, and, in particular, Non Hodgkin Lymphoma (NHL).
Aims: This retrospective single center analysis, aims to evaluate the incidence of Rituximab-related hypogammaglobulinemia (hypoIg).
Methods: In our institution, in patient affected by NHL in treatment with Rituximab-based regimens, we performed serial quantitative serum immunoglobulin (SIg) concentration at baseline, after chemotherapy, during and after Rituximab maintenance treatment. IgG, IgA and IgM deficit were respectively defined by level below 700 mg/dL, 70 mg/dL and 40 mg/dL. We considered patients as symptomatic if they developed at least two non-neutropenic infections in a 6-months observation period after or during Rituximab-based treatment.
Results: 88 patients with NHL and SIgG monitoring were retrospectively analyzed, 24% of them were relapsed or refractory after first courses of chemotherapy. The median age of patients was 61 years (range: 28-80 y).

From histological examinations, the patient were diagnosed as follicular lymphoma (FL) (n=53), small lymphocytic lymphoma (SLL) (n=9), marginal zone lymphoma (ML) (n=11), mantle cell lymphoma (MCL) (n=9), diffuse large B-cell lymphoma (DLBCL) (n=6). Patients received a median of 11 administrations of Rituximab (range: 6-27). The median follow-up of surviving patients was 3.6 years. Before treatment with Rituximab, 9/88 (10.2%) had low SIgG levels (6 FL, 1 MCL, 1 SLL, 1ML) and in 4/9 (44.4%), during R-maintenance treatment, IVIG administration was necessary. After R-based chemotherapy regimens, IgG deficiency was observed in 20/88 (22.7%), no one needed IVIG, despite 7/20 (35%) were symptomatic. After or during Rituximab-based maintenance treatment, in 22/88 (25%) IgG deficiency was observed after a median of 10 R administrations; the deficit was observed in 77% (17/22) within the fourth R maintenance administration and in no one after the sixth R administration. In this category, 12/22 (54.5%) were symptomatic and 4/22 (18.2%) required IVIG supportive treatment. In all 8 patients who needed IVIG treatment, at least two different Ig isotypes were deficient.

Summary and Conclusions: Rituximab treatment is associated with a high risk of hypoIg development. Moreover, the number of administrations is strictly related to the development of symptomatic hypoIg, and the risk of hypoIg increases in patients in Rituximab-based maintenance. The decision to introduce supportive treatment with IVIG in non-neutropenic patients was related to the number of infection episodes. HypoIg often is undiagnosed, in particular for the presence of confounding symptoms. Our study suggests that Ig levels monitoring and supportive IVIG treatment should be considered in this patients subset.

PB1777

RP6530, A DUAL PI3K DELTA/GAMMA INHIBITOR, DEMONSTRATES EFFICACY IN DLBCL CELLS IN VITRO

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Background: Diffuse Large B-Cell Lymphoma (DLBCL) is an aggressive, rapidly growing type of lymphoma that comprises of 30-40% of NHL cases. Chemotherapy remains the most widely used treatment for DLBCL thereby necessitating the need to develop targeted and safer drugs. Recent clinical trials have demonstrated a therapeutic potential for Ibrutinib, a small molecule BTK inhibitor in DLBCL, especially the ABC subtype. Given the expression pattern in DLBCL cells and overlapping pathway with BTK, treatment with a PI3K δ/γ may be a viable alternative to conventional chemotherapy. RP6530 is a novel, potent, and selective PI3K δ/γ inhibitor. RP6530 demonstrates high potency against PI3Kδ (IC_{50}=25 nM) and γ (IC_{50}=33 nM) enzymes with selectivity over α (>300-fold) and β (>100-fold) isoforms. Cellular potency has been confirmed in target-specific assays, namely anti-FcεR1-(EC_{50}=38 nM) or fMLP (EC_{50}=39 nM) induced CD63 expression in human whole blood basophils, LPS-induced CD19+ cell proliferation in human whole blood (EC_{50}=250 nM), and LPS-induced CD45R+ cell proliferation in mouse whole blood (EC_{50}=101 nM).

Aims: The objective of this study was to evaluate the effect of RP6530 in DLBCL cells.

Methods: RP6530 was tested for potency in viability, apoptosis, and Akt phosphorylation assays in the GCB and ABC subtype DLBCL cell lines, OCI-LY-1 and OCI-LY-10, respectively. Viability was assessed using the colorimetric MTT reagent after incubation of cells with RP6530 as a single agent or in combination with Ibrutinib for 72 h. Inhibition of pAKT was estimated by Western Blotting and bands were quantified using ImageJ after normalization with Actin. Apoptosis of OCI-LY-10 or OCI-LY-1 cells was determined by measuring Caspase-3 activity fluorimetrically. Primary cells from DLBCL patients were isolated, incubated with 4 μM RP6530, and analyzed for apoptosis or cytotoxicity by Annexin V/PI staining.

Results: RP6530 caused a dose-dependent inhibition in proliferation of OCI-LY-1 and OCI-LY-10 with half-maximal inhibitory values of 664 nM and 156 nM respectively Addition of 0.5 μM RP6530 caused a significant left-ward shift in the EC_{50} for ibrutinib (216 nM *vs.* 5 nM for OCI-LY-1 and 678 nM *vs.* 21 nM for OCI-LY-10). Reduction in cell viability was accompanied by corresponding inhibition of pAKT with EC_{50} of 6 & 70 nM in OCI-LY-1 and OCI-LY-10 cells, respectively. In addition, RP6530 increased apoptosis manifested by an elevation of caspase-3 activity in both cell lines. Treatment of patient-derived primary GCB-DLBCL cells with 4 μM RP6530 caused a 2-fold increase in total apoptosis as evident from Annexin V/PI staining.

Summary and Conclusions: RP6530 is a potent and selective dual PI3Kδ/γ inhibitor that inhibited growth of DLBCL cell lines with a concomitant reduction in the downstream biomarker, pAKT. Besides, RP6530 at clinically relevant concentrations potentiated the activity of ibrutinib by 30-40-fold. Additionally, the compound induced apoptosis in primary DLBCL cells. Findings provide a rationale for future clinical trials in DLBCL patients.

PB1778

Abstract withdrawn

PB1779

SOCIOECONOMIC AND ETHNIC VARIATIONS IN SURVIVAL OF PATIENTS WITH NON-HODGKIN'S LYMPHOMA IN USA

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Background: The incidence of Non-Hodgkin's Lymphoma (NHL) in the United States (US) has been increasing for the last several decades. However, US cancer mortality from NHL has seen a downtrend in the recent years. As per National Cancer Institute's (NCI) SEER factsheet the Annual percent change (APC) for NHL mortality from 1997-2009 was -3.01. This could be attributed to the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) combination regimen followed by addition of rituximab. Few studies have shown that socioeconomic status (SES) does influence diagnosis, treatment and survival of patients with NHL (Ewing et al, 2003; Bray et al, 2008; Rachet et al, 2008; Roswall et al, 2008, Wang et al, 2008; Kent et al, 2010). Several studies have been reported to demonstrate ethnic disparities in the past, however some of them looked into the elderly population alone while remaining failed to take into consideration other ethnicities (other than Hispanics, African Americans and Caucasians).

Aims: In our study we try to investigate socioeconomic and ethnic disparities in survival of NHL patients belonging to all age groups and all ethnicities.

Methods: A retrospective cohort of 22,419 NHL patients diagnosed from 2004 to 2005, belonging to all age groups and ethnicities, was identified from SEER database. The median follow up time was 4.5 years. Descriptive statistics (chi-square test) were used to analyze the differences in distribution of baseline characteristics by race. Cox proportional model was used to compare survival among different ethnic groups using PHREG procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results: Of the cohort 85.2% were Caucasians, 8.32% African Americans and 6.48% from other ethnicities. The mean age of study population was 62.2±17.3 years. A large proportion of other ethnicities (non-black and non-white) were in the highest quartile of education (32.48%) and economic status (35.86%). Hazards ratio of all cause and NHL mortality increased significantly with age, stage, unemployment rate, smoking rate, language isolation rate and decreased with increase in household income ($p<0.05$). Married population was found to have better survival compared to single and divorced/widowed population ($p<0.0001$). After confounding for all other variables, including subtypes and stage at presentation, NHL mortality was found to be higher in African Americans (HR 1.055, 95% CI 1.003-1.109) and other ethnicities (HR 1.113, 95% CI 1.053-1.175) compared to Caucasians. There was no significant difference in NHL mortality between females and males ($p=0.13$).

Summary and Conclusions: In this study we tried to assess the ethnic and socioeconomic disparities in the survival of NHL patients belonging to all age groups and all ethnicities in US. Some important findings of our study were identification of age, stage of presentation, unemployment, smoking, language isolation and household income as significant influences on overall mortality of NHL patients. We found mortality to be significantly higher in African Americans and patients belonging to other ethnicities compared to Caucasians after controlling for demographic factors, socioeconomic factors and stage at presentation. However, there was no statistically significant difference in mortality between males and females. In conclusion, our study shows a strong correlation between social deprivation and decreased survival in NHL patients belonging to all age groups. Also, Caucasians seem to have higher survival compared to African Americans and other ethnicities. However additional studies, including all other possible prognostic variables, are required to substantiate this finding.

PB1780

HEAT SHOCK PROTEIN 90 INHIBITOR NVP-AUY922 EXERTS POTENT ACTIVITY AGAINST ADULT T-CELL LEUKEMIA-LYMPHOMA CELLS

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Background: Adult T-cell leukemia-lymphoma (ATL), an aggressive neoplasm

etiologically associated with human T-lymphotropic virus type-I (HTLV-1), is chemo-resistant malignancy. Heat shock protein 90 (HSP90) is involved in folding and functions as a chaperone for multiple client proteins, many of which are important in tumorigenesis. In contrast to normal cells, tumor cells contain an abundance of catalytically active HSP90, which is found in multichaperone complexes. Therefore, HSP90 has emerged as a target of interest in cancer therapy. The HSP90 inhibitor 17-AAG, derived from geldanamycin, has shown potent antitumor activity against ATL. However, geldanamycin derivatives have several limitations, including poor solubility, formulation difficulties, and severe hepatotoxicity in clinical settings, which have prompted development of next generation synthetic HSP90 inhibitors including NVP-AUY922 (AUY922), an isoxazole-based nongeldanamycin HSP90 inhibitor that inhibits the ATPase activity of HSP90. AUY922 has shown nanomolar efficacy against a wide range of human cancer cells *in vitro* and also inhibits progression of a variety of tumors *in vivo*. Furthermore, in a phase I clinical trial of AUY922 in patients with advanced solid tumors, the agent exhibited acceptable tolerability.

Aims: We examined the effects of AUY922 on ATL cells *in vitro* and *in vivo* and explored a novel therapeutic target by investigating its molecular mechanisms.

Results: First, we analyzed the effects of AUY922 (kindly provided by Novartis) on proliferation of ATL-related cell lines [ATL-derived cell lines (KK1, SO4, LM-Y1, KOB and ST1) and HTLV-1-infected T-cell lines (MT2 and HuT102)]. Incubation with AUY922 at various concentrations (0–100 nM) for 72 hours inhibited cellular proliferation in a dose-dependent manner, as assessed by an MTS assay. The concentrations of AUY922 required to inhibit cellular proliferation of ATL-related cell lines by 50% (IC50) varied from 12.5 to 25.0 nM. Importantly, AUY922 was effective regardless of the presence of wild-type or mutant p53. We also assessed AUY922-induced inhibition of growth of peripheral blood mononuclear cells (PBMCs) obtained from both normal subjects and patients with ATL. Importantly, primary ATL cells were more susceptible to AUY922 than normal PBMCs. Also, when compared directly with 17-AAG, AUY922 was between 20- and 50-fold more active at inhibiting growth of ATL-related cell lines. FACS analysis revealed that AUY922 induced apoptotic cell death and/or G1 phase cell cycle arrest in ATL-related cell lines. To verify the molecular mechanisms, we also examined the expressions of several client proteins using Western blotting (WB) analysis. AUY922 treatment led to induction of HSP70, a surrogate marker for inhibition of HSP90 function, while it did not influence the protein level of HSP90 itself. AUY922 treatment also led to decreases in p-Akt, Akt, IKKα, IKKβ, IKKγ, Cdk4, Cdk6, Bcl-2 and survivin. In a xenograft model created with C.B-17/Icr-SCID mice, intraperitoneal administration of the vehicle or AUY922 was given after injection of HuT102 cells. In the control mice, bulky tumors grew within 4 weeks, whereas daily administrations of AUY922 significantly impaired tumor growth. To determine which molecules play important roles in AUY922-induced ATL-cell death, gene expression profiling was performed using DNA microarray analysis in 4 ATL-related cell lines (KK1, SO4, LM-Y1 and HuT102). Interestingly, decreases in 2 of the proviral integration site for Moloney murine leukemia virus (PIM) kinases, PIM-1 and -3, were commonly found in all cell lines we examined. PIM has multiple cellular functions related to cell survival, proliferation, differentiation, apoptosis and tumorigenesis. Its expression is also correlated with poor prognosis in most hematopoietic malignancies. However, its role in ATL remains unclear. Therefore, we examined the protein expression levels of PIM kinases using WB in 4 ATL-related cell lines (KK1, SO4, LM-Y1 and HuT102) treated by AUY922. Although the protein levels of PIM kinases varied in each of the ATL-related cell lines when untreated, the protein expression levels of PIM-1, -2, and -3 were decreased in all. To confirm the importance of PIM kinases in ATL cells, we evaluated the inhibitory effect of SGI-1776, a pan-PIM kinase inhibitor, on proliferation of ATL-related cell lines and primary ATL cells. SGI-1776 successfully inhibited the growth of primary ATL cells as well as ATL-related cell lines.

Summary and Conclusions: Our findings show that AUY922 may be potentially useful as a chemotherapeutic agent and PIM kinases may be a novel therapeutic target for treatment of ATL.

PB1781

APOPTOSIS INDUCING AGENTS IN CUTANEOUS T-CELL LYMPHOMA (CTCL) CELL LINES

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Background: Cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of non-Hodgkin lymphomas, derived from skin-homing mature T-cells. Mycosis fungoides (MF) and Sézary syndrome (SS) are the commonest types and together comprise 54% of all CTCL. MF evolves from patches to infiltrated plaques and eventually tumors. SS is a lymphoma-leukemia syndrome characterized by erythroderma and the presence of a malignant T-cell clone in the peripheral blood and the skin. Radiotherapy and classic anthracycline-based chemotherapy provide only short-lived remission; therefore current CTCL research efforts are focused on decoding the mechanisms of chemotherapy resistance and on identifying new pharmacological targets. Thus, several drugs have currently shown potentially significant activity either alone or in combination with conventional agents.

Aims: Few clinical studies have been conducted so far to test the most effective drug combinations. This study aims to investigate the apoptotic effects and the possible synergistic action of various, novel in CTCL treatment, drug combinations, using CTCL cell lines.

Methods: Three CTCL cell lines have been used: MyLa derived from a plaque biopsy of a patient with MF, SeAx and Hut-78 both derived from peripheral blood of patients with Sézary syndrome. All cells were cultured in RPMI 1640, supplemented with 10% FBS and 2 mM L-glutamine (37 ^{0}C,5% CO_2). Cells were treated with the following agents and their combinations for 72h: Bortezomib (10nmol/L), methotrexate (10μM) and interferon—a (100U/mL). Apoptosis and cell viability were determined by flow cytometry using the Annexin V/PI method.

Results: Hut-78 cells responded with enhanced apoptosis when treated with bortezomib and methotrexate, compared to untreated cells (control) (5.48 and 9.57 *vs* 1.32 respectively, p<0.001), while treatment with interferon-a had no significant effect on apoptosis. The methotrexate/interferon-a combination showed even higher apoptotic rates, compared to untreated cells (10.54 *vs* 1.32, p<0.001). Treatment with all three agents also significantly increased apoptosis compared to control (6.62 *vs* 1.32, p<0.001), but not at the same level as the methotrexate/interferon-a combination. Interestingly, all drug combinations led to great enhancement of late apoptosis, with the bortezomib/methotrexate/interferon-a combination presenting the most impressive effect, compared to control (67.44 *vs* 0.62, p<0.001). None of the above agents or their combinations seemed to affect early apoptosis in SeAx and MyLa cells. On the contrary, all drug combination treatments significantly increased late apoptosis in both cell lines, while the Bortezomib/methotrexate/interferon-a treatment presented the most impressive augmentation in late apoptosis rates for both cell lines (SeAx: 14.46 *vs* 0.38, p<0.001; MyLa: 76.19 *vs* 43, p<0.001).

Summary and Conclusions: Our data clearly demonstrate that CTCL cells respond to combination treatment better than to monotherapy, in terms of apoptosis' induction. We showed that methotrexate (which has a proven efficacy against CTCL), interferon-a and bortezomib have a synergistic action in CTCL, leading to enhanced sensitivity to apoptosis, in SS and MF cell lines. Although these results need to be further confirmed both *in vitro* and *in vivo*, they appear very encouraging for the integration of combination treatment in CTCL therapy.

PB1782

ANALYSES OF NUCLEAR ORPHAN RECEPTOR EXPRESSION AND CYTOTOXICITY FOLLOWING ANTI-CD 20 MONOCLONAL ANTIBODY TREATMENT IN AGGRESSIVE NON-HODGKIN'S LYMPHOMAS

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Background: Recently, we found a reduced *NR4A1* and *NR4A3* expression in aggressive B-cell lymphomas. The functional characterization of *NR4A1* in lymphoma cell lines demonstrated its pro-apoptotic effects. The standard therapy for aggressive B-cell lymphoma is a multiagent chemotherapy combined with anti-CD20 monoclonal antibody (mAb) Rituximab (Rtx). Recent studies demonstrated that monotherapy with Rtx exhibited cytotoxic activity *in vivo* and *in vitro*.

Aims: The aim was to investigate the effects of two anti-CD20 mAB (Rtx and GA101) on two CD20 positive cell lines (SuDHL4 and Raji) *in vitro* and their influence on *NR4A*s expression.

Methods: Therefore, we performed cytotoxicity assays combined with mRNA expression analysis of *NR4A1*, *NR4A2, NR4A3* and pro-apoptotic genes on two CD20 positive cell lines (SuDHL4 and Raji) treated with two anti-CD20 mAb (Rtx and GA101).

Results: Rtx treatment reduced cell viability significantly after 24h and caused a cell cycle arrest in SuDHL4 but not in Raji. In contrast, GA101 was able to reduce cell viability significantly after 3h of treatment in both cell lines. *NR4A* expression analyses demonstrated that anti-CD20 mAb treatment induces *NR4A1* and *NR4A3* expression in SuDHL4 after 3h. The highest induction of *NR4A1* (115 fold) and *NR4A3* (360 fold) expression was found in Rituximab treated SuDHL4 cells. Substantially lower, but still significant was the *NR4A1* (28 fold) and *NR4A3* (46 fold) up-regulation in GA101 treated SUDHL4 cells. By correlating the constitutive *NR4A1* and *NR4A3* expression of untreated cells with cytotoxic effects of Rtx and GA101, we observed a significant positive correlation between *NR4A1* expression and cytotoxic effects of Rtx (Spearman-Rho: 0.640 p=0.008) suggesting gene dosis of *NR4A1* might be critical for the cytotoxic effects of Rtx. Furthermore, treatment with Rtx resulted in a significant up-regulation of *BIM* (2 fold) and down-regulation of *TRAIL* (4 fold) after 3h and up-regulation of *BIK* (2 fold) after 24h in SuDHL4, whereas Raji showed only

an up-regulation of *BIK* (1.5 fold) after 24h of treatment. 12 h of GA101 treatment resulted in an up-regulation of *BIK* (2 fold) and *BAX* (2 fold) in SuDHL4 and an up-regulation of *PUMA* (1,5 fold) in Raji after 3h of treatment.
Summary and Conclusions: Our data demonstrate that Rituximab and GA101 differ in their cytotoxicity and induction of *NR4A1* and *NR4A3* expression in a cell line depending fashion. Hence, *NR4A1* and *NR4A3* might be important factors for mediating the cytotoxic effects of Rituximab and GA101 in diffuse large B-cell lymphomas.

PB1783

WHOLE-EXOME SEQUENCING ANALYSIS OF DIFFUSE LARGE B-CELL LYMPHOMAS IN THE MEDIASTINUM OF FEMALE SIBLINGS

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common malignant lymphoma in adults, comprising ~40% of cases. By means of gene-expression profiling, the morphologically indistinguishable DLBCLs can be divided into three cell-of-origin subtypes characterized by distinct molecular and clinical features. Most lymphomas arise sporadically, yet familial clustering is known, but currently poorly understood at the molecular level.
We characterize a Swiss family with two female siblings affected by DLBCLs localized in the mediastinum with the age of onset being 25 and 30, respectively. While the younger sibling, presented with CD30+ DLBCL of the activated B-cell (ABC) type with PMBL-like compartmentalizing sclerosis, achieved complete remission, her older sibling died due to disease progression 11 months after being diagnosed with a primary mediastinal DLBCL (PMBL). Pathological analyses revealed different (*e.g.* BCL2 expression, positive in the PMBL, negative in the ABC-DLBCL), but also shared molecular features, *e.g.* gain of the *JAK2* locus by FISH. The similar clinical and molecular characteristics suggested a shared biological background.
Aims: To identify the possible genetic predisposition in this family.
Methods: We performed whole-exome sequencing on matched tumor and germline (extracted from peripheral blood) DNA from both siblings and the DNA from their unaffected brother and parents. The tumor DNA, isolated from laser-dissected formalin-fixed paraffin-embedded tissue, was highly degraded and required extensive adaptations of the standard library-preparation protocol, *e.g.* treatment with PreCR Repair mix from New England Biolabs. The exome was captured using the Illumina TrueSeq 62Mb exome enrichment kit, sequencing was performed on an Illumina HiSeq2000 to produce 100bp paired-end reads. After quality control (FastQC), the reads were aligned to the reference genome hg19 (bowtie-2). GATK Unified Genotyper was used to detect germline inherited and *de novo* variants, while somatic alterations were called by Strelka and SomaticSniper.
Results: On average, 16 and 56 gigabases were sequenced per tumor and healthy DNA, respectively. In all samples, ~95% of the sequenced reads mapped to the reference genome. Removal of duplicated reads resulted in an average exome coverage of 70 and 20 fold for each healthy and tumor DNA, respectively. In this analysis, we are focusing on *a)* rare (< 10% allele frequency according to the 1000 Genomes project) germline protein altering variants that are shared by the siblings and possibly implicated in lymphomagenesis, and *b)* somatic, lymphoma-specific alterations. This approach *e.g.* revealed 960 shared rare non-silent germline alterations, including 773 missense mutations and 187 indels, affecting 721 different genes. Of these, 951 variants were inherited, while the remaining variants were acquired *de novo*. To reduce the number of germline candidates, we will consider i) genes which have been linked to lymphoma or cancer, ii) mutations which are predicted to be deleterious and iii) genes which are mutated according the Knudson's two-hit theory. Further reduction will be achieved by integrating the results of the healthy brother. The proposed bioinformatics analysis is currently ongoing and candidates will be presented.
Summary and Conclusions: The identification of alterations possibly predisposing to familial DLBCL will contribute to the understanding of the genetic basis of this complex and heterogeneous tumor. In addition, analyzing the somatic landscape of both DLBCL might provide an explanation for the different diagnosis and outcome of the siblings.

PB1784

INVESTIGATION OF RHO-KINASE GEN AND PROTEIN EXPRESSIONS IN MANTLE CELL LYMPHOMA PATIENTS

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Background: Mantle cell lymphoma (MCL) is a rare but aggressive form of B-cell non-Hodgkin's lymphoma with a heterogeneous clinical presentation. In MCL, one allele of the cyclin D1 gene is translocated from its normal localization on chromosome 11 to chromosome 14. So, this disease is characterized by overexpression of cyclin D1. There is no proven curative therapy and no standard of care has been established for initial or subsequent lines of therapy. Rho-kinase (ROCK) is a serine-threonine protein kinase with multiple downstream effects. Two isoforms of ROCK protein, ROCK1 and ROCK2 have been characterized. ROCK is involved in the migration and proliferation of tumor cells. Increased expression of the ROCK proteins contributes to the metastatic behavior of some cancers. Furthermore, ROCK activation increases the expression of cyclin D1.
Aims: The aim of this study was to elucidate the role of ROCK gene and protein expressions in MCL.
Methods: This study was performed retrospectively. A total of 60 patients with MCL and 60 controls evaluated at the Hematology Department of Gaziantep University Hospital, Turkey between 2006-2012 years were recruited into this study. Controls were from the nonneoplastic tissue sections diagnosed as reactive lymphadenopathy at the Pathology Department. Hematoxylin and eosin stained slides in the entire archive was reevaluated and selected for immunohistochemistry and PCR. p53, p63, ROCK1 and ROCK2 stainings were scored according to staining ratio in tumor cells. Ki-67 was expressed as%. To confirm the expressions of ROCK in bladder tissue, mRNA was extracted; cDNA was produced and analyzed by a BioMark HD 96.96 dynamic array system (Fluidigm, South San Francisco, CA, USA). Data were analyzed using the $2^{-\Delta Ct}$ method, compared by using the Mann-Whitney U test.
Results: In immunohistochemical studies, there was significant increase in ROCK1 (P=0.0009) and ROCK2 expression in MCL patients when compared with control group (P<0.0001). ROCK2 expression was higher than ROCK1 in MCL patients (P<0.0001). There were significant positive correlations between age and ROCK1 (P=0.044). Expressions of p53 and Ki-67 were found to be stronger in patient group than control group. There was marked increase in *ROCK1* gene expression in patients group when compared to controls (P=0.0215). However, no significant change was observed in *ROCK2* gene expression (P=0.9194).
Summary and Conclusions: In conclusion, our data showed that *ROCK* gene expressions may contribute to the development of MCL. Our results may imply that up-regulation of ROCK may represent a prognostic factor in MCL. ROCK may be a potential target for MCL diagnosis and therapy.

PB1785

THE FOXO3A-P53 AXIS: FUNCTIONAL ANALYSIS IN MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is a rare subtype of B-cell non-Hodgkin lymphoma (NHL) that comprises around 6% of all NHL and presents a poor response to current therapeutic treatments. MCL is characterized by the t(11;14) chromosomal translocation, which results in aberrant expression of cyclin D1. This genetic event is present in virtually all cases of MCL, whereas additional genetic alterations that occur in subsets of MCL have been described. Most of these alterations appear to disturb the cell cycle machinery or interfere with the cellular response to DNA damage. FOXO transcription factors are relevant tumour suppressors that mediate the expression of genes involved in several biological processes, such as cell cycle arrest, DNA repair and apoptosis.FOXO3a is the predominant member of the FOXO subfamily in lymphoid tissues, plays an essential role in lymphomagenesis and is essential for proliferation of cells of the immune system. FOXOs are negatively regulated by the PI3K/Akt pathway; interestingly, members of the PI3K pathway such as *PI3KCA* or *Akt1* are over-expressed in MCL. Several nodes of interaction have been identified between FOXO3a and p53, a major tumor suppressor in humans and mice. Our group has reported the importance of FOXO3a in the biology of MCL cells, but little is known about the relevance of the FOXO3a-p53 axis in the context of MCL biology.
Aims: We have evaluated the role of FOXO3a-p53 interaction in a panel of MCL cell lines with different p53 status and its relevance in MCL biology.
Methods: We have analysed the response of MCL cells to chemical modulators of this pathway such as Nutlin-3a, Psammaplysene A and DNA damaging agents. Cell viability assays have been conducted in a panel of cell lines, and the expression levels of FOXO3a, p53 and its targets have been analysed by qRT-PCR and western blot. Inmunoprecipitation assays have been performed to demonstrate the interaction between p53 and FOXO3a.
Results: FOXO3a is activated in response to Nutlin-3a in MCL cells, and expression of FOXO/p53 target FOXM1 is decreased. Also, coimmunoprecipitation studies show evidence of FOXO3a-p53 binding in MCL cells. DNA damaging agents modulate FOXO3a activity, FOXM1 levels and FOXO3a-p53 binding.
Summary and Conclusions: Our results show the importance of the

interaction between FOXO3a and p53 in MCL biology and suggest that this signalling route can be considered as a potential therapeutic target in MCL.

PB1786

MICRORNA LEVELS IN HEPATITIS B OR C VIRUS-RELATED INDOLENT B CELL LYMPHOMAS

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Background: Epidemiological evidence links Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) to B-cell non-Hodgkin lymphoma (B-NHL). These B-NHLs, particularly those associated with HCV, may represent a distinct sub-group with peculiar molecular features, including peculiar expression of microRNAs (miRs).

Aims: Aim of the present study was to search for miRs whose level in indolent B-NHL tissues could be associated with HBV or HCV infection.

Methods: Fourteen Formalin Fixed Paraffin Embedded (FFPE) lymph node tissues from HBV+, HCV+ and HBV-/HCV- indolent B-NHL patients were analyzed for levels of 34 selected miRs by quantitative Real-Time PCR. Reactive Lymph Nodes (RLNs) from HBV-/HCV- patients were included as non-tumor control. Statistical analysis of output data included Pearson and Spearman correlation and Mann-Whitney test and were carried out by the STATA software.

Results: MiR-92a was decreased exclusively in HBV-/HCV- B-NHLs, while miR-30b was increased in HBV+ and HCV+ samples, though only the HCV+ achieved full statistical significance. Analysis of a small subset of B-NHLs belonging to the same histological subtype (Nodal Marginal Zone Lymphoma) highlighted three miRs associated with HCV infection (miR-223, miR-29a and miR-29b) and confirmed decreased level of miR-92a in HBV-/HCV- samples also when considering this restricted B-NHL group.

Summary and Conclusions: Although caution is needed due to the limited number of analyzed samples, overall the results suggest that differences at the miR expression level exist between indolent B-NHLs developed in patients with or without HBV or HCV infection. The identification of three further miRs associated with HCV by analyzing histologically homogeneous samples suggests that variations of miR levels possibly associated with HBV or HCV may be obscured by the tissue-specific variability of miR level associated with the different histological subtypes of B-NHL. Thus, the identification of further miRs will require, in addition to an increased sample size, the comparison of B-NHL tissues with the same histological classification.

PB1787

SIMULTANEOUS HISTOLOGICAL AND MULTIPARAMETRIC FLOW CYTOMETRIC ANALYSIS OF TISSUES BIOPSY OF NON-HODGKIN LYMPHOMAS

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Background: Our internal procedure for suspected lymphoma provides more than classical histology also the flow cytometric (FC) tissue analysis.

Aims: To compare the possibility of integrate diagnosis of histology and immunophenotyping in the case of lympho nodes speciments analized by both for the diagnosis of lymphoma

Methods: we reviewed biopsies diagnosed by the pathologist and concurrently analyzed by FC from 2003 to 2013. FC data were obtained by a multi-parametric analysis as altered scatter, co-expressions and different intensities of antigens and were used by the pathologist for the final diagnosis.

Results: on the total of 655 revised samples 416 were diagnosed as hematologic neoplasm and according to the WHO classification were: 291 (70%) B-NHL, 15 (4%) T cells-NHL, 100 (24%) Hodgkin lymphoma and 8 (2%) composite lymphomas (CL). Correlation between morphologic findings and FC was found in 264 cases of 291 NHL (91%). Furthermore, the immunophenotype characterized in the lymph node was tested by FC in other types of samples such as bone marrow, peripheral blood or biological fluids at the diagnosis and/or during the follow up. The role of FC was crucial in the T cell neoplasms diagnosis, where the evidence of an atypical pattern may suggest the need for further test and make the diagnosis easyer and quicker . In the case of CLs, the multi-parametric analysis by FC has been decisive in detecting easily the coexistence of clones related to different histotypes. Among the 27 cases (9% of total) "non-concordant" 4 follicular lymphoma, hystologically diagnosed, were included becouse they were previously defined by FC as polyclonal and so confirmed by Molecular Biology. Others 23 presented cytometric data conflicting - less frequent in the course of the years – mainly due to technical aspects such as the treatment of the tissues, the fluorochromes, the antibody combinations, etc. Also inadequate samples (non significant because too small or other technical problem , unnecessary fixation, necrotic tissue etc.) for analysis (31 on 655) decreases over time.

Summary and Conclusions: Our data show as immunophenotyping of solid tissues may be used to enhance the histopathologic evaluation of lymphomas and in particular in the cases of T cell or composite lymphomas, confirming the validity of the procedures to integrate different methods for diagnosis in routine. Our review confirms also that a procedure can evolve over time with continued collaboration, shares information and common renovation (from clinical evaluation to surgery, sampling, processing, analysis, etc.) to strengthen, to enhance and to take advantage of the integrated diagnostics.

PB1788

SENESCENT OR EXHAUSTED: INTRATUMORAL TIM-3+ T-CELLS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell Lymphoma (DLBCL) is the most common subtypes of non-Hodgkin Lymphoma (NHL). Tumor infiltration with T-cells plays an important role in cell mediated immunity with different impact on patients' outcome

Aims: To evaluate DLBCL microenvironment and the impact of T-cells exhaustion on response to chemotherapy

Methods: Fresh tissue was taken from newly diagnosed 20 patients with DLBCL and we measured CD28 expression on TIM-3+ T-cells. Several techniques were employed such as cell culture of the fresh tissue, flowcytometry and ELISA.

Results: CD28 expression was positive in 75% (median) of TIM-3+ cells indicating that TIM-3 expressing T-cells are not senescent. In the other hand, the remaining 25% (median) TIM-3+ not expressing CD28,further, cytokine production was restored by IL-2 in those cells indicating that they are exhausted T-cells. patients with either exhausted or senescent T-cells accompanied with slow response on Rituximab-CHOP regimen, however, there was a direct proportion between CD8 tumor infiltrating cell rate and response to the former regimen.

Summary and Conclusions: A giant step has been made on the treatment of NHL in the last decade, however, we are still handling challenging cases need a special attention and understanding of the biology and the status of tumor microenvironment before treatment.

PB1789

ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION BIOPSY FOR DIAGNOSIS OF INTRAABDOMINAL LYMPHOMA WITHOUT ACCESSIBLE PERIPHERAL LYMPHADENOPATHY

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Background: Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNAB) is considered the procedure of choice for the diagnosis and staging of intra-abdominal non-Hodgkin's lymphoma (NHL) without accessible peripheral lymphadenopathy. However, diagnosis and subclassification lymphoma by FNAB is often challenging due to variable cellularity and lack of architecture. Recent advances of ancillary techniques such as immunohistochemical staining, flowcytometry (FCM), fluorescence *in situ* hybridization (FISH) analysis and molecular analysis allowed classify lymphoma more precisely, although sufficient information can be obtained through this procedure remained undetermined.

Aims: The present study was performed to evaluate the yield of EUS-FNAB using a standard 19 or 22-gauge needle for diagnosis and subclassification of lymphoma, assessing the feasibility of immunohistological, FCM, molecular and cytogenetic assessments.

Methods: Between April 2008 and December 2013, 88 patients with malignant lymphoma who had an intra-abdominal mass without accessible peripheral lymphadenopathy underwent EUS-guided fine needle aspiration biopsy at our hospital. All patients received positron emission tomography/computed tomography and had 2-deoxy-2-(^{18}F)fluoro-D-glucose-avid lesions in abdomen

before examination. The aspirated materials were processed for flowcytometry (FCM), molecular analysis of immunoglobulin heavy (IgH) and T-cell receptor (TCR) gene rearrangement, cytogenetic analysis by conventional G banding, and FISH analysis, in addition to standard histopathological studies. Patients' baseline data, including age, sex, laboratory examinations, imaging studies, and final diagnosis of lymphoma, were collected and examined to determine the feasibility and sensitivity for diagnosis and subclassification of lymphoma.
Results: The mean of the diameter of mass was 37mm (9.7-149mm). Among the 88 patients, FCM analysis, standard cytogenetic analysis, and FISH were successfully performed in 77 (65%), 45 (51%), and 48 (55%) cases, respectively. FCM analysis showed immunoglobulin light chain restriction in 45 cases (79%) and were diagnosed as B-cell lymphoma. FCM could not determine the T-cell clonality. Molecular analyses for TCR and/or IgH receptor rearrangements were successful in 35 patients (40%), 31 rearranged in IgH and 4 rearranged in TCR, respectively. There were 32 cases with IgH/Bcl2 fusions by FISH analysis, 26 cases in follicular lymphoma (FL) and 6 cases in diffuse large B-cell lymphoma (DLBCL), respectively. IgH/Bcl6 fusion was seen in 2 case of DLBCL and IgH/C-myc fusion was seen in 1 case of Burkitt lymphoma (BL). Finally, our cohort included 82 B-cell lymphomas (93%) and 6 T-cell lymphomas (7%). Subclassification of lymphoma in accordance with WHO system included 40 cases of FL, 39 cases of DLBCL, one case of BL, 2 cases of lymphoblastic lymphoma, 5 cases of peripheral T cell lymphoma not specified, and one case of angioimmunoblastic lymphoma. Although all of the outpatients were hospitalized until the day after biopsy, there were no serious complications related to this procedure like bleeding, perforation, ileus, and infection.
Summary and Conclusions: EUS-FNAB using a standard 19 or 22-gauge needle is safe and feasible and has high diagnostic value for subclassification of intra-abdominal lymphoma without accessible peripheral lymphadenopathy. With the use of simultaneous immunophenotyping, molecular, and cytogenetic studies, lymphoma subclassification was possible in most of the cases.

PB1790

THE HISTONE DEACETYLASE INHIBITOR ROMIDEPSIN IN COMBINATION WITH LENALIDOMIDE ENHANCES CYTOTOXICITY VIA THE DOWN-REGULATION OF PI3K/AKT AND MAPK/ERK PATHWAYS IN T-CELL LYMPHOMA CELLS

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Background: Histone deacetylase (HDAC) inhibitors are emerging as an exciting new therapeutic option for lymphoid malignancies. These drugs increase the acetylation status and modulate the activity of a wide range of non-histone proteins, and the effects on both histone and non-histone proteins may contribute to their anti-cancer activity. Romidepsin, a class I and II HDAC inhibitor has been approved by FDA for the treatment of cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL). Lenalidomide is an immunomodulatory agent highly effective for the treatment of a wide range of hematological malignancies, and a low toxicity profile.
Aims: Our goal is to evaluate if lenalidomide, in combination with romidepsin is able to enhance the apoptotic effect on T-cell lymphoma cell lines and to identify the molecular mechanisms involved.
Methods: Hut-78 (CTCL cell line) and Karpas-299 (anaplastic lymphoma cells) were treated with increasing concentrations of romidepsin (0,5 - 25 nM) and lenalidomide (1 - 100 μM) alone from 24 to 72 hours to identify the IC_{50} of each drug. The interaction between romidepsin (0,5 nM, 1 nM, 2,5 nM) and lenalidomide (2 μM, 4 μM, 10 μM) was evaluated using the Chou-Talalay method in order to determine if the combination had additive or synergistic effect. The cell cytotoxicity was assessed by MTT assay and apoptosis was studied by Annexin-V/propidium iodide (PI) and flow cytometry and confirmed by Western blot analysis for caspase activation. AKT/PI3K and MAPK/ERK signaling pathways were analyzed by Western blot.
Results: Treatment with romidepsin alone resulted in time- and dose-dependent increase in cytotoxicity in Hut-78 and Karpas-299 cell lines with an IC_{50} at 24-hour of 5.87 nM and 6.36 nM for Hut-78 and Karpas-299, respectively. Lenalidomide alone did not inhibit cells viability up to72 h of treatment in the two TCL cell lines, as we already observed in other cell lines. However, the combination of lenalidomide (10μM) with a low dose of romidepsin (2.5 nM) showed a strong synergistic interaction with combination index (CI) of 0.14 at 24 hours in Hut-78 cells, and an additive effect in Karpas-299 cells, (CI of 1.08). In Hut-78 cells sequential treatment with romidespin for 6 hours followed by washout and the next addition of lenalidomide for 24 hours enhanced the cytotoxic effect of romidepsin and confirm its irreversible effect. Noteworthy the combination did not trigger relevant decrease in the viability of normal peripheral blood mononuclear cells (PBMNCs). The apoptotic effect of the combination was confirmed by the activation of caspases -3, -9 -8 and PARP and was mediated by the increase of the pro-apoptotic protein Bim and by a decrease of the antiapoptotic proteins Bcl-xL and Mcl-1. No effect on the expression of Bcl-2 compared with each single treatment was observed. The effect of romidepsin with lenalidomide on cell cycle parameters is relatively modest. The combination of the drugs induced up-regulation of cell cycle protein p21and a slight decrease of cyclin E and cyclin D. These events were associated with the dephosphorylation of PI3K/Akt, MAPK/ERK pathways. Induction of histone acetylation (H3) and acetylated alpha-tubulin was confirmed.
Summary and Conclusions: Romidepsin with lenalidomide induced apoptosis in T cell lymphoma through signaling event involving the pro-survival pathways PI3K/AKT and MAPK/ERK. Further investigation is required to elucidate the molecular mechanisms of cell death induced by this combination in T cell lymphoma cell lines.

PB1791

PERTURBATIONS OF THE ENDOCANNABINOID SYSTEM IN MANTLE CELL LYMPHOMA; CORRELATIONS TO CLINICAL AND PATHOLOGICAL FEATURES

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Background: The endocannabinoid system (ECS) has attracted attention as potential target for therapy in inflammatory disorders and cancer. A number of agents targeting various components of the ECS have been designed and tested in clinical trials. Mantle cell lymphoma (MCL) is an aggressive disease with usually poor survival and new therapeutic options are clearly needed.
Aims: The cannabinoid receptors have been reported to be upregulated in many types of cancers, including mantle cell lymphoma (MCL) and have been suggested to constitute novel therapeutic targets. The aim of this study was to analyze the expression pattern of the cannabinoid receptors and key enzymes for endocannabinoid metabolism in a well characterized MCL patient cohort and to correlate the findings to biological features.
Methods: Tumor tissue, n=107, were analyzed for the mRNA levels of cannabinoid receptors 1 and 2 (CNR1 and CNR2) and the two main enzymes regulating the endocannabinoid anandamide levels in tissue: NAPEPLD and FAAH (participating in synthesis and degradation, respectively). The expression was correlated to clinico-pathological data.
Results: NAPEPLD was overexpressed in MCL compared to non-malignant B cells, while in 88% of MCL cases FAAH expression was reduced. CNR1 was overexpressed in 98% and CNR2 in 100% of cases. Both low CNR1 and high FAAH levels correlate to lymphocytosis (p=0,016, and p=0,022, respectively) and to leukocytosis (p=0,0018 and p=0,047). Weak to moderate CNR1 levels were a feature of SOX11 negative MCL (p=0,006). Both high CNR2 and high FAAH levels correlated to anemia (p=0,0006 and p=0,038, respectively).
Summary and Conclusions: In MCL the relative expression of NAPEPLD and FAAH is perturbed in a way suggesting accumulation of anandamide. This finding, together with high expression of cannabinoid receptors, suggests enhanced anandamide signaling. The functional consequences of endocannabinoid system perturbations are not yet understood but we find the correlation to leukemic spread of the disease potentially clinically important.

PB1792

MANAGEMENT OF PRIMARY HEPATIC NON-HODGKIN'S LYMPHOMA AND CORRELATION WITH HCV INFECTION : EXCELLENT RESULTS WITH CONVENTIONAL CHEMOTHERAPY

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Background: Primary Hepatic (PHL) non-Hodgkin's Lymphoma is a rare entity, frequently associated with a poor prognosis. PHL was first described in 1965 by Ata *el al* and in 1986 Caccamo *et al* defined PHL as a localized lymphoma, limited to the liver without extrahepatic involvement. Small series of PHL have been reported, suggesting a non-fortuitous association with Hepatitis C Virus (HCV) infection. The prognosis is believed to be dismal, with early recurrence and short survival. To date, less than 150 cases have been published.
Aims: Eleven adult consecutive patients observed in our Division from 1990 to 2013 (median age : 58 years) fulfilled the diagnostic criteria for Primary Hepatic Lymphoma. Our series of patients were derived from 1083 patients with

non-Hodgkin's Lymphoma observed in our institution in the same period (*i.e.* with a prevalence of 1.0% for PHL).
Methods: We performed a study of the viral status and the result of cytotoxic treatment. The disease occurred in middle-aged patients (median age: 58 years). The main presenting complaint was right upper quadrant abdominal pain (4/11 patients). Tumor markers (-fetoprotein and CEA) were normal in 8 patients tested. Liver scans demonstrated either a solitary nodule or multiple lesions. Pathologic examination revealed diffuse large B cell lymphoma in seven patients, two cases of follicular lymphoma, one of small lymphocytic lymphoma and one case of T cell lymphoma. Eight patients (72%) were HCV-positive. Eight patients were treated with CHOP regimen (6 CHOP and 2 R-CHOP), two patients with R-FN, while a patient with a single focal lesion underwent to surgical treatment.
Results: The complete remission rate was 100% (11/11) after frontline therapy, and only one patient relapsed but underwent remission after additional chemotherapy courses; one of these patients, who had HCV-related cirrhosis, died because of hepato-renal syndrome, and another one died because of Acute Myeloid Leukemia.
Summary and Conclusions: Our study confirms the rarity of PHL. In our Division, the outcome of patients with PHL, who are treated with combination chemotherapy, seems excellent. The frequent association of PHL with HCV infection suggests a possible role of this virus in lymphomagenesis. HCV-infection does not appear to influence the outcome.

PB1793

CONSTITUTIVE ACTIVATION OF THE CANONICAL WNT PATHWAY IN AGGRESSIVE B CELL NON-HODGKIN'S LYMPHOMA
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Background: B cell Non-Hodgkin's Lymphoma (B-NHL) represents a highly heterogeneous group of lymphoproliferative malignancies, its pathogenesis may involve in abnormalities of multiple signal transduction pathways. Canonical Wnt signaling pathway is a fundamental signaling pathway and plays an important role in normal cell growth and development. Abnormal activations of canonical Wnt signaling pathway have been found in a variety of solid tumors and hematological malignancies, including leukemia, which suggests that the canonical Wnt pathway may have a close relationship with pathogenesis of tumors. However, there is very little published information regarding potential Wnt pathway in lymphomas, especially in aggressive B-NHL
Aims: The present study was conducted using cell lines to preliminarily investigate whether canonical Wnt signaling pathway is abnormally activated in aggressive B-NHL, and to determine the emerging role of canonical Wnt signaling pathway in the process of tumorigenesis of aggressive B-NHL.
Methods: The 3 aggressive B-NHL cell lines, SUDHL-4, Raji and Namalwa were used, with normal human lymphocytes as the control. The localization of β-catenin was observed by immunocytochemical technique. The expression levels of β-catenin, glycogen synthase kinase-3β (GSK-3β) and its phosphorylated/inactive form (pGSK-3β), were detected by western blotting. The mRNA levels of canonical Wnt pathway relevant genes *CTNNB*1, low density lipoprotein receptor related protein 5(*LRP5*), *Wnt3A* and target gene *c-myc* were estimated by real-time fluorogenic quantitative PCR. After treatment of quercetin, a reported inhibitor of β-catenin, the mRNA and expression levels changes of *c-myc* were detected by real-time fluorogenic quantitative PCR and western blotting respectively. Moreover, biological characteristics including the proliferation, apoptosis and cell cycle changes after treatment of quercetin in cell lines were analyzed by CCK-8 assay and flow cytometry (FCM).
Results: The results revealed that there were different levels of β-catenin abnormal nuclear localization in 3 aggressive B-NHL cell lines compared to normal control. The total and the nuclear expressions of β-catenin proteins were also significantly up-regulated in all 3 B-NHL cell lines as compared with normal control. Different elevated levels of p-GSK3β protein expression were observed in 3 B-NHL cell lines; whereas there was no significant difference in the expression levels of GSK3β between the cell lines and the normal control. The mRNA levels of *CTNNB1, LRP5, Wnt3A* and *c-myc* gene were significantly higher in all 3 B-NHL cell lines than those in the normal control. Moreover, after treatment of quercetin, the mRNA levels and expression levels of *c-myc* were all down-regulated in B-NHL cell lines, the proliferative activity were significantly inhibited, and the majority of these cells were arrested in G2/M phase of the cell cycle, yet the apoptosis phenomenon was not observed.
Summary and Conclusions: Since β-catenin acts as the hallmark of the activation of canonical Wnt pathway, its up-regulated expression, together with other relevant molecular changes in canonical Wnt pathway, suggest that constitutional activation of canonical Wnt pathway may contribute to the pathogenesis of aggressive B-NHL.

PB1794

SYNERGISM BETWEEN ARSENIC TRIOXIDE AND MG132 ACHIEVED WITH LOW DOSES OF VALPROIC ACID AND VINCRISTINE
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Background: Arsenic trioxide (ATO) has low efficacy as a single cytotoxic drug in malignancies other than promielocytic leukaemia but may be combined with other drugs to achieve increased potency and reduced toxicity. The study of cytotoxic drugs interaction is an important aspect in the efficient design of targeted combined therapies. The method of combinatory index (CI) allows the qualification and quantification of drug interaction as synergic (CI<1), antagonistic (CI>1) or additive (CI=1) over the entire range of cytotoxic effects [0-100%]. ATO and MG132, a proteasome inhibitor that targets chemotrypsin-like activity as does Bortezomib, are antagonistic in Burkitt´s lymphoma cell line Raji.
Aims: We explored the possibility of changing the interaction between ATO and MG132 from antagonistic to synergic by adding subcytotoxic doses of two additional drugs: valproic acid (VPA) that targets histone deacetylase (HDAC), and vincristine (VCR) that disrupts the microtubule network. VPA upregulates BNIP3, a Bcl2 family member that is epigenetically silenced in various haematological malignancies and has a controversial role in autophagy and cell death.
Methods: The study was conducted in Raji cells exposed to a wide range of doses of ATO and MG132 for 24h or 72h with or without VPA and/or VCR. Cytotoxicity was assessed by flow cytometry, drug interaction was evaluated by the Chou-Talalay method, autophagy was evaluated by monodancyl-cadaverine (MDC) and UV-excited flow cytometry, mitochondria distribution was evaluated by mitotracker red and fluorescence microscopy and expression of BNIP3 was evaluated by fluorescence microscopy and RT-PCR
Results: Adding a subcytotoxic dose of 3mM VPA enhanced antagonism between ATO and MG132 (CI>1.91 against CI> 1.16 without VPA) and increased autophagy. The distribution of mitochondria was found clustered around the nuclei of Raji cells. The expression of BNIP3 as protein or mRNA was detected only in cells treated with 3mM VPA. Adding VCR at a subcytotoxic dose of 1μM blocked autophagy as compared to basal levels. In addition, the distribution of mitochondria was found elsewhere in the cytoplasm, apart from the nucleus. However, VCR did not revert antagonism between ATO and MG132 (CI>1.10), and in this case BNIP3 expression was undetectable. Adding VPA and VCR at subcytotoxic doses changed the interaction between ATO and MG132 from antagonism to synergism. (CI=0.7<1). In addition, autophagy was abrogated below basal levels, mitochondria distribution was found apart from the perinuclear area and towards cell edges and BNIP3 expression was upregulated at the protein and mRNA level.
Summary and Conclusions: We conclude that synergism between ATO and MG132 can be achieved by targeting simultaneously HDAC and the microtubule network by subcytotoxic doses of VPA and VCR. Burkitt´s lymphoma cell line sensitization was correlated to upregulation of BNIP3, inhibition of autophagy probably owing to blockage of fusion between autophagosomes and lysosomes, and altered intracellular distribution of mitochondria.

PB1795

FINE NEEDLE ASPIRATION AS A DIAGNOSTIC TECHNIQUE IN B-CELL NON-HODGKIN LYMPHOMA: THE USEFULNESS OF COMBINING CYTOMORPHOLOGY WITH FLOWCYTOMETRIC IMMUNOPHENOTYPING AND FISH ANALYSIS
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Background: The role of fine-needle aspiration biopsy (FNAB) in the primary diagnosis and subclassification of Non Hodgkin lymphoma (NHL) has been under intensive debate for a long time. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues 2008 is based on their cytomorphologic, immunophenotypic, and genetic features. These criteria can be readily applied to cytologic specimens.
Aims: This study was designed to evaluate the value of lymph node FNA specimens in the diagnosis and subclassification of NHL according to WHO classification 2008 focusing primarily on B-NHL.
Methods: he current study was prospectively conducted on 129 patients with lymphadenopathy and a clinical suspicion of lymphoproliferative disorder. The sampling procedure was performed using fine needle sampling without aspiration.Cytomorphologic examination was performed on Leishman stained smears. Representative material for cytologic examination was available for only 120 cases (93.02%). One hundred-twenty two (94.57%) specimens collected on RPMI-1640 were submitted for flowcytometric immunophenotyping (FCI) using a battery of monoclonal antibodies specific for lymphoid antigens. I-FISH analysis was performed on 20 out of 48 cases diagnosed as B-NHL (by cytomorphology and FCI) for chromosomal rearrangements t(11;14)(q13;q32)

and/or t(14;18)(q32;q21). Slides for FISH were prepared from air-dried unstained FNA smears.
Results: Using a multiparametric algorithm including clinical data, cytomorphology, FCI, and FISH analysis , definitive final diagnosis was reached in 116/129 (90%) cases. The 116 cases with definitive diagnosis included: benign lymphadenopathy (45 cases) B-NHL (48 cases), T-NHL (7 cases), Hodgkin lymphoma (8 cases), and others (8 cases). Forty eight cases had a final diagnosis of B-NHL. Of these, 33 cases (33/48, 68.8%) could be subclassified according to WHO classification 2008 as follows: SLL (3 cases), CLL (5 cases), Richter's syndrome (2 cases), MCL (4 cases), low grade FL (1 case), LPL (2 cases), B-lymphoblastic lymphoma (1 case), B-lineage ALL (1 case), Burkitt lymphoma (3 cases), DLBCL (10 cases), and DLBCL/FL3 (1 case). The remaining 15 cases (31.2%) could not be classified and were reported as B-NHL-NOS. For the sake of validation studies of FNA as a diagnostic method, a gold standard was established to control FNA final diagnoses. This comprised histopathologic examination, FISH, clinical control (LDH and β_2-microglobulin, and imaging studies), and follow-up of the clinical course (for at least 6 months), or a combination of them. The estimated diagnostic sensitivity of FNA combined with ancillary studies in distinguishing between reactive and malignant lymphoid proliferations was 98.39%, with 100% specificity and 98.89% diagnostic accuracy. Sensitivity, specificity, and diagnostic accuracy of the FNA in B-NHL diagnosis were 98.04%, 100%, and 98.89% respectively.
Summary and Conclusions: Lymph node FNA offers a simple, safe, and minimally invasive procedure for obtaining a diagnostic material that allows a rapid accurate diagnosis and subclassification of B-NHL through the integration of cytomorphology, FCI, and FISH analysis.

PB1796

CYTOGENETIC CHARACTERISTICS OF SPLENIC MARGINAL ZONE LYMPHOMA

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Background: Splenic marginal zone lymphoma (SMZL) is a well-recognized B-cell neoplasm which is characterized by splenomegaly, bone marrow involvement. The most frequent cytogenetic findings are involvement of chromosomes 1, 3, 7 and 8.
Aims: The aim of our study was to investigate the cytogenetic features of SMZL in Russian population.
Methods: In Hematological Scientific Center from January 2001 to December 2013 was observed 132 patients with SMZL.
Results: Standard cytogenetic study was conducted in 56 (65.1%) patients and in 30 (53.6%) patients were found various cytogenetic changes of the karyotype. Normal karyotype was detected in 14 (25%) patients. Mitoses were absent in 12 (21.4%) patients. Partial or full trisomy of chromosome 3 was detected in 5 (8.9%) patients, del7 chromosome - 7 (12.5%), trisomy of chromosome 12 - 3 (5.3%), trisomy of chromosome 18 - 4 (7%) cases and in 4 patients were found the combination of trisomy of chromosome 18 and 3. Translocation t(14;19)(q32;q13) was detected in three cases, in 2 patients were found translocation with rearrangement of immunoglobulin heavy chain gene, one - 17p inversion and translocation t(9;13)(p23;q21) in 1 patient.
Summary and Conclusions: Standard cytogenetic studies in SMZL weren't always revealing various cytogenetic changes of the karyotype. The main reason for failure is the lack of metaphases, due to the low proliferative activity of leukemic cells. A normal karyotype was determined in ¼ patients. Cytogenetic damage of chromosome 7 revealed del7 in 12.5% of cases, which is slightly less than those described in the literature. In patients with SMZL were found rare translocation: translocation t(14;19)(q32;q13) in 3 patients, translocation with BCL6 gene rearrangement in 2 patients. Past research has shown that t(14;19)(q32;q13)-positive SMZL was distinct variant and characterized by rapid progression after splenectomy, poor responses to chemotherapy and short survival. So t(14;19)(q32;q13) may be regarded as a poor prognostic factor.

PB1797

THE SPHINGOLIPIDS METABOLISM INDEXES AND RESPONSE TO CHEMOTHERAPY BY LYMPHOMAS

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Background: The involvement of sphingolipids, and their metabolites, such as ceramides in cancerogenesis was demonstrated in solid tumors as well as in hematological malignancies. As is known, the appearance of multidrug resistant phenotype (MDR) - one of the major causes of failures in the treatment of malignant diseases. According to recent years' data, ceramide signaling plays an important role in tumor progression and development of chemoresistance.
Aims: The present study was to examine the modification of acid ceramidase (aCD) activity and ceramides level as an indicator of the drug resistance in patients by lymphoma.
Methods: The study included 123 patients with B-cell nonHodgkin's lymphoma (NHL), which were admitted to the Hematology Center of Armenia. After obtaining informed consent were evaluated clinical and hematological parameters of all patients. Patients' blood was taken before and after the treatment. For the normal control was used blood from 31 healthy donors of Hematology center. Ceramide has been determined using high performance liquid chromatography (HPLC). The aCDase activity determination was conducted according to the fluorogenic methods (Bedia C. *et al.*, 2010).
Results: According to obtained data two of 105 newly diagnosed and untreated NHL patients (1.9%) and seven of 18 previously treated patients and drug-resistant (38.9%) had detectable high levels of ceramides and twofold increased activity of aCD. Due to failure of therapy, 7 patients died in the treatment first trimesters, the remaining 2 patients 6 and 8 months ago. Development of MDR in NHL is in part driven by the inherent genetic heterogeneity and instability of the tumor cells. Our results suggest that ceramides level and ceramidase activity is a potential pharmacologic target for the NHL treatment. The inhibition of ceramides expression or ceramidase activity might represent a novel strategy to sensitize B-cell NHL patients to chemotherapy.
Summary and Conclusions: Sphingolipids metabolites can be considered promising therapeutic tools alone or in combination with other compounds, as well as valid targets in the attempt to lymphoma treatment and overcome drug resistance.

Indolent Non-Hodgkin lymphoma - Clinical

PB1798

Abstract withdrawn

PB1799

LOW TOXICITY IN BENDAMUSTINE PLUS RITUXIMAB. COMBINATION: RESULT OF A RETROSPECTIVE SINGLE CENTRE ANALYSIS

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Background: Recent trials showed efficacy of bendamustine plus rituximab compared to gold standard therapy in Non Hodgkin's Lymphoma.
Aims: In our study the objective is to confirm also the less toxicity and consequentely the improved quality of life of this alkylating agent in frail patients.
Methods: : In our institution from 2011 to 2013 we treated with bendamustine 33 Non Hodgkin's Lymphoma(NHL) patients, performance status 1-3, (25 indolent, 8 aggressive) with age > 65 years (29 patients relapse/refractory) and while 4 first line patients<65 with severe dilatative cardiopathy. Of 33 patients (13 female and 20 male) the median age was 70 (range 52-88) and 14 (42%) patients were older than 75 years of age. Bendamustine was administered intravenous (minimum 70-maximum 90mg/m2/day) for 2 consecutive days in combination with rituximab 375mg/m2 for 1 day every 28-days cycle for a minimum of 4 courses to a maximum of 8, for a total of 156 courses. The patients comorbidities were:hepatopathy HCV/HBV correlated,hypertensive or ischemic or dilatative cardiopathy , chronic bronchitis, diabetes, chronic renal impairment without dialysis.We admistred routinely primary prophylactic granulocyte colony-stimulating factor support and antimicrobic therapy.
Results: At a median of 12 months' follow-up, 25 patients were evaluable for efficacy and safety. The overall response rate at that time was 90%, with 57% complete responses, 30% partial response, 12% stable disease and 3%early death (1 patients 77 yrs old, died in neutropenic fever after 2st cycle for staphylococcus haemoliticus sepsi) Adverse events were : in 31 patients (94%) prophylactic use of granulocyte colony-stimulating factor (G-CSF) reduced to grade 1neutropenia without infectious complications or therapy delayed or dose reductions applied, while cytopenias grade 4 occurred in 2 patients (6%)reverible with G-CSF and supported with red cell and platelet transfusions ;the fever infections that requiring IV antiobiotics were in 2 patients(6%)in particular 1 patients presented an E.Coli pneumonia and the other patient a staphylococcus haemoliticus sepsi;skin allergic toxicities as rash and pruritus in 3 patients (9%) of which in 2 reversible stopping concomitant cotrimoxazole and allopurinol (grade 1)and 1 patient urticaria (grade 2) treated with short and low doses steroid therapy, gastrointestinal toxicity as nausea, vomiting in 1 patients (3%) grade 2 corrected only with antiemetic medical treatment. In our updated analysis, no cases of myelodysplastic syndrome, secondary neoplasms or medically interaction with several drug taken by elderly patients,had been observed
Summary and Conclusions: The quality of life assessment questionnaire proposed to the patients showed willingly acceptance of this medical treatment without changing life personal routine. Frail NHL patients (elderly or severe co-morbidities) who would not be suitable to receive intensive chemotherapies and might be at increased risk of toxicities could benefit of bendamustine plus rituximab combination.

PB1800

DOES THE PATIENTS WITH FOLLICULAR LYMPHOMA IN LEUKEMIC PHASE HAVE WORST PROGNOSIS IN THE ERA OF IMMUNOCHEMOTHERAPY?

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Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin`s lymphoma and it is rarely presented with the FL cells in bloodstream. Introduction of the monoclonal antibody (rituximab) in the combination with conventional chemotherapy has made major improvement of the overall survival (OS).
Aims: The aim of the study was to analyze prognostic significance of the leukemic phase of FL at presentation in pts treated with immunochemotherapy.
Methods: The study included 162 newly diagnosed pts with FL (75 male/87 female, mean age 52yrs, range 20-74). According to the clinical stage 15 pts had I and II stage (9,3%), 135 pts had advanced III and IV stage (83,3%), while 12 pts (7,4%) had leukemic phase of FL. Regarding to FLIPI, low score was present in 26pts (16,1%), intermediate in 52pts (32,1%) and high in 84pts (51,8%). Low and intermediate FLIPI 2 score was present in 80pts (49,4%), and high score in 82pts (50,6%). In the first line therapy, 61pts (37,7%) received chemotherapy (CHOP/CVP), and 101pts (62,3%) received immunochemotherapy (R-CHOP/ R-CVP). In the group treated with chemotherapy (CH), 4pts had FL in leukemic phase, while in the group treated with immunochemotherapy (IHT) 8pts had FL in leukemic phase.
Results: Treatment response (CR, PR) in the group treated with CH was achieved in 88,5%pts, and 95% in the group treated with IHT. Pts treated with CH had significantly shorter OS in comparison to the pts treated with IHT (Log Rank 19,76, p <0.001). In the group of 4 pts in leukemic phase of FL treated with CH, 2pts died, while in the other group of 8pts, only 1pt with same characteristics died. According to the FLIPI score, there was no statistical difference in the OS (Log Rank 5,05; p=0,17) in all analyzed 162pts. However, there was high statistical difference in OS according to FLIPI 2 score (Log Rank 14,95, p <0,001) in analyzed group. In the group treated with IHT pts with low and intermediate FLIPI 2 had significantly longer OS in comparison to the pts with high FLIPI 2 score (Long Rank 12,8; p <0,001). According to the FLIPI 2 score, patients with FL in leukemic phase had similar OS to the pts with same values of FLIPI 2 score without leukemic presentation (Log Rank 0,05; p=0,83). We have tested a prognostic model for the pts treated with IHT which includes high FLIPI 2; presence of leukemic phase; number of infiltrated lymph nodes>4; ECOG performance status (ECOG PS); and elevated lactate dehidrogenase (LDH). In the proposed model which was statistically significant (χ^2 14,74; p= 0,012), FLIPI 2 index was of major impact on the OS, followed by ECOG PS (HR 7,89; 95% CI; 1,7-36,5).
Summary and Conclusions: Negative prognostic impact of the leukemic phase of follicular lymphoma is successfully overcome with current treatment recommendations of IHT. However high FLIPI2 score plus ECOG PS indicates special consideration in terms of individualized treatment approach followed by possible maintenance IHT.

PB1801

SAFETY AND USE OF BENDAMUSTINE FROM THE BENDAMUSTINE EXPANDED ACCESS TRIAL IN CANADA (BEND-ACT)

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Background: Bendamustine is widely used in the treatment of indolent non-Hodgkin's lymphoma (iNHL) and chronic lymphocytic leukemia (CLL).
Aims: The purpose of the current study is to evaluate additional safety data of bendamustine in up to 100 patients with iNHL relapsing from a rituximab regimen or CLL.
Methods: An expanded access trial with the primary objective of safety was conducted at sixteen centres across Canada and enrolled patients from March 2012 to June 2013. Eligible patients were those at least 18 years old, able to provide informed consent with relapsed/refractory iNHL and were previously treated with a rituximab-containing regimen or had previously untreated CLL, ECOG performance status 0-2, and good organ function. Patients with iNHL received up to 8 cycles of bendamustine (120mg/m^2) on days 1 and 2 every 21 or 28 days; patients with CLL were given bendamustine 100 mg/m^2 on days 1 and 2 every 28 days for up to 6 cycles. Algorithms were followed for dose adjustments for toxicities. Patients were followed for up to 6 weeks after completion of treatment.
Results: Ninety patients started on treatment (74 iNHL; 16 CLL). The mean age was 64 y (range 40–90 y), 44% of the CLL patients were at least 70 years old. For iNHL patients, 77% were treated on a 28-day schedule, with a median of 6 cycles of treatment and 24.3% of patients received 8 cycles. Median time on treatment was 137 days (28–224 days). For CLL patients, the majority received at least 3 cycles and median time on treatment was 91 days (28–168 days). All patients reported at least one adverse event (AE). Grade 3 or 4 toxicities resulted in dose delays in 31.1% of patients with hematological toxicities being the most common reason (24.4.%). Withdrawal from treatment secondary to treatment emergent AEs occurred in 32.2% of patients. Withdrawals were mainly due to rash, hematological, gastrointestinal, respiratory and infectious reasons. Serious AEs were reported in 36.7% of patients and included fever (10%), gastrointestinal events (6.6%), febrile neutropenia (5.6%), pneumonia (5.5%), renal failure (3.3%), hypotension (2.2%), syncope (2.2%), and tumor lysis syndrome (2.2%). The cycle length did not result in any obvious difference in the rate of SAEs. Overall mortality was 4.4%. AEs associated with death included *Pneumocystis jiroveci* pneumonia, multi-organ failure (this was the only fatal AE deemed probably related to treatment), cardiac arrest, respiratory failure, and abdominal pain.

Summary and Conclusions: In this expanded access trial, hematological toxicities were the most frequent reason for dose delays and infections or fever were the most common reason for serious AEs. Overall, the toxicity and safety profile of single agent bendamustine is consistent with other published studies in a similar patient population.

PB1802

RETROSPECTIVE ANALYSIS OF PRIMARY CHEMOIMMUNOTHERAPY WITH BENDAMUSTINE IN COMBINATION WITH RITUXIMAB IN PATIENTS WITH INDOLENT AND MANTLE-CELL LYMPHOMAS

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Background: Induction therapy for advanced indolent lymphoma (iNHL) and elderly mantle-cell lymphoma (MCL) patients consists of rituximab (R) in combination with chemotherapy; typically cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP). Bendamustine plus R (BR) has been shown to be effective in treating patients with relapsed or refractory disease. In a recent German study (Rummel *et al*. 2013)[1] for previously untreated patients with iNHLs, BR demonstrated increased progression-free survival (PFS) and was better tolerated than R-CHOP, thereby challenging R-CHOP's status as a standard first-line therapy.
Aims: This retrospective, Nordic multicenter study (BRiL study) aimed to evaluate the efficacy of BR as first-line chemotherapy in a population-based setting in patients with follicular lymphoma (FL), MCL and other iNHLs, determined via complete remission (CR) and overall response rate (ORR). PFS, overall survival and toxicity were also assessed.
Methods: Patients aged >18 years, with a histological diagnosis of iNHL (FL, MCL, splenic marginal zone lymphoma [SMZ], mucosa-associated lymphoma [MALT], plasmacytic lymphoma [PL, including Waldenström´s lymphoma], and low-grade lymphoma not specified [iNHL NOS]), who had received at least two cycles of BR (minimum dose of 70 mg/m^2) as first-line treatment were included. Previous R monotherapy and/or radiotherapy, and R maintenance following BR treatment, were allowed. Informed consent was obtained according to country regulations.

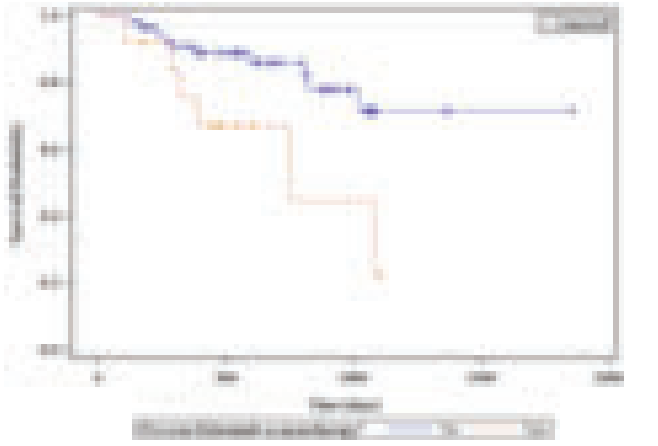

Figure 1. Kaplan-Meier plot showing PFS (days) in patients with FL. FL, follicular lymphoma; PFS, progression-free survival

Results: The study included 116 patients (n = FL, 71; MCL, 15; SMZ, 8; MALT, 9; PL, 6; iNHL NOS, 7); mean age 65.7 years (median, 67.0). Fifteen patients had previously received R monotherapy. After treatment with BR, CR was achieved in 56.9% of all patients, 57.7% of patients with FL, and 46.7% of patients with MCL. CR in patients who had previously received R monotherapy was lower than in those who had not (40.0% *vs.* 60.0% respectively). ORR was 85.3% for all patients, 87.3% of patients with FL, and 66.7% of patients with MCL. At the end of the study, 89% of patients were alive. Stem cells were harvested in four patients, all had >2x10^6/kg CD34 cells. R maintenance improved PFS. The incidence of adverse events was similar to previously reported trials with BR. Thirteen patients died but only two of the deaths were related to treatment: one patient died during treatment with BR due to sepsis, and one patient experienced progressive multifocal leukoencephalopathy and died during R-maintenance treatment. The remaining patients died due to progressive lymphoma (n=6), secondary malignancy (n=2), cardiac arrest (n=1), and other cause (n=2). During R maintenance another patient suffered from herpes encephalitis but survived. An update on side effects from R-maintenance treatment will be presented.
Summary and Conclusions: The results of this study confirm, at a population-based level, the efficacy and tolerability of BR as first-line chemoimmunotherapy for iNHL and MCL. The estimated 3-year PFS of 70% for patients with FL without previous R monotherapy (Figure 1) is similar to the comparable group in the German study.[1]
This study was funded by Mundipharma AB, Mundipharma Oy, Mundipharma AS, and Norpharma a/s. Writing assistance was provided by Watermeadow Medical, sponsored by Mundipharma International Limited.

Reference
1. Rummel *et al*. *Lancet* 2013;381:1203-10.

PB1803

RISK OF SECOND MULTIPLE MYELOMA AFTER NON-HODGKIN AND HODGKIN LYMPHOMA

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Background: Multiple Myeloma (MM), non Hodgkin (NHL) and Hodgkin (HL) Lymphoma are lymphoproliferative diseases (LD) arising from B-cells at several stages of differentiation. Although previous studies have reported an elevated risk of second malignancies following these disorders, few studies have investigated the development of two lymphoproliferative diseases in the same patient.
Aims: We assess long-term risk of MM emergence as a second malignancy in patients with NHL and HL.
Methods: From 2003 to 2013 a cohort of 419 NHL and 277 HL patients, at first diagnosis, were observed from a single center Institution and occurrence of MM was pointed out. Standardized Incidence Ratio (SIR) was calculated as the ratio of observed to expected number of incident MM.
Results: In total only two and one second MM occurred among NHL and HL patients, respectively. The cumulative risk of developing a MM following NHL and HL was respectively 0.43% (1/230) and 0.36 (1/277) among males and about 0.53% (1/189) and 0% (0/140) among females. SIR for male and female NHL patients was 0.83 (p=0.56) and 1.46 (p=0.79). Therefore, in HL group male and female SIR values were respectively, 0 (p=0.39) and 2 (p=0.61).
Among patients with secondary MM, one patient had a previous diagnosis of DLBCL (stage IIA) and was treated according to R-CHOP scheme for six cycles, the second patient was diagnosed with gastric MALT and was treated with four CHOP cycles and the last one was affected by classical HL (stage IIIA) and received six courses of ABVD-like treatment followed by involved field radiotherapy. All three patients obtained complete response after first line treatment for lymphoma and developed MM after the recognition of MGUS. One of them showed M-component since the time of LNH (DLBCL) diagnosis while the other two patients developed it during the follow-up. The average time from first lymphoproliferative diseases and MM diagnosis was 7.6 years (range 5-13), while the average time from MGUS and MM transformation was 3.3 years (range 2-5). Information for light-chain restriction of NHL and MM was available only for the patient with previous MALT diagnosis and the light-chain restriction was different in that case. Among these three patients who developed MM, one is on PR after only three course of liposomal doxorubicin, bortezomib and dexamethasone (PAD), one is on CR after thalidomide plus dexamethasone followed by autologous stem cell transplantation. For the last patient receiving only one administration of VTD (bortezomib, thalidomide and dexamethasone), it was not possible to evaluate response.
Summary and Conclusions: The occurrence of MM and lymphoproliferative diseases in the same patient is very rare. It is generally accepted that normal plasma cells are terminally differentiated, specialized cells arising from B-cells, and it is theoretically possible that the monotypic plasma cells are further differentiated or transformed neoplastic B-cells. But the two processes may arise independently from the same stem cell or from different B cells purely coincidentally. Although it would be rare, a possibility of independent of two infrequent malignancies in the same patient exists and seems to be more convincing theory.

PB1804

PROGNOSTIC SIGNIFICANCE OF BONE MARROW INVOLVEMENT PATTERN IN WALDENSTRÖM MACROGLOBULINEMIA: ANALYSIS OF A SERIES OF 46 PATIENTS

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Background: WHO classification defines Waldenström macroglobulinemia (WM) as a lymphoplasmacytic lymphoma with bone marrow involvement and an IgM monoclonal gammopathy of any concentration. With a typically indolent course, factors associated with a worse prognosis include advanced age, cytopenias, functional status, elevated beta-2 microglobulin level and, for some authors, a diffuse pattern of bone marrow involvement.
Aims: Our aim was to analyze the clinical and biological features of patients with a WM diagnosis in our center, with special attention to the clinical and prognostic significance of bone marrow involvement pattern.
Methods: We studied 46 patients diagnosed between 1992 and 2012. Data collected at diagnosis were age, sex, hyperviscosity and B symptoms, organomegaly, lymphadenopathy, blood count, peripheral expression, beta-2 microglobulin, serum IgM, serum LDH, lymphoplasmacytic infiltration in bone marrow aspirate, tumor burden and predominant marrow pattern (interstitial, nodular or diffuse) in bone marrow trephine, and cytogenetics. We also collected treatment schemes and response, transformation to aggressive lymphoma, overall survival (OS), and mortality. Statistical analyzes were performed using SPSS and Statxact programs.
Results: Mean age was 71 years (range 41-92), male 72%. The presence at diagnosis of lymphadenopathy, splenomegaly, hepatomegaly and peripheral expression was 33%, 17%, 11% and 9%, respectively. Serum LDH and beta-2 microglobulin were found elevated in 11% and 26% of cases, respectively. The average values of tumor burden in bone marrow biopsy and lymphoplasmacytic infiltration in aspirate were 40% (range 10-95) and 37% (10-90). Only one case of transformation to aggressive lymphoma was observed. The predominant pattern was interstitial in 21 cases (46%), nodular 17 (37%) and diffuse 8 (17%). In 7 of 17 cases with a nodular pattern, paratrabecular aggregates were also present. A diffuse pattern was correlated with lower platelet counts (p=0.004) and lower values of hemoglobin (p=0.07), and the interstitial pattern was more frequent in younger patients (p=0.033). The median OS was 43.5 months (range 1-247). The type of marrow involvement pattern did not influence the OS and, specifically, the diffuse pattern was not associated with a worse prognosis. We observed a strong statistical correlation between older age and worse OS (p=0.006).
Summary and Conclusions: In our experience, in line with other authors, advanced age is a major adverse prognostic factor in WM. However, the pattern of bone marrow involvement does not influence the OS, and the diffuse pattern does not carry a worse prognosis when compared with other patterns.

PB1805

TEN YEAR SINGLE CENTRE EXPERIENCE IN TREATMENT OF 34 PATIENTS WITH OCULAR ADNEXAL NON HODGKIN LYMPHOMAS

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Background: Ocular adnexal Non Hodgkin Lymphomas (NHL) consists 2% of all NHL and are in most cases (up to 68%) primary extranodal neoplasms. The most frequent histological subtype is extranodal marginal zone B cell lymphoma (MALT-Mucosa-Associated Lymphoid Tissue) or ocular adnexal MALT lymphoma (OAML), and all other subtypes, including diffuse large B-cell lymphoma (DLBCL, ~ 8%), are much less common. Only few cases of primary ocular adnexal T-cell lymphoma have been reported.
Aims: To investigate clinical parameters of patients (pts) with ocular adnexal NHLs, as well as to compare efficacy between different treatment modalities and to investigate disease and treatment outcome.
Methods: 34 pts with NHL eye involvement on presentation, diagnosed in the Clinic of Hematology, Clinical Centre of Serbia between January 2004 and January 2014, were enrolled. Clinical characteristics and the disease dissemination on presentation were researched. We compared various initial treatment modalities effectiveness, as well as treatment and disease outcome.
Results: In our group 27 pts (79.41%) were diagnosed with OAML, six (17.65%) with DLBCL, and one (2.94%) with extremely rare intraocular peripheral T-cell lymphoma (PTCL). The highest disease incidence rate was in the eight decade, with almost equal distribution among sexes (M/F ratio 1.12). Overall median age was 61.5 years (36-81), males 60 (36-79), females 63 (48-81). The median interval between onset of symptoms and diagnosis in the group of pts with OAML was 5.5 months (1-36) in comparison with median interval in the group of pts with DLBCL (2 months, 1-25). The most frequent sign on presentation was palpable tumor (23.53%). On presentation 19 pts (55.88%) had orbital involvement, conjuctival six (17.64%), lacrimal gland and eyelid three (8.82%) pts each, involvement of the iris two (5.88%) and uveal involvement one (2.94%) patient. Pts with OAML were predominantly (85%) staged as IE CS (Ann Arbor lymphoma staging system), as was a patient with PTCL. Most pts with DLBCL (66.67%) presented with disseminated disease. Chemotherapy (per oral Chloramucil) was the most common initial therapy for OAML pts (33.33%), followed by radiotherapy, surgery and combined approach (22.22% each). All pts with DLBCL received chemotherapy with monoclonal CD 20 antibody, Rituximab (R), and in most cases, intrathecal prophylaxis. The patient with intraocular PTCL received intensive chemotherapy as well. 5-year overall survival (5-y OS) for entire group was 89% and 5-y progression free survival (PFS) was 59%. In the group of pts with OAML, 5-y OS was 91% and 5-y PFS 50%**.** Median OS was 55 months (1-120). In the group of pts with DLBCL 5-y OS was 100%. Median OS was 46.5 months (1-60). Twelve pts (35.29%), all diagnosed with OAML, relapsed. Within this subgroup no significant difference in PFS, in terms of initial treatment option, was registered (p=0.8484, DF 4). Median PFS was 31 months (3-81). Two pts initially diagnosed with OAML have had subsequent transformation in DLBCL. No relapses were registered in the group of pts with DLBCL. Three pts died (two with OAML and one PTCL), of whom two deaths were related to NHL progression.
Summary and Conclusions: Within our group of 34 pts, all DLBCL pts achieved durable complete remissions with R-chemotherapy, whereas in the group of pts with indolent lymphoma (OAML), not treated with systemic chemotherapy, one third relapsed. Our results suggest superiority of initial chemotherapy use in treatment of patients with ocular adnexal NHLs.

PB1806

Abstract withdrawn

PB1807

EVALUATION OF BENDAMUSTINE-RITUXIMAB ROUTINE USE FOLLOWED BY RITUXIMAB MAINTENANCE THERAPY IN RELAPSED/REFRACTORY INDOLENT NHL: INTERMEDIATE RESULTS OF PROSPECTIVE OBSERVATIONAL PROGRAM BEN-RUS-004O

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Background: Combination bendamustine-rituximab (BR) has shown promising results with acceptable toxicity in patients with heavily pre-treated relapsed or refractory indolent B-cell non-Hodgkin lymphoma (iNHL).
Aims: To evaluate the efficacy and safety of bendamustine in combination with rituximab in patients with relapsed and refractory iNHL [clinicaltrial.gov, NCT02072967].
Methods: Starting from May 14, 2012 42 patients (pts) were enrolled at 25 study centres located in 19 Russian cities. Pts received bendamustine 90 mg/m^2 i/v on days 1 and 2 and rituximab – 375 mg/m^2 i/v on day 1 of each 28-day cycle. The 1st assessment of tumor response is performed after 3 BR cycles, in case of complete response (CR), partial response (PR) or stabilization disease (SD) treatment lasts up to 6-8 cycles followed by rituximab maintenance therapy.
Results: 36 pts were evaluated up to date (6 pts received less than 3 cycles of chemotherapy). Histological subtypes were: 23 patients – follicular lymphoma I-II cytological types (55%), 13 pts – small lymphocytic lymphoma (31%), 4 – marginal zone lymphoma (9%), 2 – Waldenstrom's disease (5%). Median age - 59 years (29 - 80) . The majority of pts had later-stage disease, stage III or IV – 34 (81%). 34 patients (48%) had B-symptoms, equal number - 15 (35%) cases of severe condition common scale ECOG (≥ 2) and large tumor masses. In pts with follicular lymphoma, FLIPI subgroups were: low risk (0-1 factor) assigned 6 (26%) patients, an intermediate risk (factor 2) - 9 (39%) pts, and high-risk group (factors 3-5) included 8 (35%) cases. All patients were heavily pre-treated: median of disease duration for all patients prior to study entry was 45 months (9-162). 17 (40%) pts had primary resistant lymphoma, 41% pts – 1st relapse , and 19% pts with two or more relapses of lymphoma. 38% pts had over three lines prior therapy. Thus, the group of patients included into this study is related to unfavourable prognosis. At median follow-up 8 months, in the group of indolent NHL overall response rate (ORR) was 80,5%, complete response (CR) was 28% (10 pts), unconfirmed complete response (CRu) was 5,5% (2 pts), partial response (PR) 38% (17 pts), stabile disease (SD) 5,5% (2pts), and disease progression (PD) 14% (5 pts). In the group of primary resistant lymphoma (17 pts) ORR was 70,5%, CR was 23,5% (4pts), PR – 47% (8 pts), SD – 6% (1 pt), PD – 23,5% (4pts). In the group of relapsed lymphoma (19 pts) ORR was 89,5%, CR

– 31,6% (6 pts), CRu – 10,5% (2 pts), PR – 47,4% (9 pts), SD – 5,25% (1 pt), and PD - 5,25% (1 pt). BR is more active in the group of relapsed lymphoma *versus* the group of primary refractory lymphoma (Table 1). Safety analysis was available for 36 pts. Overall, the combination BR was well-tolerate. The most common grade 2/3 adverse events were leucopenia (9%) and infections (9%).

Table 1. Efficacy outcomes.

Summary and Conclusions: Based on this intermediate results, BR combination is highly active and well tolerated in heavily pre-treated pts with relapsed lymphoma and in primary refractory pts.

PB1808

HAIRY CELL LEUKEMIA: MULTICENTER RETROSPECTIVE ANALYSIS IN TURKEY

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Background: Hairy Cell Leukemia (HCL) constitutes 2-3% of all adult leukemias. This is characterized by infiltration of the bone marrow, liver, spleen and rarely lymph nodes by malignant B cells with hairy extension.
Aims: In this multicenter study, we aimed to present therapeutic outcomes and survival of 93 patients with HCL.
Methods: A retrospective analysis of clinical, laboratory and treatment results of 93 cases, who were diagnosed as having HCL in 13 centers in Turkey between October 1990 and July 2011 are presented in this report.
Results: Sixty-five of the patients were males and 28 were females, with a median age of 55. Splenomegaly was present in 92.6% of cases at diagnosis. At diagnosis, 57% of the patients had leukopenia, 17.2% leukocytosis, 77.4% anemia, 75.3% thrombocytopenia, 15.1% had cytopenia, 34.4% had bi-cytopenia, and 47.3% had pancytopenia. 63% of the patients showed a "dry tap" in bone marrow aspiration. Of the patients with an indication for treatment, 89.5% (77/86) were treated with cladribine as first-line treatment, while in 7% (6/86) of the patients splenectomy was preferred, and in 3.5% (3/86) interferon was preferred. Four patients died before being treated, and 3 could not be followed. Of the patients who were treated with cladribine, 80.5% (62/77) had a complete response (CR), 16.8% (13/77) had a partial response (PR); with a total response rate of 97.3%, 2 patients (2.6%) had died during treatment. Of the patients who were treated with a splenectomy, a CR was obtained in 66.7% (4/6), and a PR was obtained in 33.3% (2/6). No patients died in this group. A partial response (PR) was obtained in patients treated with IFN. The relapse rate was 16.9% (13/77) in patients treated with cladribine and in whom a CR or PR was obtained. In 66.7% (4/6) of the patients treated with a splenectomy and all of the patients treated with IFN, second line of treatment was given as a result of relapse. As second-line of treatment, in 95% (19/20) of the patients cladribine was used and rituximab was preferred in only one patient (5%). A CR was obtained in the patient treated with rituximab, while CR was obtained in 68.4% (13/19) of the patients treated with cladribine, while a PR was obtained in 31.5% (6/19) of them. A second relapse developed in 6 of these patients (6/19). In third - line of treatment, 3 of this 6 patients were given cladribine again (50%), while 2 were given rituximab (33.3%) and 1 was given (16.6%) deoxycoformycin. In third-line treatment, a CR was obtained in 66.6% (2/3) of the patients, PR was obtained in 33.3% (1/3), while CR was obtained in 100% (2/2) of the patients treated with rituximab. A PR was obtained in the patient treated with deoxyicoformicyn. The 28-month median OS was 91.7% in all patients followed-up in this study. In patients who were given cladribine as first-line treatment, OS was observed in 96% in a median 25 months of follow up. In the secondary cancer evaluation of these patients, one had a diagnosis of lung cancer before HCL was diagnosed, and an acute leukemia was diagnosed in one patient 3 months later HCL was diagnosed.
Summary and Conclusions: Cladribine treatment was confirmed as a safe and effective therapy in this study, where patients with HCL were followed up for a long time period, and demographic characteristics of patients with HCL are presented. Also, a long time period of follow up is important for evaluation of development of a secondary malignancy.

PB1809

PRIMARY GASTOINTESTINAL NON-HODGKIN'S LYMPHOMA: RETROSPECTIVE EVALUATION OF 50 PATIENTS

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Background: Primary gastrointestinal non-Hodgkin's lymphoma (PGI-NHL) is a heterogeneous entity different from the nodal counterpart regarding definition, clinicopathological characteristics, histological subgroups, diagnosis, staging, prognosis and treatment.
Aims: The aim of this study is to define the epidemiologic and clinicopathological characteristics of and the prognostic factors related to PGI-NHL, and to evaluate treatment results combined with survival analysis retrospectively.
Methods: Clinical records of 50 patients presented in our center between 1985-2011, diagnosed with PGI-NHL according to the 'relaxed criteria' described by Lewin et al, and followed-up for at least 1 year, were reviewed retrospectively. Histopathologic classification was done according to WHO, 2008. Patients are staged according to the Lugano staging system. MIPI is used for the stratification of prognostic risk groups. Treatment modalities are heterogeneously chosen by the caring physician with regard to clinical characteristics, disease localization, histological subgroup and stage, according to current guidelines. Disease or therapy related toxicities were assessed according to National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03.
Results: Twenty-four patients were male, and the median age was 56 years (22-80). The most common disease localization was the stomach (70%). Abdominal pain was the main symptom (64%). The most common histological subgroup was diffuse large B cell lymphoma (74%) followed by MALT lymphoma (16%). Localized disease was more common (66% *vs* 34%). Bulky disease had negative influence on PFS and DFS (p=0.021 and 0.026, respectively). Bone marrow and/or ≥2 extranodal involvement had adverse effect on OS, PFS and DFS rates (for bone marrow involvement p=0.007, 0.017 and 0.020, respectively; for ≥2 extranodal involvement p=0.001, 0.003 and 0.007, respectively). MIPI (0-1 *vs* ≥2) has been found to separate the study population into prognostically distinctive risk groups with different OS, PFS and DFS rates (p=0.009, 0.011 and 0.010, respectively). Multivariate analysis showed that MIPI was the strongest predictor of overall survival. Compared with single-modality conservative treatment (Campylobacter jejuni eradication, chemotherapy, autologous hematopoetic stem cell transplantation), combination with surgery resulted in better OS rates in intestinal lymphomas (p=0.031). Despite that, in gastric lymphomas surgery has largely been replaced by conservative treatment modalities which are comparable to combination treatments in terms of survival. Over a median follow-up of 31 months, 36 of the patients were alive, 7 were dead and the remaining seven were lost to follow-up. The most common etiology of death was disease progression (n:5). The projected 5-year overall, progression-free and disease-free survival rates were 82.2%, 69.3% and 65.8%, respectively, with gastric lymphomas having better survival rates than intestinal lymphomas.
Summary and Conclusions: Considering the challenging heterogeneity, a uniform definition, histologic classification and staging system is important for comparative interpretations. Although the number of patients with primary gastric lymphoma allowed for a detailed analysis, a larger study group is needed for intestinal lymphomas. This study shows that MIPI is an effective model in PGI-NHL patients enabling identification of patient groups with different prognoses. Clinicopathological characteristics and parameters which have a statistically significant or nearly significant effect on survival need to be evaluated in multidisciplinary, prospective, multicenter trials with a patient population of greater number treated with standardized regimens according to current guidelines.

PB1810

USE OF BENDAMUSTINE AS FIRST LINE TREATMENT IN SELECTED POPULATION OF NON HODGKIN'S LYMPHOMA: TUSCANY REGION EXPERIENCE

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Background: Bendamustine was introduced in Italy from 2008 and in our region, starting from 2011 thanks to regional regulation, was possible to use Bendamustine in first line therapy as 'off-label' drug.
Aims: The aim of this study was to collect all consecutive patients treated in first line with Bendamustine in 7 Tuscany centers.
Results: From June 2011 to December 2012, 72 patients were prospectively included in the study. Diagnosis was: Lymphocytic in 25 patients (34%), Follicular in 18 (25%), Diffuse large B cell in 11 (15%), Mantle cell in 10 (14%), Lymphoplasmocytic in 5 (7%) and MALT in 3 (5%). Thirty-nine patients were treated with Bendamustine 90 mg/m2 for two days, 28 with 70 mg/m2 and 5 with 120 mg/m2. In 14 patients Bendamustine was used alone, in 56 in combination with Rituximab or other drug. The median age was 69 years (range 45-89), in DLBCL was 81 years and 78 years in MCL. Considering the advanced age of this population we applied the geriatric score assessment and 25% of patients were unfit or frail. The median number of cycles performed was 4 (range 2, 6). All patients but 2 were evaluable for response, 35 obtained a complete remission, 27 a partial remission with an overall response rate of 88%, stable disease in 2 patients and progressive disease in 6 patients. According to histotype to note that all but two indolent lymphoma obtained a response; in aggressive lymphomas 4/11 DLBCL and 5/9 MCL reached a complete remission but 4 DLBCL and 2 MCL experienced rapid progressive disease. Treatment was well tolerated, we observed: grade 3-4 neutropenia in 18 patients, no grade 3-4 anemia or thrombocytopenia, grade 3-4 infection in 3 patients. According to extrahematological toxicity no grade 3-4 were reported. Skin rush grade 1 was reported in in 5 patients. After a median follow-up of 14 months the overall survival was 83%; 10 patients died. Progression free survival, after a median observation period of 10 months, was 60%; sixty-two patients are alive, 31 in continuous complete remission, 3 relapsed and 28 with disease under control.
Summary and Conclusions: In conclusion in this 'real life' negatively selected population, the use of Bendamustine showed a very high response rate particularly in indolent lymphomas, promising results, also, are observed in aggressive disease.

PB1811

RAPID TRANSFORMATION OF FOLLICULAR LYMPHOMA, GRADE 1, ASSOCIATED WITH AN UNCOMMON C.796G>A PG266R MUTATION OF THE P53 GENE AND LOSS OF CD45 (LCA) EXPRESSION AT DIAGNOSIS

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Background: Transformation of low-grade follicular lymphoma (FL) to aggressive diffuse large B-cell lymphoma (DLBCL) has been associated with acquiring new genetic events including p53 mutations during the disease course. In addition, the presence of p53 mutation at the time of diagnosis of follicular lymphoma may identify a high-risk group of patients with rapid disease progression and poorer survival (O'Shea *et al.*, Blood. 2008; 112:3126).
Aims: We report a case of rapid follicular lymphoma transformation associated with an uncommon p53 mutation, and loss of CD45 expression at diagnosis.
Methods: Histologic examination, immunohistochemistry, flow cytometry, molecular diagnostics including PCR for IgH and light chain gene rearrangements, and fluorescent *in situ* hybridization (FISH) for t(14;18) translocation were used as diagnostic methods for this case. p53 mutation analysis was performed for the exons 4-8 of the gene using PCR and automated direct sequencing techniques. Additional immunohistochemical studies were performed to assess p53 and c-myc protein levels.
Results: A 66-year old patient with a previous history of rheumatoid arthritis treated with rituximab and a TNF-alfa-blocking agent , presented with B-symptoms and a large tumor mass in abdomen with infiltration of the omentum. At presentation, LDH was 3,8 microkat/L and s-albumin 34 g/L. Bone marrow was negative. A diagnosis of stage III low grade follicular lymphoma was made with tumor cell proliferation <5%. The tumor cells were positive for t(14;18) assessed by FISH. The patient initially received rituximab (4x) without response and subsequently CHOP-21 x 6 with stable disease for 3 months. Because of clinical progression, the patient thereafter received bendamustin followed by local radiation therapy. Thirty five months after initial diagnosis, a new biopsy from abdomen confirmed transformation to DLBCL with high tumor cell proliferation (90%). In both, diagnostic low grade FL and DLBCL (transformed FL), a rare c.796G>A pG266R mutation of the p53 gene (exon 8) was detected, which is reported for first time in FL. This mutation is rarely detected in other lymphoma types accounting for <0.7% of all lymphomas (IARC database, R17). In addition, a silent mutation in exon 4 of the p53 gene was found in both samples. Moreover, aberrant loss of CD45 (LCA) in FL cells was seen in the diagnostic lymph node and DLBCL cells. By immunohistochemistry, overexpression of p53 protein (100% positive tumor cells) was detected in both samples. No change in the levels of c-myc protein was seen.
Summary and Conclusions: Our findings from this case report demonstrate for first time that mutations of the 266R codon of p53 gene may be involved in FL transformation. Therefore, this case along with previously reported data for the potential predictive value of p53 mutations in FL at diagnosis, support the use of immunohistochemical and molecular testing of p53 gene in all newly diagnosed low grade FL.

PB1812

TREATMENT OF HAIRY CELL LEUKAEMIA: LONG TERM FOLLOW UP AND TOXICITY

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Background: Hairy cell leukaemia (HCL) is Characterised by infiltration of the bone marrow, liver and spleen, by a malignant B-cell. First treatment choices were splenectomy and alpha-interferon with a Median survival of 4 years. The purine analogues, introduced in the 1980s, transformed this prognosis.
Aims: Aim of our study was to analyze the outcome of patients with active hairy-cell leukemia with different lines of therapy.
Methods: We retrospectively analysed 16 HCL patients followed in East Kent Hospitals between 1997 to 2013 (12 male, 4 female). Cases were identified by searching for "Cladrabine and Pentostatin" in the pharmacy data base.10/16 patients received Cladrabine (2-CdA) -SC infusion at a dose of 0.14 mg/kg/d over 5 consecutive days, 5/16 patients received Cladrabine -IV infusion at a dose of 0.1 mg/kg/d over 7 consecutive days and 1/16 patient received Pentostatin.
Results: At 6 months after treatment, complete response (CR) occurred in 14/16 patients (87.5) and partial response (PR) occurred in 2/16 patients (12.5%). Six patients (37.5%) have relapsed with a duration of response ranging 3-7 years. All six patients have been re-treated with 2-CdA (5/6) and Pentostatin (1/6) followed by Rituximab, and all achieved at least a partial remission. 3/6 relapsed and had recurrent disease and 1 patient has been refractory to vemurafenib. 75% of patients (12/16) suffered from Neutropenic sepsis requiring hospital admission.
Summary and Conclusions: With a median follow-up of more than 65 months, 13/16 patients (81.3%) continue to be in remission. Although only two deaths have occurred due to refractory disease, the relapse rate is high. Patients who relapse may be re-treated with 2-CdA, but subsequent remissions may be of shorter duration and alternative treatments are needed for patients with recurrent disease. Our data also showed that there is a markedly increased incidence of Neutropenic sepsis indicating that Cladrabine is not a gentle chemotherapy.

Aggressive Non-Hodgkin lymphoma - Clinical

PB1813

THE ROLE OF HIGH DOSE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION AS FIRST LINE TREATMENT IN PATIENTS WITH AGGRESSIVE NON-HODGKIN LYMPHOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: Rituximab-cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) has been the mainstay of treatment in patients with aggressive non-Hodgkin lymphoma (NHL). Despite the improvement in survival rate with the addition of rituximab, there are still a significant proportion of patients who cannot be cured with conventional therapy. One of the strategies to improve survival rate is consolidation treatment with high dose chemotherapy (HDT) followed by autologous stem cell transplantation (ASCT), especially in young patients with high risk aggressive NHL. Randomized controlled trials that addressed this issue yielded conflicting results.
Aims: In order to examine the effect of HDT and ACST on overall survival, we performed a systematic review and meta-analysis.
Methods: Systematic review and meta-analysis of randomized controlled trials comparing HDT and ASCT after achievement of complete remission (CR) or partial remission (PR) to standard dose chemotherapy, with or without rituximab as first line treatment in patients with aggressive NHL. The Cochrane Library, MEDLINE, conference proceedings and references were searched until December 2013. The primary outcome was overall survival (OS). Secondary outcomes were relapse rate, overall response (ORR), CR and secondary malignancies. For dichotomous data, relative risk (RR) with 95% confidence intervals (CIs) were estimated and pooled and hazard ratios (HR) for time to event data were estimated and pooled. We used fixed effect model to pool results.
Results: Our search yielded 20 trials conducted between the years 1987 and 2011, including 4488 patients. In five trials rituximab was added to both arms. Median age of patients ranged between 31 to 51 years old. Seven trials included only patients with intermediate-high and high risk age-adjusted international prognostic index (aaIPI). Ten trials were judged to be at low risk for selection bias (allocation concealment and sequence generation).Data from 19 trials were available for analysis of OS. Treatment with HDT and ASCT did not improve OS as compared to standard dose chemotherapy, HR 1.09, 95% confidence interval (CI) 0.98-1.21, I^2 for heterogeneity 32%. Also, no survival benefit was shown in a sub- analysis of patients with intermediate-high and high aaIPI (HR 1.08 95% CI (0.95-1.24), I^2=66%, 10 trials). However, HDT and ASCT was associated with an increased rate of CR [RR 1.07 95% CI (1.02-1.11)], and a decreased rate of relapse, RR 0.58 95% CI (0.49-0.69). No statistically significant difference was observed regarding the incidence of secondary malignancies.
Summary and Conclusions: HDT and ASCT as first line treatment for aggressive NHL is not associated with increased OS, yet it improves CR rate and decreases relapse rate. The possibility to salvage relapsing patients with HDT and ASCT may contribute to the lack of effect of this treatment on OS when given as first line therapy.

PB1814

USEFULNESS OF 99M TC-TETROFOSMIN SCINTIGRAPHY IN THE FOLLOW UP IN PATIENTS WITH MALIGNANT LYMPHOMA IN THE ERA OF 18F-FDG PET-CT

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Background: In patients with malignant lymphomas the precise staging and follow up is very important for treatment and prognosis of these patients. 99mTc-Tetrofosmin is currently used to study myocardial perfusion, but also has been reported to be localized in various types of malignant tumours, including lymphomas, giving their localization and proliferation activity. With previously used visualized techniques it has been extremely difficult to differentiate metabolically active tumour tissue from post-therapy fibrosis. Nowadays 18F-FDG PET-CT is a method of choice for these applications.
Aims: In this study, we aimed to evaluate the role of 99mTc-Tetrofosmin scintigraphy for therapy control of lymphoma patients in the era of 18F-FDG PET-CT.
Methods: We investigated 103 patients (56 men and 47 women) with Hodgkin's (HL) and non-Hodgkin's lymphoma (NHL), aged 19-73 years and treated with standard chemotherapy protocols. Planar and SPECT images were carried out 30 minutes and 3 hours after intravenous injection of 740 MBq 99m Tc-Tetrofosmin and analyzed qualitatively and quantitatively. The scintigraphic results were compared with the data of conventional methods (clinical examination, x-ray, CT). Additionally, patients underwent 18F-FDG PET-CT scan. Beta-2-microglobulin was measured by radioimmunoassay.
Results: 99mTc-Tetrofosmin scintigraphy was positive in 42 patients with HL and NHL before treatment. From 58 detected lesions 40 had lymph node involvement. Increased uptake of the radiotracer was demonstrated in mediastinal, neck, supraclavicular, axillar and inguinal lymph nodes. Extranodal localization was detected in 18 lesions. The tumour/background ratio ranged from 1.5 to 2.1. In six patients with false negative scintigraphy, CT investigation showed enlarged abdominal lymph nodes. True negative 99mTc-Tetrofosmin scan after chemotherapy was registered in 37 patients. Focal pathological tetrofosmin uptake was seen in ten patients and 21 lesions were identified. Fourteen lymph node lesions were detected in mediastinal, neck, supraclavicular, axillar and inguinal area. Seven lesions had extranodal localization – lungs and bones. False negative scintigraphy was found in 8 patients, whereas CT scan showed lesion with subdiaphragmatic localization. The presence of B-symptoms and elevated beta-2-microglobulin were found in these patients. The patients with inconclusive 99mTc-Tetrofosmin scan were referred to PET-CT. Additional 22 PET-CT positive nodal and extranodal subdiaphragmatic lesions were detected.
Summary and Conclusions: 99mTc-Tetrofosmin is a promising tracer in determining disease activity in supradiaphragmatic lesions. This method that has high sensitivity and low radiation burden, is appropriate in lymphoma patients especially with supradiaphragmatic localization of the lesions. In patients with small subdiaphragmatic lesions (<15 mm) and inconclusive 99mTc- Tetrofosmin scintigraphy, PET-CT is method of choice.

PB1815

BENDAMUSTINE PLUS RITUXIMAB FOR RELAPSED OR REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA: A RETROSPECTIVE STUDY

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Background: After standard R-CHOP therapy, patients with relapsed or refractory diffuse large B cell lymphoma (DLBCL) are generally treated with aggressive salvage chemotherapy followed by high dose therapy with autologous stem-cell transplantation (ASCT). However, for patients who are not eligible for intensive chemotherapies and ASCT because of comorbidities and/or advanced age or relapse after heavy salvage regimens, treatment options are very limited and prognosis is poor. Based on the demonstration of efficacy and safety of bendamustine plus rituximab (BR) in patients with indolent and mantle cell non-Hodgkin lymphomas in terms of increased progression-free survival and fewer toxic effects than R-CHOP, additional studies are being carried out to assess the activity and safety of this combination in patients with DLBCL not eligible for intensive chemotherapy regimens.
Aims: To analyze a group of patients with relapsed or refractory DLBCL treated with combination BR between July 2010 and January 2014, and to evaluate overall response rate (ORR), progression-free survival (PFS) and treatment safety.
Methods: Of 22 patients registered, 19 (14 males and 5 females) are currently available for this analysis. Patients gave informed consent. The median age was 71 years (range 54-82); ECOG performance status was 1 (n=6, 27.2%), 2 (n=15, 68.1%) and 3 (n=1, 4.5%); R-IPI scores before starting therapy were good (n=13, 59%) and poor (n=9, 41%). The median number of prior chemotherapy regimens was 2 (range 1-5); lactate dehydrogenase was elevated in 12 patients (54.5%) and normal in 10 (45.4%). The Ann Arbor clinical stage at baseline was IV (n=8, 36.3%), III (n=8, 36.3%), and II (n=6, 27.2%). Rituximab was administered at the standard dose of 375 mg/m^2 and bendamustine at a dose between 70 and 120 mg/m^2 (mean 90 mg/m^2) on days 1 and 2 of each 28-day cycle for up to six cycles. Nine patients (41%) completed six cycles of treatment and the median number of cycles received per patient was 5 (range 1-6).
Results: ORR was 47.3% (complete response: n=7 patients, 36.8% and partial response: n=2 patients, 10.5%), with stable disease in 1 patient (5.2%) and progression disease in 9 patients (47.3%). At the median follow up of 6.4 months (range 1 to 37.4 months), the median PFS was 7 months (95% CI 4.4 - 26.6) for all patients. Four patients showed remission lasting longer than 24 months. Grade 3 / 4 toxicities observed were: lymphopenia (42.1%), neutropenia (36.8%), anemia (15.7%), and thrombocytopenia (10.5%). Infection by cytomegalovirus (CMV) was observed in two patients (10.5%). Common non-hematologic adverse events included nausea (31.5%), fever

(21%), fatigue (15.7%), cutaneous rash (5.2%), and diarrhoea (5.2%). One patient experienced transfusion-dependent anemia one year after the end of therapy, and a diagnosis of myelodysplastic syndrome refractory anemia type was made.
Summary and Conclusions: BR can be considered to have a role in the treatment of patients with relapsed/refractory DLBCL with limited therapeutic options, in that it can induce long-term remission in some patients with an acceptable toxicity profile. However, the high percentage of non-responding patients in this study suggests that larger trials are needed to assess the efficacy of this combination.
Disclosures: Off Label Use: bendamustine in salvage therapy in aggressive B-cell NHL.

PB1816

Abstract withdrawn

PB1817

ASSOCIATION OF POLYMORPHISM RS1625895 GENE TP53 WITH EFFECTIVENESS OF R-CHOP TREATMENT OF DLBCL
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Background: In response to DNA damage the p53 protein functions to induce cell cycle arrest, DNA repair or apoptosis. Deficiency of p53 function is one of the adverse prognostic factors in NHL. In DLBCL incidence of *TP53* mutations and 17p deletion in the onset of the disease is rare. Peller *et al.* (1997) described rs1625895 gene TP53 associated with changes in apoptosis of leukocytes in patients with cancers.
Aims: The purpose of the present study was study association of rs1625895 with with effectiveness of R-CHOP treatment of DLBCL.
Methods: We studied the single nucleotide polymorphism rs1625895 in 106 unrelated patients with DLBCL treated by R-CHOP. Clinical response to treatment was assessed according to Cheson Criteria. Genotyping was carried out with use of PCR-RFLP. Overall survival (OS) and relapse-free survival (RFS) probabilities were estimated with the use of the Kaplan–Meier method and were compared between patients with a mutation and those without mutant alleles by means of the log-rank test. Multivariate analyses were conducted with the use of the Cox model with forward selection to identify independent prognostic variables influencing the OS and RFS.
Results: For rs1625895 genotype distribution in DLBCL patients was as follows: genotype G/G - 75,5%, G/A - 22.6% and A/A - 1.9%.
The response rate in subgroups of patients with rare allele and homozygous genotype G/G was 73.1% and 50.0%, respectively (p=0.0396). Odds ratio response to R-CHOP therapy in patients with genotype G/G, was 0.37 [95% CI: 0,15; 0,99, P <0,05]. In patients with DLBCL with genotype G/G rs1625895 5 - year OS was 42.5% *vs* 65.4% in patients with genotypes G/A and A/A (p =0.014). In the subgroup of patients with homozygous genotype G/G rs1625895 relapse-free survival was 36.3%. It was significantly lower (p=0.030) than in the subgroup of patients with genotypes G/A and A/A - 57.7%. In multivariate analysis by Cox regression method was shown that rs1625895, along with the index IPI, can serve as a predictor of the likelihood of achieving OS and DFS.
Summary and Conclusions: The present study showed that genotype G/G rs1625895 gene *TP53* is associated with a high probability of failure R-CHOP therapy of DLBCL patients. Future studies to confirm these data are required.

PB1818

OUTCOMES AFTER 90YTTRIUM-IBRITUMOMAB TIUXETAN-BEAM IN DIFFUSE LARGE B-CELL LYMPHOMA: A META-ANALYSIS
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Background: High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a standard therapy in patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) who are chemo-sensitive. The combination of carmustine, etoposide, cytarabine, and melphalan (BEAM) is commonly used as a conditioning regimen. The addition of yttrium-90 (^{90}Y)-ibritumomab tiuxetan (Zevalin®) to BEAM (Z-BEAM) is increasingly being used to improve outcomes and overcome refractory disease.
Aims: We conducted a literature review and meta-analysis in order to evaluate the clinical effects of Z-BEAM followed by ASCT in patients with DLBCL
Methods: A literature search was conducted for randomized controlled trials and observational studies of Z-BEAM as a conditioning regimen for ASCT in adult patients with DLBCL. Extracted data included baseline patient demographics, overall response (ORR), complete response (CR), overall survival (OS), progression-free survival (PFS), non-relapse mortality (NRM), median time to ANC and platelet engraftment, and rate of myelodysplastic syndrome. Mixed effects models were used to determine estimates.
Results: Twelve studies (N = 409) were included in the meta-analysis. The 2-year OS and PFS were 85.5% (n=409) and 67.7% (n=366), respectively. Outcomes were superior in patients with non-transformed lymphoma. Post-transplant, ORR and CR rates were 72.6% and 68.5%, respectively. The primary toxicity was neutropenia (49.9%). Two-year OS was significantly associated with pre-transplant ORR (p=0.002, tau square=0) and with pre-transplant CR rate (p=0.04, tau square=0.15). There was no significant association between PFS and pre-transplant response.
Summary and Conclusions: Z-BEAM is safe and effective as a conditioning regimen in relapsed/refractory DLBCL.

PB1819

GOOD PROGNOSIS OF PATIENTS WITH PRIMARY INTRAOCULAR LYMPHOMA TREATED WITH SYSTEMIC HIGH DOSE METHOTREXATE AND INTRAVITREAL METHOTREXATE INJECTION: A STUDY OF 23 PATIENTS
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Background: Primary intraocular lymphoma (PIOL) is a rare subtype of malignant non-Hodgkin's lymphoma which occurs either alone or in association with brain involvement. Diagnosis of PIOL can be challenging and the optimal treatment has yet to be defined.
Aims: This study is to assess the clinic-biologic features, diagnostic tools and treatment outcome in a cohort of patients with PIOL.
Methods: Retrospective case analysis of medical records and review of cytology of a consecutive series of 23 patients presenting with PIOL who were treated with systemic high dose methotrexate and intravitreal methotrexate injection at the National Taiwan University Hospital between Jan 2003 and Jul 2013 were investigated. Clinical data, including patient's age at the onset of disease, gender, medical history, clinical findings, laboratory investigations, mode of diagnosis, interval between the onset of symptoms and final diagnosis, type of therapy, disease course and duration of survival were obtained.
Results: Of 23 patients enrolled, 17 (73.9%) were female. The median age of onset of symptoms was 64.5 years with range from 36 to 76 years. All the patients were immune-competent and human immunodeficiency virus negative. The commonest symptoms reported by patients were blurred and/or floaters. The diagnosis of PIOL of these patients was based on the identification of abnormal lymphoid cells in vitreous fluid and 6 patients (26.1%) had bilateral eye involvement. The median time from the initial ocular presentation to diagnosis was 6 months (range 1.6–26.6 months). Eight patients had concurrent brain involvement at diagnosis, including three patients with parenchyma lesions. None had systemic involvement. Among 15 patients receiving flow cytometry examination, all patients were diagnosed as having B-cell lymphoma with monoclonality of light chain. All patients received both systemic chemotherapy with high-dose methotrexate (MTx, 8 gm/m^2 on day 1) and intravitreal injection with MTx as the induction regimen. Nineteen patients (79.2%) achieved first complete remission (CR1) with a median interval of three months. Among them, eight (42.1%) relapsed with a median remission duration of 7.8 months (range 2.8-21.4). Fortunately, seven (87.5%) patients achieved second CR after salvage chemotherapy with intravitreal MTx, systemic BOMES (BCNU 65 mg/m^2 on day 1 and day 2, oncovin 2 mg on day 1 and day 8, methotrexate 1.5 gm/m^2 on day 8, etoposide 50 mg/m^2 on days 1-5 and methylprednisolone 200 mg on days 1-7) or whole brain radiotherapy. The most common adverse effects of high-dose MTx were manageable and reversible, including hepatotoxicity (grade I, 9 patients; grade II, 6 and grade III, 6) and nephrotoxicity (grade I, 2 and grade II, 2). With a median follow-up duration of 81.8 months (range 4.4-117.3) the five-year overall survival (OS) rate was 64.7% and the median OS was not reached. A total of 5 patients died of lymphoma (21.7%). There was no survival difference between the patients with and without CNS involvement.
Summary and Conclusions: Diagnosis of PIOL requires both clinical suspicion and careful cytologic examination. The frontline treatment with high-dose MTx and intravitreal chemotherapy yielded a substantial remission rate and longer survival. It is proved to be an effective regimen with manageable side effects.

PB1820

EFFICACY AND TOXICITY OF MOGAMULIZUMAB FOR RELAPLSED/ REFRACTORY ADULT T-CELL LEUKEMIA-LYMPHOMA: A RETEOSPECTIVE ANALYSIS IN SAGA PREFECTURE, JAPAN

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Background: Adult T-cell leukemia-lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by human T-cell lymphotropic virus type I, and incurable by conventional chometherapy. CC chemokine receptor 4 (CCR4) is a chemokine receptor expressed on T-helper type 2 and regulatory T cells (Treg). CCR4 is also expressed on tumor cells from about 90% of patients with ATL. Mogamulizumab is a defucosylated humanized anti-CCR4 antibody that show potent antibody-dependent cell cytotoxicity (ADCC). In the phase II study, mogamulizumab has been shown to be effective and well tolerated against relapsed ATL patients. However, its efficacy and toxicity has not been evaluated in clinical practice.
Aims: We investigated and analyzed the efficacy and toxicity of 20 patients with relapsed/refractory ATL.
Methods: Relapsed or refractory ATL patients treated with mogamulizumab at Saga University Hospital and Karatsu Red Cross Hospital from April 2012 to December 2013, were evaluated. Overall survival (OS) was analyzed from the date of the first dose to death or to date of last follow-up.
Results: A total of 20 patients (13 male, 7 female) was evaluated. Median age was 70 years (range, 46-84). The disease subtypes at initial therapy included 17 acute, 2 lymphoma, 1 unfavorable chronic type ATL. The responses to prior therapy were 1 CR, 5 PR, 4 SD, and 10 PD. Mogamulizumab was intravenously administered once a week for 8 weeks at a dose of 1.0mg/kg, and 5 patients (25%) completed the schedule. Of the remaining 15 patients, 9 discontinued treatment because of disease progression, 1 because of skin rash, and 5 because of others. The median number of mogamulizumab infusion was 4 (range, 1-8). Eight of the 20 patients (40%) achieved objective response, including 4 with CR (20%) and 4 (20%) with PR. The response in the remaining12 patients was SD in 2 patients (10%) and PD in 10 patients (50%). Response rate for relapsed ATL and refractory ATL was 17% (1 in 6 patients), and 50% (7 in 14 patients), respectively. Responses according to disease site were 88% (of 17 patients, 12 CR and 3 PR) for peripheral blood, 38% (of 8 patients, 3 PR) for skin, and 27% (of 15 patients, 1 CR and 3 PR) for nodal lesions. Median OS were 5 months (range, 0.3-16.2). Adverse events at grades 3 to 4 were 8 lymphopenia (40%), 2 skin rash (10%), and 1 thrombocytopenia (5%).
Summary and Conclusions: In the present study, mogamulizumab showed efficacy similar to that shown in the phase II study for refractory ATL patients, however, the response rate for relapsed ATL patients was low. OS was also shorter than that of the phase II study. Further clinical investigations are required to examine whether concomitant use of this novel agent with other drugs with different mechanism of action would be more effective for ATLs. Though allogeneic hematopoietic cell transplantation (allo-HCT) is a promising treatment to achieve long-term survival for patients with relapsed or refractory ATL, mogamulizumab may be the good option for those who are ineligible for allo-HCT. In the congress, we will also discuss about the effect of mogamulizumab for graft-*versus*-host disease after allo-HCT.

PB1821

Abstract withdrawn

PB1822

IDENTIFYING LYMPHOMA ANTIGEN RECEPTOR SEQUENCES BY IMMUNE REPERTOIRE PROFILING

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Background: Neoplastic T and B cells rapidly expand, leading to T and B-cell receptor (TCR and BCR) repertoires dominated by one rearrangement. Techniques that use amplification of rearranged receptors from unselected populations of lymphocytes to identify malignancy, including high-throughput sequencing and the biomed primers, assume that neoplastic clones are present at conspicuously high frequency. However clones reacting to an antigen also expand. Differentiating high-frequency reactive clones from lymphomas is important for both diagnosing and monitoring lymphomas.
Aims: This project aims to use high-throughput sequencing of the antigen receptor repertoire to identify source-specific thresholds that differentiate high-frequency clones from neoplastic clones.
Methods: We developed a method that amplifies rearranged antigen receptor CDR3 sequences and uses high-throughput sequencing (HTS) to sequence tens of thousands of chains simultaneously. Because the technology utilizes gDNA the frequency of sequenced CDR3 chains is representative of the relative frequency of each CDR3 sequence in the sample population of lymphocytes. To demonstrate the potential of HTS of T and B-cell receptors to contribute to diagnosing and monitoring neoplasia in mature lymphomas; we amplify the TCR repertoire of 98 and the BCR repertoire of 60 index samples to identify high-frequency *TRB* or *IGH* rearrangements. Concurrently, we sequence the *TRB* and *IGH* repertoire in control blood, bone marrow, skin, and reactive lymph tissue from 7, 8, 6, and 14 samples respectively. Clones in the index samples are classified as neoplastic if occurring at a proportion greater than 7 standard deviations above the mean frequency of the most abundant rearranged *TRB* or *IGH* in matched control samples.
Results: Using control samples, we defined the expected frequency of highest-copy clones by source type (Table 1). Using our definition of neoplastic clone, Eighty-four percent of our 158 index samples have a tractable rearrangement.

Table 1. Average and standard deviation of highest copy TCR clones in control samples.

Summary and Conclusions: We find that for most disease diagnoses, high-throughput sequencing identifies a tractable clone and T-cell and B-cell receptor repertoire analysis may be useful for clinical laboratory evaluation of patients with T and B-cell neoplasms.

PB1823

NUMBER NEEDED TO TREAT AND COST UTILITY ANALYSIS OF GRANULOCYTE-COLONY STIMULATING FACTORS FOR PRIMARY PROPHYLAXIS OF CHEMOTHERAPY INDUCED FEBRILE NEUTROPENIA IN DLBCL PATIENTS IN THE NETHERLANDS

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Background: Myelosuppression is a frequent side effect of (R)CHOP chemotherapy. Severe neutropenia can leave patients vulnerable to febrile neutropenia (FN), with associated in-hospital mortality rates in non-Hodgkin's Lymphoma (NHL) of up to 9.4%. Severe neutropenia and FN also have the potential to delay chemotherapy cycles and mandate dose reductions. Compromising the relative dose intensity of (R)CHOP-21 chemotherapy may result in poorer survival outcomes in diffuse large B-cell lymphoma (DLBCL). International guidelines by EORTC, ESMO, ASCO and NCCN recommend using granulocyte-colony stimulating factor (G-CSF) for primary prophylaxis (PP) to reduce the incidence of febrile neutropenia (FN) for patients at an overall FN risk of 20% or more. For adult patients with NHL treated with (R)CHOP-21 like chemotherapy, FN incidences between 17% and 50% have been reported, demonstrating the eligibility of this regimen for PP with G-CSF as indicated in international guidelines.
Aims: 1.To estimate the number needed to treat (NNT) to avoid an FN episode for different PP options in comparison to no prophylaxis in patients receiving 6 cycles of standard (R)CHOP-21 chemotherapy for DLBCL. 2. To assess the cost-utility in the Netherlands of PP with once-per-cycle pegfilgrastim compared to no prophylaxis, PP with daily filgrastim (11- or 6-days per cycle) and secondary prophylaxis (SP) with pegfilgrastim.
Methods: A decision-analytic model was constructed from the Dutch healthcare-payer perspective. Costs were obtained from official list prices (January 2014) or literature and included drugs (lowest list prices for each product), drug administration and FN-related hospitalization costs. G-CSF relative effectiveness inputs were based on a recent meta-analysis and previous NHL studies. Survival and utility variables were modeled from available data for NHL patients in the US SEER cancer registry and published literature. Univariate sensitivity analyses evaluated the robustness of the model for all input variables.
Results: PP with pegfilgrastim provides the most effective primary prophylaxis of FN with a NNT to avoid an FN episode of 4.7 (see Table 1). In the cost-utility analysis PP with pegfilgrastim was dominant (more effective and cost saving) compared to PP with 11-days filgrastim and cost-effective compared to no prophylaxis, PP with 6-days filgrastim and SP with pegfilgrastim. (see Table 2) The sensitivity analyses revealed that baseline

FN risk, G-CSF effectiveness in reducing FN and long term survival assumptions were the most sensitive parameters, and showed that the model was robust. Even when in a scenario analysis the price of daily G-CSF was reduced by up to 90%, PP with pegfilgrastim remained cost-effective compared to the daily G-CSF options.

Table 1. Number needed to treat to avoid an FN episode.

Table 2. Cost utility of PP pegfilgrastim in comparison to other strategies.

Summary and Conclusions: For DLBCL patients treated with (R)CHOP in the Netherlands, primary prophylaxis of FN with pegfilgrastim is a cost-effective use of healthcare resources.

PB1824

HIGH-DOSE METHOTREXATE, HIGH-DOSE CYTARABINE AND TEMOZOLOMIDE FOR THE TREATMENT OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL), SIX YEARS SURVIVAL IS REACHED

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Background: Treatment of primary central nervous system lymphoma (PCNSL) associates with low response rates and poor survival using conventional radio and chemotherapy. Due to its favorable toxicity profile, Temozolomide has emerged as a new option for treatment of PCNSL in young patients. In this study we report a series of (PCNSL) patients treated with an innovative regimen.
Aims: To evaluate a new intensive chemotherapy with Temozolomide, trying to assess response and progression free survival and overall survival taking into consideration the toxicity profile. The study was performed at Al Mowassa Charity hospital in Damascus (SYRIA).
Methods: 40 patients with histologically confirmed PCNSL median age 52 years (range 20-65) years were included. Biopsies were cultured and a Karyotyping was made in 32 patients. An induction chemotherapy was started Methotrexate 3 gr/m² over 12 hours on day1 , Cytarabine 3 gr/m² every 12 hours on day 1 and Temozolomide 150 mg/m² from day 2 through day 6 with a total of 6 cycles were given on a monthly basis.
Results: Among the 40 patients included in the study a complete response was observed in 34 patients (85%) and a partial response in the remaining 6 patients (15%). Disease progressed in 8 out of 40 patients (20%) while 32 patients are still living at five years making the overall survival reaching (80%). Grade II nephrotoxicity was observed in 2 patients while grade III and IV hematotoxicity was observed in 5 patients. The study was updated in January 2014 and we still have the same progression free survival but we lost a patient died from heart attack making the overall survival rate 77% at 6 years.
Summary and Conclusions: high-dose of both Ara-c and MTX combined with Temozolomide appears to be a good choice in the treatment of PCNSL, in the light of good response and overall survival rates

PB1825

PROGNOSTIC SIGNIFICANCE OF INTERIM POSITRON EMISSION TOMOGRAPHY SCAN IN PATIENTS WITH MATURE T-CELL AND NK-CELL LYMPHOMA

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Background: Interim positron emission tomography (PET) scan has been found to be effective in Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL). The prognostic value of interim PET in mature T-cell and NK-cell lymphoma remains uncertain. Recently, the Deauville five-point scale (5-PS), which visually assess the uptake of lesions in comparison with background mediastinal and liver uptakes, has been validated in the large numbers of patients with HL and DLBCL. However, the prognostic impact of 5-PS on clinical outcomes has not been investigated in mature T-cell and NK-cell lymphoma.
Aims: The aim of this study was to determine the prognostic role of interim PET, assessed by Deauville 5-PS, in patients with mature T cell and NK cell lymphoma and treated with systemic chemotherapy.
Methods: We consecutively enrolled patients with newly diagnosed mature T-cell and NK-cell lymphoma, treated with CHOP/CHOP-like or non-anthracycline-based chemotherapy and had the baseline PET data with ³1 evaluable hypermetabolic lesion between 2006 and 2012 in two Korean institutions. Patients treated with upfront chemoradiotherapy before interim PET scan were excluded. Interim PET scan was performed after 3 cycles of chemotherapy, before 1 week of the next cycle. Interim PET response was visually assessed by 5-PS and four point or higher was regarded as positive. All PET assessment was performed by 2 nuclear medicine specialists at each institution, and the discrepancy of assessment was resolved by the agreement through discussion.
Results: A total of 35 patients was included in this analysis. The median age was 60 years (range, 31-79) and 26 (74%) were male. Histologic subtypes included were PTCL, not otherwise specified in 10 (29%), extranodal NK/T cell lymphoma in 8 (23%), angioimmunoblastic T cell lymphoma in 7 (20%), anaplastic large cell lymphoma, ALK negative in 4 (11%), and others in 6 (18%). 22 patients (63%) were presented as advanced stage disease and 9 (26%) had B symptoms. ECOG performance status was ≥ 2 in 7 (20%), serum LDH level was elevated in 16 (46%), and bone marrow was involved in 5 (14%). Thus, 14 patients (40%) were classified as high risk (≥ 2 factors) by the prognostic index for PTCL (PIT). 31 patients (89%) completed planned systemic chemotherapy±involved-field radiotherapy and 25 (71%) achieved complete response by systemic chemotherapy. 10 patients (29%) underwent consolidative autologous stem cell transplantation (ASCT). Using 5-PS, interim PET scan was visually scored as follows; 1 point in 10 patients (29%), 2 in 6 (17%), 3 in 8 (23%), 4 in 7 (20%), and 5 in 4 (11%). Among these, 11 patients (31%) had 4 point or above were considered positive for interim PET scan. With a median follow-up of 43.4 (range, 4.3-89.8) months, progression-free survival (PFS; median, 5.2 *vs* 38.0 months, respectively; *P*=0.001) and overall survival (median, 12.6 months *vs* not reached, respectively; *P*=0.004) was significantly worse in patients with positive interim PET than those with negative results. In multivariate analysis for PFS, high risk of PIT (HR, 3.67; 95% CI, 1.13-11.99) and positive interim PET (HR, 4.02; 95% CI, 1.32-12.23) were independently associated with faster disease progression.
Summary and Conclusions: This study represents that Deauville 5-PS is useful to predict early outcomes of patients with mature T-cell and NK-cell lymphoma. Patients with positive interim PET are associated with extremely poor early outcomes. Further studies regarding response-adapted stratification based on interim PET might be needed in mature T-cell and NK-cell lymphoma.

PB1826

SIMILAR PROGNOSIS OF TRANSFORMED AND DE NOVO DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH IMMUNOCHEMOTHERAPY

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Background: Transformed diffuse large B cell lymphomas (TL) have historically been associated with an aggressive course and a poor prognosis. However, in the last decade some studies have shown an important improvement in their prognosis. Furthermore, diffuse large B cell lymphomas (DLBCL) are heterogeneous, with major groups defined by the cell of origin (COO). There are few studies aiming to characterize the COO in TL and its prognostic implications.
Aims: To compare the clinical and biological features (including the COO), as well as the prognosis between TL and DLBCL treated with rituximab and chemotherapy.
Methods: We revised the records of 163 patients with DLBCL with those from

31 with TL treated between 2003 and 2012 in our institution. The COO was established by the Hans' algorythm, which uses the imunohistochemical expression of CD10, BCL6 and MUM1 on tissue sections to classify DLBCL into germinal center B-cell like (GCB) and non-GCB. Only patients diagnosed by tissue biopsy were included. Patients not treated with immunochemotherapy, HIV positive, those whose biopsy specimen was unavailable for revision and those with specific subtypes of DLBCL such as primary cutaneous DLBCL, leg type and primary mediastinal DLBCL were excluded from the study.
Results: 29 patients with TL and 101 with DLBCL were included in the study. Of the 29 TL, 9 had a previous diagnosis of follicular lymphoma (FL), 6 of marginal zone lymphoma, 4 chronic lymphocytic leukemia (CLL) and 10 showed both DLBCL and FL in the same lymph node (composite lymphoma). There were no differences in the clinical or analytical parameters between TL and DLBCL. TL was diagnosed at advanced stages in 68% of cases while DLBCL was in 53% (p=0.198). Data on the COO was available for 23 TL and 76 DLBCL. All TL evolving from FL (7/7) and composite lymphomas (9/9) were GCB while all TL from marginal lymphoma (4/4) and CLL (3/3) were non-GCB. Overall, 16 out of 23 (70%) were GCB. 32 out of 76 (42%) DLBCL were CGB. TL were more frequently of GCB subtype than DLBCL (p=0.031) and CD10 was expressed more frequently in TL (p=0.002). TL and DLBCL were treated with RCHOP in 21 and 89 cases, respectively. Five TL cases received consolidation with autologous stem cell transplantation (SCT) in first remission and 2 were submitted to allogeneic SCT. No DLBCL patient received SCT in first remission. There were no differences between TL and DLBCL in the rate of complete remission with first line treatment (62% *vs.* 66%), nor in overall survival (OS, 63%, [95%CI 43%,83%] *vs.* 61% [95%CI 50%,72%]), or in progression-free survival (PFS, 61% [95%CI 42%,80%] *vs.* 60% [95%CI 49%,71%] respectively). Excluding the 7 patients with TL who received SCT, the OS and PFS in TL treated only with immunochemotherapy were 58% (95%CI 35%,81%) and 55% (95%CI 33%,77%), respectively, without differences with those observed in DLBCL patients. Analyzing only GCB lymphomas, there were no differences between TL and DLBCL in both OS (61% *vs.* 62%) and PFS (66% *vs.* 63%).
Summary and Conclusions: Patients with TL and DLBCL from this series had similar clinical and biological characteristics at diagnosis. GCB was more frequent in TL than in DLBCL. The prognosis of TL and DLBCL was similar in patients treated with immunochemotherapy.
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PB1827

TREATMENT WITH YTTRIUM-90 (90Y)-IBRITUMOMAB TIUXETAN (ZEVALIN®) IN DIFFUSE LARGE B-CELL LYMPHOMA: A META-ANALYSIS

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most frequently diagnosed non-Hodgkin's lymphoma (NHL). Current treatment paradigm in first line, is based on immunochemotherapy and, in case of relapse or refractory, on a salvage therapy with high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). Yttrium-90 (^{90}Y)-ibritumomab tiuxetan, off label, offers a new therapeutic approach for DLBCL, both as first-line induction or consolidation and in relapsed or refractory disease. Data on this subject is increasingly being reported as well in consolidation as alone, in first line or after.
Aims: To assess efficacy and find the best place of Yttrium-90 (^{90}Y)-ibritumomab tiuxetan in DCLL, we conducted a literature review and meta-analysis of randomized clinical trials and observational studies on the effect of (^{90}Y)-ibritumomab tiuxetan treatment in this setting, except in ASCT.
Methods: The primary goal was to assess the effect of (^{90}Y)-ibritumomab tiuxetan on overall response rate (ORR) and complete response rate (CRR) then assess the 2-year overall survival (OS_{2y}) and 2-year progression-free survival rates (PFS_{2y})
Results: Sixteen studies were identified (399 patients) with DLBCL receiving (^{90}Y)-ibritumomab tiuxetan as consolidation or as treatment alone. The ORR and CRR were 74.8% (n=374) and 67.4% (n=365), respectively. Outcomes were superior when (^{90}Y)-ibritumomab tiuxetan is used in consolidation after immunochemotherapy induction at first line of treatment. OS_{2y} and PFS_{2y} were 87.5% and 82.8%, respectively. The primary toxicity was neutropenia (49.9%).
Summary and Conclusions: The use of (^{90}Y)-ibritumomab tiuxetan in patients with DLBCL is safe and effective.

PB1828

MANAGEMENT OF HEPATITIS B VIRUS (HBV) INFECTION IN PATIENTS (PTS) RECEIVING CANCER CHEMOTHERAPY IN A REAL WORLD CLINICAL SETTING

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Background: HBV infection is highly prevalent in Chinese patients (pts) with B-NHL, compared with the general population. Rituximab (R) and chemotherapy (chemo) have been associated with HBV reactivation, which may influence the continuation of therapy and can result in fatal liver dysfunction. Therefore, standardized management of HBV reactivation in pts receiving chemo is needed.
Aims: To describe the management status of B-NHL pts with HBV infections in real practice in China.
Methods: Pts with previously untreated DLBCL and FL who were eligible to receive R-chemo were enrolled in a multicenter, prospective, single-arm observational study in China. Data were collected and analyzed from medical records 120 d after the last R dose was administered. HBV infection management in pts was evaluated, including HBV infection and liver function screening before R-chemo, viral replication monitoring during and after R-chemo, antiviral prophylaxis use and HBV reactivation rates. HBsAg-positive (pos) pts had HBV reactivation if HBV DNA increased ≥ 1 log10 from baseline, if HBV DNA appeared (above the lower limit of detection) or if HBeAg appeared in HBeAg-negative (neg) pts. HBsAg-neg/HBcAb-pos pts had HBV reactivation if there was appearance of either HBsAg or HBV DNA (above the lower limit of detection). These analyses are preliminary.

Tablel 1. Antiviral treatment, HBV infection monitoring and HBV reactivation in R-chemo-treated DLBCL/FL pts.

Results: HBV screening data were available from 269/309 enrolled pts. At baseline (BL), 26 pts were HBsAg pos, 74 were HBsAg neg/HBcAb pos and 169 were double neg. HBV infection status was undefined for 40 pts. 20 HBsAg-pos, 7 HBsAg-neg/HBcAb-pos, 1 double-neg and 4 undefined pts had a history of hepatitis. HBV DNA levels were evaluated in 18/26 HBsAg-pos, 34/74 HBsAg-neg/HBcAb-pos, 33/169 double-neg and 7/40 undefined pts. 8/18 HBsAg-pos and 3/34 HBsAg-neg/HBcAb-pos pts were positive for HBV DNA. HBsAg-pos pts had a median log HBV level of 3.9 copies/mL (n=7). HBV DNA levels were below the level of quantification in 1 HBsAg-pos and 3 HBsAg-neg/HBcAb-pos pts who were HBV DNA pos. Levels of ALT, AST and total

bilirubin were tested in 99.0%, 98.7% and 98.4% of pts, respectively. Data on antiviral prophylaxis treatment, monitoring and HBV reactivation are in Table 1. Most pts who received antiviral prophylaxis were HBsAg pos-only 69% of HBsAg-pos pts were treated. By 120 d after the last R dose, some pts had already stopped prophylaxis. Not all pts were monitored for serologic markers, HBV DNA and liver function during induction therapy, and monitoring declined further after the last R-chemo dose. HBV reactivation occurred in 3 HBsAg-pos, 3 HBsAg-neg/HBcAb-pos and 1 double-neg pts. In contrast, investigators reported 1 case of HBV reactivation each in HBsAg-neg/HBcAb-pos, double-neg and undefined pts, based on clinical experience.

Summary and Conclusions: This study reports HBV load in previously untreated DLBCL/FL pts receiving R-chemo as first-line therapy in China. Most Chinese physicians acknowledge the importance of HBV screening before initiation of R-chemo. However, improvements in HBV infection monitoring and antiviral treatment duration are required. Even with monitoring, HBV reactivation is not defined consistently by physicians, which may lead to underreporting of reactivation as shown herein. By the time of analysis, some early reactivation had emerged, but late reactivation, especially after stopping antiviral treatment, should be monitored long term. Finally, standardized monitoring, follow-up strategies and education are needed for proper management of HBV infection in B-NHL pts receiving chemo.

PB1829

COMPARISON OF GEMCITABINE *VS.* NON-GEMCITABINE BASED SALVAGE CHEMOTHERAPY IN RELAPSED/REFRACTORY AGGRESSIVE LYMPHOMA

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Background: Despite advances in immuno-chemotherapy, outcomes in relapsed/refractory (R/R) lymphoma remain dismal. The treatment approach, in eligible patients, is intensive salvage chemotherapy followed by transplantation. The optimal salvage regime remains a matter of debate with wide institutional variations. (R)-DHAP (cytosine arabinoside, cisplatin, dexamethasone, ±Rituximab) remains the most commonly used regimen. Gemcitabine based regimens are, at least, non-inferior to (R)-DHAP and allow effective mobilization of haematopoietic stem cells even in heavily pre-treated patients. The combination of ifosfamide, gemcitabine and vinorelbine (IGEV) has a good profile of tolerability and efficacy in R/R Hodgkin' lymphoma (HL) and lends itself to use in an outpatient setting; but data for its use in non-Hodgkin' lymphoma (NHL) are lacking.

Aims: To assess feasibility, safety and efficacy of (R)-IGEV for the treatment of patients with R/R HL and NHL.

Methods: In a retrospective cohort study, we analysed the outcome of 46 consecutive patients treated for R/R lymphoma at our institution between January 2010 and October 2013. Patients received either IGEV or DHAP for HL and additional Rituximab for B-NHL. Primary outcomes were overall response rate (ORR); PFS (from first salvage to progression, relapse, or death from any cause); and overall survival (OS). OS and PFS were estimated using the Kaplan-Meier method and compared using the log-rank test. In addition, the likelihood of proceeding to transplantation, on an intent-to-treat basis, was assessed.

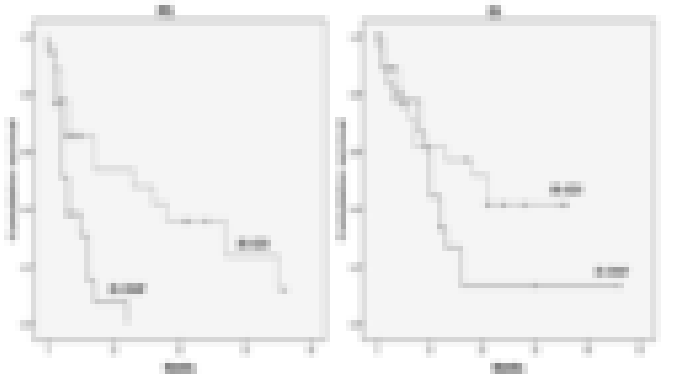

Figure 1.

Results: We analysed 46 consecutive patients treated at our institution for R/R HL and NHL between January 2011 and October 2013. Median age at salvage was 57 (19-78) yrs, 67% of the patients were male. At diagnosis, 86% of patients had stage III/IV disease, and 15 patients had a diagnosis of HL. In the (R)-IGEV group the ORR rate, after 2 courses, was 44% with a CR rate of 36% (as assessed by PET-CT). ORR and CR rates in the (R)-DHAP group were 27% and 25% respectively. After a median follow-up of 26 months, OS in the (R)-IGEV and (R)-DHAP groups was 21 months (95% CI, 10.1-31.8 months) and 10 months (95% CI, 8.6-11.4 months) (p=0.179) respectively. The percentage of patients that proceeded to transplantation was 50% in both groups. Median PFS in the (R)-IGEV and (R)-DHAP groups were 13 months (95% CI 1-24 months) and 3 months (95% CI 1.7-4.2 months) respectively (p=0.001). Principal grade 3/4 toxicities in both groups were cytopenias. Overall, the toxicity profile of the two regimens was comparable. Significantly, of a total 78 courses of IGEV, 53% were successfully administered in an outpatient setting. All courses of (R)-DHAP required in-patient stay.

Summary and Conclusions: In summary, (R)-IGEV represents a safe and effective outpatient salvage therapy for R/R lymphoma. In our cohort, patients in the (R)-IGEV group had a statistically superior PFS compared to patients in the (R)-DHAP group. Our data also suggest that subsequent transplantation rates are comparable in both groups. In addition, (R)-IGEV lends itself to administration in an outpatient setting thus avoiding potential delays because of inpatient bed availability, enhancing patient experience, and has health economic benefits.

PB1830

SIGNIFICANT SURVIVAL IMPROVEMENT OF THE ELDERLY PATIENTS AND WOMEN WITH LOW/INTERMEDIATE RISK MIPI MANTLE CELL LYMPHOMA OVER THE PERIOD OF 14 YEARS

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Background: Mantle cell lymphoma (MCL) is an aggressive type of B-cell non-Hodgkin lymphoma (NHL) with poor prognosis. In recent years the outcome of patients with MCL has been improved mainly by implementation of rituximab (R), high-dose araC (HDAC) into induction regimen, consolidation with autologous stem cell transplant (ASCT) and R maintenance.

Aims: A single center retrospective analysis of MCL patients treated since 1999 to 2012.

Methods: We retrospectively analyzed 185 consecutive patients with confirmed diagnosis of MCL treated at the Charles University General Hospital since 1999 to 2012. The whole cohort comprised 71% men and 29% women with median age of 66 years (both men and women). Most patients had advanced-stage disease (stage IV= 80% patients) and adverse prognosis according to the mantle-cell lymphoma prognostic index (MIPI): MIPI high (MIPI-H)= 48.1%, MIPI intermediate (MIPI-I)= 23.8% and MIPI low (MIPI-L)= 24.9%. Out of all 185 patients, 2 were not treated. Three most common types of induction therapy included R-CHOP (n=52), Nordic protocol (alternating 3 cycles of R-Maxi-CHOP and 3 cycles of R-HDAC, n=45) and R-COP (n=26). 45 patients received ASCT. Most patients (86.9%) had rituximab as part of induction.

Results: Overall response rate for all patients was 81.9% (CR/uCR= 57.9%, PR= 24%). Median progression-free survival (PFS) and overall survival (OS) was 2.84 years, and 6.27 years, respectively. The median follow-up was 4.4 years. The analysis revealed several interesting findings. First, patients (pts) diagnosed in 2006-2012 (2006+) *vs* 1999-2005 (1999+) had significantly better PFS (median 2.04 *vs* 3.38 years, p= 0.0222) and trend toward better OS (median 7.2 *vs.* 4.3 years, p= 0.11). Curiously, the age-stratified analysis revealed that only elderly patients (≥ 60 years) in 2006+ group had significantly improved PFS (median 2.74 *vs.* 1.63 years, p= 0.0086) and trend toward improved OS (median 4.2 *vs* 2.3 years, p=0.0625) compared to those in 1999+ group. The younger patients in 2006+ compared to 1999+ group had similar PFS (median 5.52 *vs.* 5.51 years, p= 0.884) and OS (median undefined in both subgroups, p= 0.784). The reason for the improved outcome of the elderly patients in recent years is probably a consequence of introduction of rituximab maintenance. In the elderly groups 1999+ and 2006+ two of 44 and 32 of 66 pts received rituximab maintenance, respectively. The elderly pts 2006+, who received rituximab maintenance (compared to those, who did not) had higher PFS (median 4.5 *vs* 2.23 years, p= 0.0055) and OS (median undefined *vs* 3.41 years, p= 0.0018). Second, MIPI discriminated well into 3 subgroups of patients only in the whole cohort. However, in the age-stratified analysis there remained only two relevant MIPI subgroups: MIPI-I merged with MIPI-H (in younger pts) or with MIPI-L (in elderly pts). Finally we observed trend toward better OS in the subcohorts of women with MIPI-L and MIPI-I compared to men (MIPI-L median undefined *vs* 8.48 years, p= 0.0692; MIPI-I median 10.51 *vs* 4.56 years, p= 0.0657). PFS was significantly prolonged in women with MIPI-I compared to men (median 8.31 *vs* 5.59 years, p= 0.539), but did not differ in women with MIPI-L (median 8.04 *vs.* 2.44 years, p= 0.0237).

Summary and Conclusions: On the large single center cohort of unselected MCL patients we demonstrated significant outcome improvement of elderly patients but not younger patients during the last 7 years. We found that MIPI discriminated only two prognostic subgroups when used in age-defined cohorts of patients. Finally, women with low and intermediate risk MIPI appeared to have better outcome compared to man.

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PB1831

CLINICAL IMPLICATIONS AND PROGNOSTIC SIGNIFICANCE OF POSITRON EMISSION TOMOGRAPHY (PET/CT) IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) AFTER R-CHOP CHEMOIMMUNOTHERAPY

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Background: About 30-40% of patients with DLBCL are primary refractory or relapse after first line treatment with the R-CHOP regimen. Among responders according to the IWC criteria, many patients still have post-treatment residual masses. Therefore, PET/CT is increasingly used for the identification of patients with active disease at the end of treatment, although relevant data are scarce in the literature.
Aims: The retrospective analysis of PET/CT findings in patients with DLBCL after conventional response to R-CHOP and the assessment of their impact on outcome in comparison to established prognostic factors.
Methods: From 2004 to 2013, 151 patients with DLBCL who achieved CR, CRu, or PR by conventional radiographic imaging (according to IWC) underwent posttreatment PET/CT. All patients had been treated with 4-8 cycles of R-CHOP or similar anthracycline-containing immunochemotherapy regimens. PET/CT was performed at the treating haematologist's discretion, but availability was also an issue, particularly during the earlier years of the study. The primary end-point of the analysis was progression free survival (PFS). PET/CT scans were interpreted according to the International Harmonization Project criteria.
Results: The main baseline patients' characteristics were: median age 61 (18-89), 65% male, 55% stage III/IV, 17% ECOG PS ≥2, 23% ≥2 E-sites, 53% abnormal LDH, IPI-L 42%,IPI-LI 21%,IPI-HI 24%, IPI-H 14%. Among the 151 patients, 117 (77%) became PET(-) (including 11 patients with indeterminate findings) and 34/151 (23%) remained PET(+). The 4-year PFS was 86% *vs* 40% for PET(-) and PET(+) patients respectively (p<0.0001). Among PET(-) patients, 107/117 remained in complete remission for a median follow-up of 25 months from the date of PET/CT. Among PET(-) patients, 10/117 relapsed. PS ≥2 was associated with inferior PFS in PET(-) patients (4-year PFS 89% *vs.* 69% for patients with PS 0-1 *vs.* 2-4; p=0.02). Marginally significant trends towards inferior PFS among PET(-) patients were also observed for males, multiple extranodal involvement and bone marrow involvement (0.10<p<0.15). Among 34 PET(+) patients, 16 progressed or relapsed for a 4-year PFS of 40%. Biopsy-proven false positive results and the use of consolidative irradiation (although limited) may have contributed to this promising figure (details to be presented at the meeting).
Summary and Conclusions: DLBCL patients who become PET(-) at the end of R-CHOP had a moderate risk of disease progression in the order of 15%. The risk increased with worsening PS at diagnosis, while the significance of several other adverse prognostic factors needs further investigation. DLBCL patients in PET(+) PR who respond to R-CHOP had 60% risk of relapse, but 40% of them remained disease-free despite the limited subsequent use of radiotherapy. Our findings could facilitate the design of follow-up and guide potential consolidative treatment in patients with DLBCL.

PB1832

AN ANALYSIS OF THE PHARMACOKINETICS AND TOXICITY OF HIGH-DOSE METHOTREXATE IN PATIENTS WITH HISTOLOGICALLY AGGRESSIVE NON-HODGKIN LYMPHOMA AT HIGH RISK OF CENTRAL NERVOUS SYSTEM DISEASE

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Background: CNS relapse in aggressive NHL is an infrequent yet devastating cause of treatment failure and patients (pts) at high risk [aaIPI >2 or multiple/specific extranodal sites of involvement (testis, breast, kidney, adrenal, BM, or epidural)] at our centres receive 2 courses of intravenous high-dose methotrexate (HD-MTX) as CNS parenchymal prophylaxis. HD-MTX can however result in significant toxicity leading to treatment delay so identification of predictors of toxicity is crucial.
Aims: The aim of this study was to examine HD-MTX pharmacokinetics, the incidence and predictors of delayed MTX clearance, hepatic and nephrotoxicity.
Methods: We conducted a retrospective analysis of pts receiving ≥1 cycle of HD-MTX for histologically aggressive NHL. Clinical and pharmacy databases were reviewed from 2004-2013 inclusive. Data collected included patient sex, age, diagnosis, MTX dose and number of cycles, rate of MTX infusion, peri-treatment radiological contrast exposure, baseline bicarbonate level as a measure of urine alkalinisation (mmol/L), nadir bicarbonate level during MTX exposure, peak serum and 24hr post-infusional MTX levels (μmol/L), time to clear MTX defined as level ≤0.05 (μmol/L) and hepatic and renal toxicity as defined by CTCAE v4.0.
Results: A total of 90 pts (54M, 36F) met the inclusion criteria with median age of 61.7yrs (19.8-82.6); 36 (40%) were >65yo (cut-off age for receiving MTX > 1g/m^2). A total of 161 cycles were delivered; 72 at 3g/m^2, 65 at 1g/m^2 and 24 at other doses (0.5, 1.5 or 2g/m^2). 71/90 (79%) pts received both planned cycles. 88% of 32 pts having 1g/m^2 in cycle 1 had 1g/m^2 in cycle 2, only 61% of 44 pts having 3g/m^2 had 3g/m^2 in cycle 2; 9% had a dose reduction, 30% did not have a second cycle. Most HD-MTX was given as a 24-hr infusion, only 4 pts had a 3hr infusion with 75% having significant toxicity or delayed clearance prohibiting cycle 2. The median peak MTX level (μmol/L) was 34.95 (5.18-125.95) in pts having 3g/m^2 (29/72 courses recorded) and 15.38 (1.21-22.19) in pts having 1g/m^2 (33/65 courses recorded), with median 24h post-infusion level of 0.37 (0.10-12.66) and 0.28 (0.03-3.51) respectively. The 24h MTX level correlates better with delayed clearance then end of infusion level does (R 0.77 *vs.* R 0.17). If 24h level >2.0, then 5/6 (83%) had delayed clearance; <2.0 then only 21/80 had delayed clearance, P=0.009. Median time to clear MTX was 57h (25h-137h) with median length of hospital stay of 98h (64h-385h). Delayed clearance, defined as MTX level >0.05 μmol/L at 72h, was observed in total of 39 cycles (24%). Grades 1-3 hepatotoxicity was observed in 26.7%, 10.6% and 6.8% of cycles respectively. Grades 1-4 nephrotoxicity was observed in 53.4%, 8.7%, 2.5% and 0.6% of cycles respectively. We analysed our postulated risk factors as predictors of delayed clearance or nephrotoxicity ≥G2 independently for cycles 1 and 2 because these are likely to represent different populations given most pts who have toxicity in cycle 1 usually don't have a second cycle. The only significant finding was an association between MTX 1g/m^2 in cycle 1 and delayed clearance but not nephrotoxicity. Overall, 74% (14/19) pts with nephrotoxicity ≥G2 had delayed clearance, but only 49% (14/39) pts with delayed clearance had nephrotoxicity ≥G2.
Summary and Conclusions: HD-MTX associated toxicity resulted in suboptimal delivery of CNS prophylaxis in 21% of our patients. A 24h level of >2.0 predicts for much greater risk of delayed clearance and could be a red flag for more aggressive intervention. Unlike previous reports of age as a predictor of toxicity, age-based dose adjustment diminishes this risk association. Patients having dose-adjusted MTX at 1g/m2 were more likely to have delayed MTX clearance but not nephrotoxicity. Other MTX doses, low serum bicarbonate as an indirect assessment of poor urine alkalinisation and rate of infusion were not associated with toxicity.

PB1833

COMPARISON BETWEEN CONTRAST-ENHANCED COMPUTERIZED TOMOGRAPHY (CECT) AND 18FLUORO-DEOXY-GLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) FOR INITIAL AND END-OF-THERAPY STAGING OF T-CELL LYMPHOMA

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Background: T-cell lymphoma are rare lymphoprolipherative disease, characterized by disappointing outcome. Even if T-cell lymphomas are usually considered as aggressive disease, very few data are available on the role of fluoro-deoxy-glucose positron emission tomography (FDG-PET) in T-cell non Hodgkin Lymphoma (NHL).
Aims: We retrospectively reviewed 96 FDG-PET scans performed since 2004 to 2014 in 38 T-cell NHL pts and we focused on 58 scans for which a simultaneous standard contrast-enhanced computerized tomography (CECT) was available for comparison, in order to evaluate the role of FDG-PET in optimizing this challenging disease management.
Methods: Patients' median age was 54 years (range: 25-77) and 21/38 pts (55%) were females. As regards histology, 19 pts (50%) had peripheral T-cell lymphoma not otherwise specified, 5 pts (13%) had angioimmunoblastic T-cell lymphoma, 13 pts (34%) had anaplastic T-cell lymphoma and 1 pt (3%) had nasal-type T-cell lymphoma. Baseline FDG-PET scans were 29, while 29 FDG-PET were performed for end-of-therapy disease reassessment.
Results: At baseline staging, PET and CECT pointed out the same disease sites of involvement in 7% (2/29) of cases. PET identified a higher number of disease sites compared to CECT in 66% (19/29) of the pts; on the contrary, in 24% (7/29) of cases PET showed a lower number of disease sites than CECT. In 1 pt (1/29, 3%) we observed a PET positivity, with CECT negativity after diagnostic lymphnode biopsy. Additional sites identified by PET were both nodal and extra-nodal: new nodal sites in 19 pts, spleen in 2 pts, bone in 3 pts, nasopharinynx, pancreas, muscle, bowel and testicle in 1 pt each. FDG-PET results led to change the an Ann Arbor stage in 44.8% (13/29) of cases, with an up-staging in 37.9% (11/29) of pts. At end-of-therapy restaging, 62% (18/29)

pts had a negative FDG-PET scan; CECT was concordant and consisting with complete response in 78% (14/18) of cases, while in the remaining 22% (4/18) of cases CECT showed a partial response with residual lesions. In the 11/29 (38%) final PET positive pts, CECT was concordant and pointed out a partial response in 7/11(64%) pts, while was negative in 4/11 (36%) cases. A survival analysis according to final PET result was not performed due to the low number of final PET positive pts. No statistically significant difference was found in PET behaviour between different histological subtypes.
Summary and Conclusions: In conclusion, in our hands FDG-PET allowed a more accurate baseline disease extension evaluation: it could identify additional sites, both nodal and extra-nodal, in two-thirds of pts. A change in clinical stage according to FDG-PET was observed in 44.8% of cases, with an upstaging in the majority of pts. A high level of concordance was observed between PET and CECT at restaging. Nevertheless, PET pointed out minimal residual disease in 22% of final CECT negative pts and complete remission in 36% of final CECT positive pts, allowing to optimize response evaluation in small but critical subgroups of pts.

PB1834

HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN FIRST LINE TREATMENT FOR HIGH-RISK DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) IN THE RITUXIMAB ERA: AN INTENTION TO TREAT-ANALYSIS

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Background: The combination of Rituximab and CHOP (R-CHOP) is considered to be the standard treatment for patients (pts) with newly diagnosed diffuse large B-cell lymphoma (DLBCL). Treatment results are still unsatisfactory in a significant proportion of patients, particularly in those with a high-risk disease defined by the IPI score. The use of high-dose chemotherapy with autologous stem-cell transplantation (ASCT) is standard clinical practice for patients with relapsed/refractory DLBCL, while its significance as consolidation in first-line treatment remains unclear.
Aims: We analyzed safety and effectiveness of R-CHOP followed by salvage chemotherapy and ASCT for patients with young (<65 years) high-risk DLBCL, defined by an age-adjusted IPI score of 2/3, for whom from 2004 on our institutional guidelines recommended ASCT as consolidation. We analyzed prognostic factors in this group.
Methods: The treatment program consisted of 4 cycles R-CHOP-14 followed by 3 cycles of a DHAP-like salvage regimen, R-MICMA (Sorà et al, Cancer 2006; 106: 859), and consolidation with Busulfan-Melphalan supported with ASCT. We observed 76 consecutive patients (median age 50 years, range 15-64 years; 32 females and 44 males) diagnosed between May 2004 and January 2013 with DLBCL who had an age-adjusted IPI score of 2 or 3. Response was assessed according to Cheson criteria (Cheson et al, JCO 1999; 17:1244).
Results: Nine of 76 patients (12%) were not eligible for the treatment program that included ASCT. Reasons were important comorbidities in 6 pts (1 cardiac, 2 neurologic, 1 hepatic, 1 hematologic, 1 renal) and start of another treatment regimen (CODOX-M/IVAC in the suspicion of a Burkitt lymphoma) in 3 pts. Response after 4 cycles R-CHOP was CR/CRu in 40/67 pts (60%), PR in 21/67 pts (31%) and NR in 6/67 (9%). Sixty-one patients went on to salvage chemotherapy with R-MICMA, while 6 pts in CR/CRu continued R-CHOP, and 53 pts were transplanted. Reasons not to proceed to transplant were progressive disease (3 pts), infections (3 pts), mobilization failure (1 pt) and patient's decision (1 pt). The 3-year EFS and OS of the entire group of 76 patients were 67% (95% CI, 55-76) and 71% (95% CI, 59-80%), respectively. The 3-year EFS and OS of transplanted patients were 70% (95% CI, 55-80) and 76% (95% CI, 62-85). Factors associated with inferior EFS were age-adjusted IPI score (2 *vs.* 3, p=0.004) and disease status after 4 cycles R-CHOP (p=0.01) in univariate and multivariate analysis. These differences were also retained in the group of patients who received ASCT, with a three-years EFS of 78% in pts with an age-adjusted IPI score 2 *vs* 46% in pts with an age-adjusted IPI score 3 (p=0.003), suggesting that ASCT is insufficient for highest risk patients.
Summary and Conclusions: Our findings of an intention-to-treat, single centre experience indicate that 88% of patients with high-risk DLBCL and age <65 years are eligible for a treatment strategy that includes ASCT, and 70% will eventually receive ASCT as part of their first-line treatment. Consolidation with upfront ASCT for high-risk DLBCL is a feasible and promising therapy also in the Rituximab era, but there are still subsets of patients that continue to have a poor prognosis despite ASCT, and addition of new biologic drugs, as tyrosine kinase inhibitors, have to be tested to improve outcome in these patients.

PB1835

POST-TRANSPLANT MONOMORPHIC BURKITT'S LYMPHOMA: CLINICAL CHARACTERISTICS AND OUTCOME OF A MULTICENTER SERIES

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Background: Post-transplant lymphoproliferative disorders (PTLD) are lymphoid neoplasms that develop after stem-cell or solid organ transplantation (SOT) associated with immunosuppressive therapy (IS) and Epstein-Barr virus (EBV) infection. Burkitt's monomorphic PTLD (BM-PTLD) is an uncommon and not properly described subtype of PTLD.
Aims: To gain insight into this rare lymphoma, the histological and clinical features of a series of adult diagnosed with BM-PTLD in four transplant centers were retrospectively reviewed.
Methods: All patients diagnosed with BM-PTLD between February 1996 and November 2013 were recorded and analyzed. Patients with PTLD negative for C-MYC breaks and/or diagnosed with unclassifiable lymphoma with intermediate features between DLBCL and BL were excluded.
Results: Ten patients with the diagnosis of BM-PTLD were included. Seven patients were female and the median age at diagnosis was 41 years (range, 25-52 yrs). All patients underwent SOT (7 kidney, 2 liver, and 1 lung). Median time of the diagnosis of PTLD from transplant was 94 months (range, 27-197 mos), and all patients were receiving IS, 7 with tacrolimus and three with cyclosporine. The PTLD was diagnosed in stage I-III in 4 patients and in stage IV in 6. Serum LDH was increased in 6/8 patients, and $\beta_2$microglobulin in 5/8. Immunohistochemistry analyses (n=8) revealed that all cases were CD20, CD10, and BCL-6 positive and negative for BCL-2. Ki67 expression was >95% in all cases. FISH showed the presence of the MYC translocation in all cases analyzed, while BCL2 and BCL6 translocations were excluded in all cases examined (5/5). *In situ* hybridation for EBV was positive in 3 of 5 cases. Six patients were treated with immunochemotherapy, 4 with R-CHOP along with CNS intrathecal prophylaxis and 2 with the Burkimab regimen (Ribera *et al.*, Cancer 2013;119 1660) that contains high-dose chemotherapy along with rituximab. One additional patient received 4 courses of rituximab with the withdrawal of IS. Finally, 3 patients diagnosed in the pre-rituximab era were treated with CHOP (n=2) and one with a combined regime (etoposide, cyclophosphamide, methotrexate, bleomycin, and vincristine). All patients that finished the initial treatment (n=8) obtained a complete remission (CR). Three patients died, two during the first course of treatment due to PTLD progression and a sepsis, and another one due to unrelated causes. Two patients that relapsed 12 and 9 months after R-CHOP and rituximab monotherapy, respectively, were salvaged with the Burkimab regimen achieving a second CR. After a median follow-up of 35 months (range 1 to 120), 7 patients remain in CR. The 3-year progression-free survival and overall survival was 55% and 80%, respectively, for the whole series, and of 63% and 100% for the 7 patients receiving rituximab-containing therapies.
Summary and Conclusions: In this retrospective series BM-PTLD appears as an infrequent and aggressive variety of PTLD usually diagnosed late in the evolution after transplant and in advanced stage of the disease. This lymphoid proliferation seems to have an excellent response to the immunochemotherapy combinations, including R-CHOP or to more intensive regimens like Burkimab. For those patients not candidate to intensive regimens, R-CHOP could be an excellent therapeutic alternative. A nation-wide collection of cases of BM-PTLD is now ongoing.

PB1836

CLINICAL RELEVANCE OF SOME LYMPHANGIOGENIC AND INFLAMMATORY CYTOKINS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Pathological angio- and lymphangiogenesis, consisting in the new blood and lymphatic vessels development, within and around a malignant tumour, are differently regulated and bear different significance for neoplastic development, including DLBCL. VEGF-C,VEGF-D and VEGFR-3 are crucial for lymphangiogenesis, but the relationship between their plasma levels and clinical course of DLBCL is unknown. Similarly, there are no reports on the role of inflammatory cytokine IL-17B and its receptor IL-17BR in DLBCL.
Aims: The aim of our study was to assess the plasma levels of VEGF-C, VEGF-D, VEGFR-3, IL-17B and IL-17BR in DLBCL patients upon diagnosis in order to evaluate the predictive value of those cytokines as to the achievement of complete remission (CR) after standard R-CHOP treatment, and their prognostic value regarding the overall survival (OS).
Methods: The study was performed in 55 untreated DLBCL NOS type patients after their written consent (38 M, 17 F), aged 23-88 yrs (mean 65.4), and 30 healthy control individuals (9 M, 21 F), aged 39-88 yrs (mean 61.1). For each patient blood count, serum LDH activity, b-2microglobulin, albumin and CRP concentrations were determined. The clinical stage of the disease and the general

condition were determined according to the Ann Arbor classification, ECOG and the IPI system. The patients received 6-8 courses of the standard 21-R-CHOP regimen, and 24 of them (43,6%) achieved CR. The cytokines plasma concentrations were established by ELISA method. Results were processed statistically with the Mann-Whitney and the Wilcoxon tests, the Spearman rank correlation coefficient and the chi^2 test. The Kaplan-Meier survival curves were used for estimation of OS probability and compared with the log-rank test.
Results: Lymphangiogenic cytokines plasma levels did not significantly differ between patients and controls, except VEGFR-3 which was higher in patients than in controls (75.8±42.1 and 45.7±16.7ng/ml; respectively; p=0.0007). VEGFR-3 and VEGF-C values were significantly higher in patients with IPI (3-5) than with IPI<3 (63.5±40.6 and 83.5±41.8ng/ml for VEGFR-3; p=0.048, and 2309.0±1729.5 and 2895.6±1140.5pg/ml; p=0,02 for VEGF-C). VEGFR-3 was positively correlated with β-2microglobulin (R=0.37; p=0.01). VEGF-D was correlated positively with β-2microglobulin (R=0.35; p=0.02) and LDH (0.35; p=0.01) and negatively with serum albumin concentration (R=-0.44;p=0.001). The concentration of IL-17BR was significantly higher in patients than in controls (2,7±3,3 and 1,4±1,1pg/ml; respectively; p=0.03). There was a significant relationship between the concentrations of IL-17B and IL-17BR, and this relationship was stronger in the control group (R=0.89; p<0.0001) than in DLBCL patients (R=0.50; p=0.0001). We did not find a relationship between the plasma concentration of any cytokine and the achievement of CR. The OS probability curves did not significantly differ between the patients with concentrations of any evaluated cytokine above *versus* below the median value, but the Cox regression model showed negative prognostic impact of high VEGF-D concentration on OS (HR=1.005, 95% CI, p=0.008).
Summary and Conclusions: Relationship between plasma levels of lymphangiogenic cytokines and some markers of disease activity may reflect an influence of lymphangiogenesis on DLBCL progression. The negative impact of VEGF-D value on OS warrants further studies. Higher IL-17BR plasma concentration and its weaker correlation with IL-17B in patients than in healthy controls might indicate a deregulation of the IL-17BR expression in DLBCL

PB1837

COMPARISON OF PROGNOSTIC IMPACT OF ABSOLUTE LYMPHOCYTE COUNT (ALC), ABSOLUTE MONOCYTE COUNT (AMC), ALC/AMC PROGNOSTIC SCORE AND ALC/AMC RATIO IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The combination of absolute lymphocyte count (ALC) and absolute monocyte count (AMC) at diagnosis have prognostic relevance in patients with diffuse large B cell lymphoma (DLBCL).
Aims: Aims: The present study was designed to investigate prognostic significance of ALC and AMC and to determine whether ALC/AMC ratio or ALC/AMC prognostic score is better predictor of outcome in DLBCL.
Methods: We retrospectively analysed prognostic significance of ALC and AMC, ALC/AMC ratio and ALC/AMC prognostic score at diagnosis in 222 DLBCL patients treated with R-CHOP. ALC/AMC score was determined according to Wilcox's model. Namely, the patients were stratified into three risk groups: low risk (normal AMC and ALC), intermediate (high AMC or low ALC), and high risk (high AMC and low ALC).
Results: ROC analysis showed that optimal cut-off values of AMC and ALC/AMC ratio with the best sensitivity and specificity were 0.59x109/L and 2.8, respectively. Cut-off of ALC was determined according to the literature data (1x109/L). Median lymphocyte count was 1.4x109/L and the range was 0.2-13x109/L. Median of monocyte count was 0.5x109/L and the range was from 0.1 to 3.9x109/L. Median of ALC/AMC ratio was 2.8, and range was from 0.33 to 57. Distribution of patients according to ALC/AMC prognostic score was as follows: low risk 91(41.0%), intermediate risk 99(44.6%) and high risk 32(14.4%) patients.Low ALC, high AMC, low ALC/AMC ratio and high ALC/AMC prognostic score were in significant association with lower rate of therapy response and survival. In contrast, these parameters were not in significant correlation with relapse rate. The patients with low ALC, "high" AMC, low ALC/AMC ratio and high ALC/AMC prognostic score at diagnosis had significantly shorter EFS and OS. In multivariate analysis all tested parameters (ALC, AMC, ALC/AMC prognostic score and ratio) are independent risk factors along with "bulky" disease and IPI.
Summary and Conclusions: All tested parameters (ALC, AMC, ALC/AMC score and ratio) may be useful prognostic factors in DLBCL patients. ALC/AMC score has a slight advantage as it allows the classification of patients into three prognostic groups. Further studies are needed to determine which of these parameters has the highest predictive value.

PB1838

90Y-IBRITUMOMAB TIUXETAN CONSOLIDATION AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IMPROVES SURVIVAL OF INTERMEDIATE/HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS NOT RESPONDING ADEQUATELY TO FIRST

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma entity, accounting for approximately 25-30% of new cases of non-Hodgkin's lymphoma. Up to now the prognosis of high-risk patients, namely those with an elevated IPI, bulky disease, involvement of central nervous system or testes, is poor due to early relapses. Aggressive salvage treatments to improve their outcomes were unsatisfactory. Therefore a consolidation therapy after early salvage regimen with autologous stem cell trasplant (ASCT) could reduce the relapse rate and prolong survival.
Aims: This analysis was performed to evaluated whether the additional yttrium-90-ibritumumab tiuxetan (90Y-IT) after early salvage treatment is able to improve the outcome of high-risk DLBCL.
Methods: We retrospectively assessed 37 patients affected by intermediate-high risk DLBCL not in complete remission after 3 cycles od R-CHOP chemotherapy. All were addressed to early salvage treatment with ASCT and 20 eligible patients underwent additional 90Y-IT consolidation. Salvage treatment consisted of 3-4 courses of R-DHAP followed by stem cell mobilization with cyclophosphamide (3 g/m2) and triple ASCT with a high-dose of cytarabine conditioning regimen (3-4 g/m2 days 1-4).
Results: After completion of the salvage treatment, in the 90Y-IT group 45% achieved a complete remission (CR) and 55% a partial remission (PR) compared to 94% and 6% in the other group. After 90Y-IT consolidation all achieved a CR. Adverse events after 90Y-IT treatment consisted of moderate transient hematologic toxicity. During the 3-year median follow-up period, 50% in the 90Y-IT group relapsed, compared to 82.3% in the other cohort (p=0.002). Progression and disease free survival (Figure 1) were significantly longer in the 90Y-IT group. However, probably due to the relatively short follow-up period, no difference in overall survival (OS) was observed.

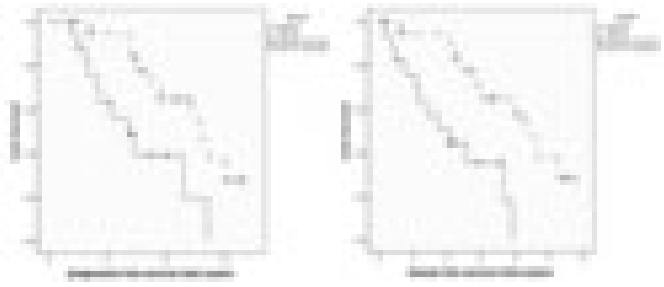

Figure 1.

Summary and Conclusions: 90Y-IT consolidation after early salvage chemotherapy improves treatment responses and reduces the percentage of relapses without significant additional toxicities. However, the follow-up time was not long enough to observe a difference in OS. Prospective confirmatory data is needed.

PB1839

MYC PROTEIN EXPRESSION IS THE POOR FACTOR IN DIFFUSE LARGE B CELL LYMPHOMA TREATED WITH RCHOP

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous group of neoplasma. It is increasing important to search the predictive biomarkers which could identify cases who will fail to RCHOP.
Aims: This study aims to investigate the prognostic significance of the MYC protein expression in DLBCL patients treated with RCHOP.
Methods: A total of sixty patients with DLBCL from 2008 to 2013 were included. Formalin-fixed paraffin-embedded(FFPE) tumor samples were analyzed for MYC protein expression and divided into high or low MYC group. The MYC protein expression and the international prognostic variables were evaluated.
Results: The high MYC protein expression predicted a shorter 3-year estimated overall survival (OS) and progression-free survival (PFS) *versus* the

low MYC protein expression (57% *vs.* 96%, P<0.001 and 50% *vs.* 96%, P= 0.001, respectively). Multivariate analysis confirmed the prognostic significance of the MYC protein expression for both OS (HR, 11.862; 95% CI, 1.462-96.218; P= 0.021) and PFS(HR, 6.233; 95% CI, 1.292-30.071;P=0.023). MYC protein expression with IPI score distinguished patients into three risk groups with different 3-year OS rates ($X^2$23.079; P<0.001) and distinct 3-year PFS rates ($X^2$15.862; P<0.001).

Summary and Conclusions: the MYC protein expression is an important independent inferior prognostic factor for survival in patients with DLBCL treated with RCHOP. The combinative model with IPI score and MYC protein expression could stratify DLBCL patients into prognostically relevant subgroups more effectively than either the IPI or the MYC alone.

PB1840

PROGNOSTIC SIGNIFICANCE OF LYMPHOCYTE/MONOCYTE RATIO (LMR) AT DIAGNOSIS IN A COHORT OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) TREATED WITH IMMUNOCHEMOTHERAPY: A SINGLE CENTRE EXPERIENCE.

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Background: Diffuse large B-cell lymphoma (DLBCL), the most common subtype of lymphoid neoplasm, is characterized as an aggressive lymphoma with heterogeneous clinical behaviors. A large number of studies have therefore focused on the search for surrogate biomarkers which are immunologically relevant and can serve as prognostic factors. Lymphopenia, a surrogate marker of immune suppression, was found to predict survival in DLBCL. Monocytes, which are considered immunologically relevant and are regarded as a surrogate marker of the tumor microenvironment, were also recently reported to be a prognostic factor in DLBCL. Although the introduction of rituximab combined with chemotherapy has greatly improved survival outcomes in DLBCL patients, there is limited available data about prognostic value of lymphocyte/monocyte ratio (LMR) in DLBCL patients (pts) in the rituximab era.

Aims: We investigated the prognostic role of LMR in terms of complete remission (CR) after first line treatment, overall survival (OS) and progression free survival (PFS) in a cohort of pts with DLBCL and treated with immunochemotherapy at our istitution.

Methods: We retrospectively analyzed data from a total of 133 DLBCL pts treated at our institution from January 2007 to July 2013. The median age of all pts at diagnosis was 64 years (range of 18–92 years). Pts were treated with immunochemotherapy with or without anthracycline (RCHOP or RCVP). We analyzed different LMR cut-off values and we found that the most discriminative LMR was 2.4; the absolute monocyte count (AMC) and absolute lymphocyte count (ALC) were derived from pre-treatment CBC counts. Survival curves according to Kaplan-Meyer method were employed to estimate PFS and OS. Log rank test was used to analyze differences. OS, PFS and relapse rate were defined by the standard criteria.

Results: The median follow-up time of the surviving pts was 24 months. 72 pts (54.1%) had localized disease (Ann Arbor stage I–II) and 61 extended disease (Ann Arbor stage III–IV); among them, 16 pts and 8 pts had extranodal localization, respectively. Based on the aaIPI score, 13 patients were in the aaIPI 0 group (9.7%), 50 pts in the aaIPI 1 group (37.6%), 48 pts in the aaIPI 2 group (36%) and 22 pts in the aaIPI 3 group (16.5%). An LMR value<2.4 was associated significantly with an advanced clinical stage (p=0.007), and a higher IPI score (p=0.004) and higher aaIPI (p=0.002). In the entire cohort, 90 pts obtained CR after first line treatment, while 43 pts did not obtain CR. CR rate (55% *vs* 61%) and 2 year-OS (85% *vs* 87%) were similar for pts with LMR<2.4 and for pts with LMR>2.4. Among 40 pts with an LMR>2.4 only one relapsed (6 months after CR), while 7/50 pts with LMR<2.4 relapsed after 4-22 months, with 2 year-PFS of 86% *vs* 96%, as shown in Figure 1 (p=0.07).

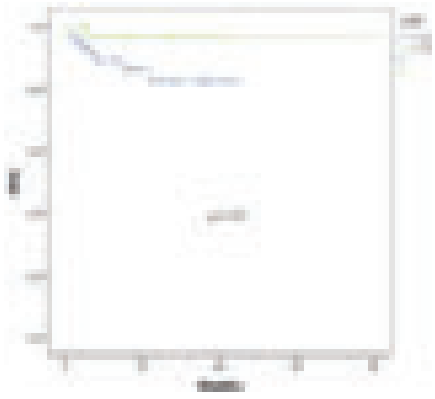

Figure 1.

Summary and Conclusions: Recent results have clearly shown that via CCL5, lymphoma B cells can recruit monocytes which in turn support the survival and proliferation of neoplastic B cells and suppress the proliferation of normal T cells. Our results confirm the hypotesis that the evaluation of LMR at diagnosis is a simple and robust tool to better define clinical response and long-term outcome of DLBCL patients receiving immunochemotherapy. Further study are needed to assess the role of LMR in DLBCL at diagnosis, supporting the idea that it could be incorporated into the IPI stratification.

PB1841

HIGH-DOSE METHOTREXATE CONSOLIDATION IN POOR-RISK DIFFUSE LARGE B-CELL LYMPHOMA IS ASSOCIATED WITH IMPROVED PROGRESSION FREE SURVIVAL

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Background: While the majority of patients with Diffuse large B-cell lymphoma (DLBCL) can expect to be cured with R-CHOP chemotherapy, the outcome for patients with poor risk disease is less certain with approximately 50% of patients eventually succumbing to their disease. Dose intense regimens have largely failed to improve outcomes, and are associated with increased toxicity. However certain regimens may have a role in younger poor risk patients.

The association of poor-risk disease with central nervous system (CNS) recurrence has led many centres to advocate the use of systemic high-dose methotrexate (HD-MTX) consolidation in poor-risk patients. Since 2007 our centre has been consolidating patients deemed to be at high-risk of CNS recurrence including those with IPI≥3, involvement of a high-risk site (testis, ovary, breast, kidney, extradural or skin) or those with two involved extra nodal sites and a raised lactate dehydrogenase with two cycles of intravenous methotrexate 3g/m^2 and rituximab 375mg/m^2 following the completion of R-CHOP. HD-MTX is only administered to patients in CR after completion of R-CHOP with adequate performance status.

Aims: In this study we examine the effect of HD-MTX on overall survival (OS) and Progression Free Survival (PFS) in patients with poor-risk DLBCL.

Methods: Patients were included in the study if they had DLBCL diagnosed according to WHO 2008 criteria, an R-IPI ≥ 3, received two or more cycles of R-CHOP-like chemotherapy, and had adequate clinical information that included baseline clinical characteristics, treatment regimens and clinical outcome. Patients were excluded if they were too frail to receive chemotherapy or failed to achieve a CR following R-CHOP therapy. A total of 92 patients met the entry criteria of which 17 received HD-MTX. Survival correlates were analysed by Cox regression using SPSS.

Results: While patients receiving HD-MTX were younger (median age 69 v. 74), the proportion over 60 years was similar in both groups; (HD-MTX 83% v. standard 85%). The proportions with advanced stage (100% v 85%) raised LDH (82% *vs.* 80%) and ECOG>2 (17% *vs.* 11%) were also similar, however the HD-MTX group had fewer patients with ECOG >1 (41% v. 60%). All patients received Rituximab containing chemotherapy regimens, with both groups receiving a median of six cycles (range 3-6).

The median follow up of surviving patients was 2.5 years. Patients who received HD-MTX had improved 5 year PFS: 65% *vs.* 34% (HR 0.50, p=0.048) and a trend to improved OS 73% v. 44% (HR 0.50, P =0.082) This was mainly attributable to a reduction in the five year systemic rela:pse rate: 17.6% *vs.* 38.7% (HR 0.46, P=0.063). No difference in the rate of CNS recurrence was evident however events in both groups were uncommon. The regimen was well tolerated with no severe toxicity (Figure 1).

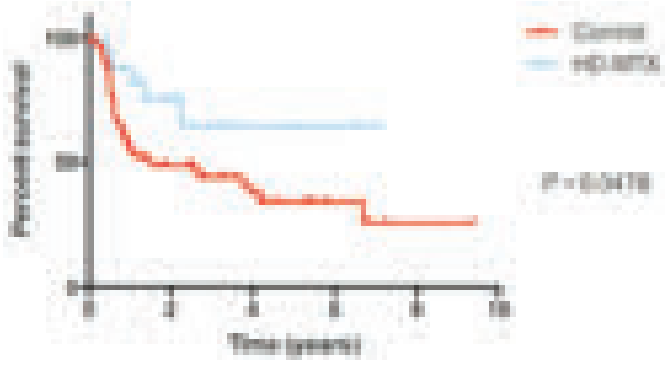

Figure 1. Progression free survival, IPI 3, 4, 5.

Summary and Conclusions: The use of HD-MTX is associated with improved PFS and a trend to improved OS when used as consolidation therapy in patients with poor-risk DLBCL, including the elderly. We speculate that the low rates of toxicity observed in our study may be due to sequential rather than concurrent administration of HD-MTX, and careful patient selection. Given the inherent limitations of this retrospective cohort study, further prospective studies are warranted to validate this approach in poor-risk patients with DLBCL.

PB1842

TRANSFORMED FOLLICULAR LYMPHOMA TREATMENT OUTCOME DURING THE RITUXIMAB ERA

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Background: Follicular lymphoma transformation (tFL) to Diffuse Large B Cell lymphoma occurs in 16% to 60% of cases and is historically associated with a median survival of less than 2 years and there are no prospective studies specifically evaluating treatment approaches in patients with tFL and there is little published data on the outcome of tFL in the Rituximab era.
Aims: Retrospective study to evaluate treatment outcome of patients with tFL diagnosed between 2003 and 2012 after adapting specific treatment algorithm.
Methods: Patients diagnosed with tFL, between 2003 and 2012 identified from the institutional lymphoma database were included. Data collected included age, gender, stage and prior treatment, interval to tFL diagnosis, IPI score and tFL treatment. Only cases which fulfil the diagnostic criteria of DLBCL based on WHO criteria were evaluated.

Table 1. Patient clinical charateristics.

Male/Female	20/9	69/31%
Median Age/yrs	51	Range (27-72)
Denovo tFLvsKnown FL	15 *vs* 14	52% *vs* 48%
Stagel,II *vs* III,IV	6/23	20/80%
BM invol. NegvsPos	16/5	55/17%
Not available	8	
R-IPI 0-2 *vs* 3-4	25/4	86/14
B.Symptoms (+/-)	10/19	34.5/66.5%
Relapse Disease (+/-)	11/18	38/62%

Table 2. Consolidation treatment.

tFL	No	%
Rituximab Maintenance	8	27.5
Zevilan	3	10
Auto-SCT	8	27.5
AllO-SCT	8	27.5
Both-SCT	2	7

Figure 1. Our institutional transformed follicular lymphoma treatment algorithm.

Results: Over this period, 29 (7.25%) patients with biopsy proven tFL were identified from a total cohort of 400 patients with FL, 15 patients (51.7%) presented with de novo tFL and 14 (48.2%) had known FL with median time to transformation of 6.7 years. Twenty (65.5%) patients were male with a median age at transformation of 54 years (range 27-72) and 80% had stage III/IV disease at transformation. The median follow-up time from tFL diagnosis is 3.3 years. All patient received Rituximab - chemotherapy (R-CHOP or R-CHOP like), with 11 patients (38%) requiring second line therapy to attain remission. Consolidation therapy algorithm was adapted based on age, performance status and donor availability. 16 patients (55%) were transplant eligible, 8 patients (27.5%) received allogeneic SCT and 8 patients received autologous(A) SCT and 2 patients were allo-transplanted after relapsing post-ASCT . Thirteen (45%) patients were not SCT eligible and were consolidated with either Zevalin 3 (10%) or Rituximab maintenance 8 (27.5) patients. The median survival from transformation was 98 months with three deaths, (two from disease progression and one from transplant related mortality (Tables 1, 2 and Figure 1).
Summary and Conclusions: Compared to historical published data the median OS of 98 months in this small cohort of patients is encouraging and suggests that Rituximab therapy may have changed the natural history of tFL. Fourteen of 16 patients transplanted remain alive and disease free and these outcomes should be confirmed in a larger series.

PB1843

SECONDARY CNS INVOLVEMENT IN MALIGNANT LYMPHOMA: DATA FROM A PROSPECTIVE REGISTRY

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Background: Secondary CNS involvement of malignant lymphoma (SCNSL) is extremely rare, and its optimal management is yet to be defined. In a prospective German registry we evaluated clinical characteristics, treatment and outcome of patients diagnosed with SCNSL since July 2011.
Aims: Collection of data on diagnosis making and treatment strategies in SCNSL in the clinical routine.
Methods: Patients with secondary CNS involvement of indolent or aggressive non-Hodgkin`s lymphoma (confirmed histologically or cytologically) with or without systemic involvement at time point of CNS involvement were eligible. Informed consent was obtained from all patients.
Results: Data of the first 69 patients (median age 60 years, range 26-80) were analysed. Eight (12%) patients had simultaneous CNS and systemic involvement at first diagnosis (cohort I), and 61 (88%) had CNS involvement at relapse (cohort II), 11 (16%) of whom simultaneously had active systemic disease. Histology at first diagnosis was aggressive B-cell lymphoma in 48 (70%) and mantle-cell and T-cell lymphoma in 2 patients (3%) each; 17 patients (25%) had indolent B-cell lymphoma. Primary therapy in cohort II was R-CHOP in 35 patients (51%), CHOP in 9 (13%), R-CHOP/CHOP combined with other systemic chemotherapy in 8 (12%) and other systemic chemotherapy in 7 (10%). CNS prophylaxis was given to 10 (14%) patients: intrathecal (i.th.) chemotherapy alone in 3, high-dose methotrexate (HDMTX) intravenously alone in 3, whole-brain radiotherapy in 1 and a combination of those in 3; 2 patients (3%) have not received any anti-lymphoma therapy. Median time from first diagnosis to CNS relapse (cohort II) was 16 months. CNS lymphoma localisation was brain parenchyma in 39 patients (57%), meninges in 18 (26%), spinal cord in 2 (3%) and combination of those in 10 (14%). Therapy for CNS involvement was systemic chemotherapy in 37 patients (54%), systemic + i.th. chemotherapy in 30 (43%) and i.th. chemotherapy alone in 2 (3%). Systemic therapy was HDMTX-based in 62 patients (90%) and high-dose cytarabine-based in 36 (52%); high-dose chemotherapy with autologous stem-cell transplantation was given to 23 patients (33%). Median follow-up time from inclusion in the registry was 17 months. In cohort I, 3 patients progressed/relapsed after 7.0-11.5 months and one died after 16 months following diagnosis of CNS involvement. Median progression-free survival (PFS) and overall survival (OS) from CNS involvement in cohort II was 6.7 months (95%CI 3.7-9.5) and 15.7 months (95%CI 7.0-24.3), respectively. Median PFS and OS of patients with parenchymal involvement were significantly longer (both not reached) as compared to patients with meningeal involvement with 5.5

(95%CI 2.9-8.0) and 8.2 (95%CI 5.2-11.1) months, respectively (P<0.001 for PFS and 0.007 for OS).
Summary and Conclusions: With modern and systemically more potent anti-lymphoma therapy the frequency of isolated CNS relapse seems higher than previously reported reflecting reduced ability to clear the CNS as compared to systemic disease. Despite frequent use of CNS-penetrating systemic chemotherapy outcome of patients with SCNSL, particularly with meningeal involvement, remains poor.

PB1844

SIGNIFICANT EFFECT OF RITUXIMAB MAINTENANCE IN FIRST LINE TREATMENT IN MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is a relatively rare malignancy with unfavorable outcomes. Introduction of anti-CD20 rituximab maintenance (RM) treatment into therapeutic protocols should be beneficial.
Aims: To verify the importance of rituximab maintenance in MCL first line treatment.
Methods: Prospective single center observation of newly diagnosed MCL patients: n=57, median age 66 (47-82), 68% males, 84% Ann Arbor stage IV., prognostic MIPI index media 6 (2-10), 92% R-CHOP-like and 8% R-COP induction therapy protocol, 46% intensified with Autologous stem cells transplantation (SCT), 82% complete and 18% partial remissions prior to start of RM administration. Rituximab maintenance was administered as 375mg/m2 dose every 3 months for 2 years and the median number of administered doses was 7 (2-8), seven patients are still on treatment, eight patients discontinued the RM prematurely due to MCL relapse/progression, refusal of further treatment, or health insurance company obstacles. Progression free survival (PFS, time since diagnosis to relaps/progression) was analyzed separately in a group of patients with and without Autologous SCT and in respect to RM administration.
Results: In a group of non-intensified patients without Autologous SCT the probability of 2-years PFS and median PFS was 26% and 20 months if RM was not given (n=16) *versus* 86% and median PFS not yet reached at the median follow-up of 37 (14-65) months if RM (n=15) was administered (p=0,01). In Autologous SCT patients the probability of 2-years PFS and median PFS was 75% and 46 months if RM was not given (n=12) *versus* 95% and median PFS not yet reached at the median follow-up of 50 (22-82) months if RM (n=14) was administered (p=0,02). No statistically significant differences were observed when comparing age, gender, MCL stage, MIPI index, complete remission ratios prior to RM. RM administration was statistically significant predictor for prolonged PFS in the multivariable analysis (Figure 1).

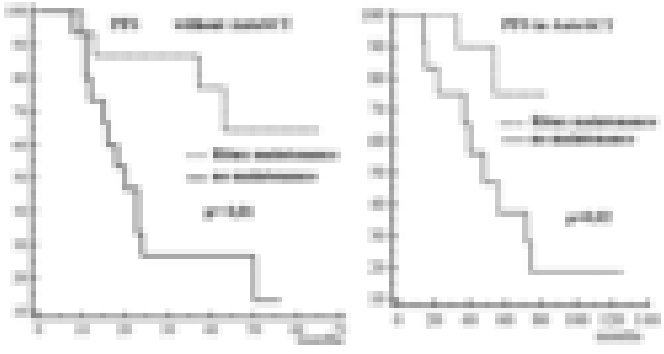

Figure 1.

Summary and Conclusions: Even though the numbers of our analyzed patients are small, we can observe that in newly diagnosed MCL patients responding to the initial induction therapy (R-CHOP-like, R-COP), the administration of RM significantly prolongs PFS in both Autologous SCT treated patients and in those non-intensified. The data are not mature enough to asses the overall survival.

PB1845

TREATMENT OF PERIPHERAL T-CELL LYMPHOMA WITH AN INTENSIVE PROTOCOL ACEP (ADRIAMYCIN,CYCLOPHOSFAMIDE,ETOPOSIDE AND PREDNISOLONE) AND IFOSFAMIDE SHOWING AN IMPORTANT OVERALL SURVIVAL AT 6 YEARS

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Background: Peripheral T-Cell Lymphoma (PTCL) is a group of lymphoid malignancies which has never been treated with any confidence as opposed to its counterpart B-Cell Lymphomas. Despite the studies, which were retrospective, the results in the majority of cases were disappointing, taking into consideration the aggressive clinical course of the disease, so survival did not exceed 2 years in median.
Aims: To assess the response, progression free survival and overall survival rates at 5 years using a new intensive combination chemotherapy
Methods: Enrolled patients were diagnosed with PTCL, confirmed by a referenced pathologist, treated with the new chemotherapy ACEP X 6 (Doxorubicine 75 mg/m² on day 1 + Cyclofosfamide 1200 mg/m² on day 1 + Etoposide 300 mg/m² on day 1 and Prednisolone 60 mg/m² from day 1 through day 5) and Ifosfamide X 4 (Ifosfamide 4 grams/m² on day 1) which were given after the completion of the first 6 cycles of ACEP . The study was performed at Al-Bairouni University Hospital and the study was approved by the Syrian Association of Clinical Oncology.
Results: 25 patients underwent the treatment. Most of them showed a complete response after the completion of the first six cycles (17/25) forming 68% of patients, while another 5 patients became complete responders after the completion of treatment. Consequently, 22 patients are still living after 5 years, with an overall survival rate of 88%, the study was updated in December 2013 and we still have 88% overall survival rate at 6 years.
Summary and Conclusions: (ACEP) and Ifosfamide appears to be a good choice in PTCLs, in light of the good response and overall survival rates, taking into account the acceptable toxicity profile. However, a larger sample is needed to make it acceptable new combination chemotherapy for PTCLs patients.

PB1846

A RISK OF DERMOPATHY AND OPOTUNISTIC INFECTION AFTER TREATMENT WITH ANTI-CCR4 ANTIBODIES FOR ADULT T-CELL LEUKEMIA

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Background: Human T-cell leukemia virus type I (HTLV-I) is a human retrovirus that is an etiologic agent of adult T-cell leukemia/lymphoma (ATL/ATLL). The poor outcome of adult ATL is mainly because of an intrinsic resistance of the leukemic cells to conventional or high doses of chemotherapy and severe immunosuppression. The CC-chemokine receptor 4 (CCR4) is expressed in almost ATLL cells. Thus, anti-CCR4 antibodies can be used as a treatment strategy for ATLL. Mogamulizumab (MOG), which is a defucosylated anti-CCR4 monoclonal antibody, showed good results even in patients with recurrent ATLL in phase I or II studies. MOG has strong antibody-dependent cellular cytotoxicity (ADCC). The common adverse events in the phase II study were infusion reaction and skin rashes. In our study, MOG inhibited the growth of ATL cells and induced remission. CCR4 is expressed not only in ATL cells but also in non-ATL regulatory T cells (Treg). After treatment with MOG, the population of Treg decreased significantly, which boosted the antitumor activity. MOG treatment was associated with the risk of viral infection as an opportunistic infection and skin disturbance (dermopathy).
Aims: In the present study, we observe the effects of MOG as CCR4-specific ADCC against CCR4-positive ATL cells. However, CCR4 is not only on ATL cells but also on endogenous Treg. The decrease in the number of Treg after MOG monotherapy has been expected to boost the antitumor activity and to be involved in the development of immune disorders, including autoimmune diseases. On the other hand, we observed opportunistic viral infection including active CMV infection and skin disturbance associated with a rapid decrease in CD4+ T cells.
Methods: In the present study, we treated 14 patients with ATL who were resistant to chemotherapy using MOG monotherapy, and we observed CCR4-specific ADCC against CCR4-positive ATL cells. In this study, CMV infection was detected using C7- horseradish peroxidase (C7-HRP) methods.
Results: All patients showed CR with a marked decrease in the number of ATL cells. These results suggested that MOG was effective in chemotherapy-resistant ATL patients. ATL cells were not found in her peripheral blood, and no rearrangement of TCRab gene was observed, which indicated molecular CR. Skin disturbance (dermopathy) was found 9 cases of 14 ATL patients treated with MOG monotherapy. The skin rash spread throughout her body with bulla and skin biopsy was performed, and diagnosed as Stevens–Johnson syndrome (SJS) in 2 patients of them. Administration of steroid and intravenous immunoglobulin was performed, and SJS gradually improved. Further, In other patients, grade 2 and 3 dermopathy was found, and the patients was treated with glucocorticoid steroid. CMV infection was detected using C7-HRP methods. CMV infection was diagnosed in 5 patients of 14 MOG treated ATL patinets. One of the patients died because of severe CMV infection despite of adequate treatment.
Summary and Conclusions: Thus, physicians should be aware of CMV infection and development of immunocompromised condition after MOG monotherapy. Specific anti-CMV treatment for CMV reactivation should be recommended. Moreover, We have to find out the skin rash as soon as possible after treatment

with MOG and it should be recommended quick administeration with glucocorticoid. Futher study for the dermopathy should be considered.

PB1847

HOW TO TREAT B-CELL LYMPHOMA, UNCLASSIFIABLE, WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA AND BURKITT LYMPHOMA. SINGLE CENTRE EXPERIENCE

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Background: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (DLBCL/BL) is a separate entity in the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues 2008. It is nowadays a provisional category of a spectrum of features between two borderline lymphomas – difusse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). These lymphomas have morphological and genetic features of both DLBCL and BL, so they cannot be clearly included in either one of those two entities. DLBCL/BL generally has an agressive clinical course and no guidelines for treatment are available yet.
Aims: To compare efficacy of different treatment options.
Methods: Retrospectively we reviewed clinical data from our institution's register between years 2010-2013. Subsequently we analysed therapy outcome due to chosen regimens. The treatment strategy decision was made depending on patient's age, clinical stage of the disease and the IPI score. All of the histology samples were reviewed by experienced pathologists, in majority of the cases including FISH and genetic abnormalities diagnostic tests.
Results: Since January 2010 to December 2013 we registered 30 patients with newly diagnosed DLBCL/BL, constituting approximately 3.4% of all non-Hodgkin lymphomas (n=881). For comparison, in the same time period, we registered 301 cases of DLBCL (34%) and 16 cases of BL (1.8%). There were 14 men and 16 women, average age being 65 years (38-86y). One patient (86y) was treated by paliative radiotherapy only and died within 1 month due to lymphoma progression. 10/30 patients (33%), average age 53y (38-66y), clinical stage I-IV, average IPI score 3 (0-5), median LDH 5.27 µkat/l (2.79-107), were treated with intensive approach, when R-CHOP treatment was enhanced with 3-4 cycles R-CODOX-M with or without R-IVAC up to 5-7 chemotherapy cycles total. Two patients from this group died, one (53y) had confirmed concurrent BCL2-IGH and MYC rearrangement (double-hit lymphoma), was primarily chemoresistant, and died within 4 months from diagnosis due to lymphoma progression. The second one (60y) died due to treatment toxicity and concurrent illness (sepsis and ischemic cerebral stroke) immediately after treatment completion. The remaining 8 patients (80%) from a group of intensively treated are nowadays in complete remission lasting 3-31 months depending on the treatment completion date. 19/30 patients (63%), average age 71y (57-86y), clinical stage I-IV, average IPI score 3 (0-5), median LDH 4.99 µkat/l (2.5-40.14), got usual R-CHOP based chemotherapy, in 9 cases (47%) including 1-2 cycles of high dose methotrexate or high dose cytarabine finally. 3 patients (16%) from this group died during the treatment or within 6 months after treatment completion due to lymphoma progression. 3 patients (16%) from this group relapsed within one year and recieved another chemotherapy regimens. 13 patients (68%) from this group are in complete remission or the treatment is nowadays still ongoing. We haven't evaluated the disease free survival (DFS) and overall survival (OS) yet, because of short follow-up.
Summary and Conclusions: Despite short follow-up, our data suggest that R-CHOP based chemotherapy is less effective than it's combination with intensive regimens containing R-CODOX-M and R-IVAC. The most important factor that influenced the treatment strategy was patients' age. For setting the proper treatment guidelines bigger patients cohorts and a longer follow up of the survivals are required.

PB1848

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER AFTER KIDNEY TRANSPLANTATION

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Background: Post-transplant lymphoproliferative disorder (PTLD) is a heterogeneous group of lymphoid lesions. Although rare, it is an important long-term complication in kidney transplant recipients.
Aims: To describe demographics and prognosis in a cohort of PTLD patients.
Methods: The Norwegian Nephrology Registry has a complete data set for renal recipients since 1969. The register was linked to the Norwegian Cancer Registry of Norway to identify cases of PTLD. Clinical data were retrieved from local hospitals. Demographic data, transplant history, outcomes and pathology were characterized using SPSS statistics.
Results: Fifty-five kidney transplant recipients with PTLD were identified, at a median of 7.1 years since transplantation. Mean age at time of lymphoma was 54.4 years, 80% were men, and median follow-up time for all patients was ten months, and six years for patients alive at last follow up. Sixty percent had a previous rejection, of whom 37% had been treated with ATG. Thirty-six percent of the lymphomas were nodal, 24% involved GI-tract, 18% CNS, and 21% other organs. Sixty-seven per cent were Diffuse Large B-Cell Lymphomas, 11% were polymorphic PTLD and 14% were others. Thirty-seven patients died with a five year overall survival of 34% (Figure 1).
Summary and Conclusions: Post-transplant lymphoma disorder affects kidney transplant recipients after several years with heterogenous clinical manifestations. PTLD is strongly associated with previous treatment for graft rejection. Early death was more common than what is seen in non-PTLD lymphomas, probably due to co-morbidity and increased infectious complication. Better supportive care and an individualized treatment approach seems important.

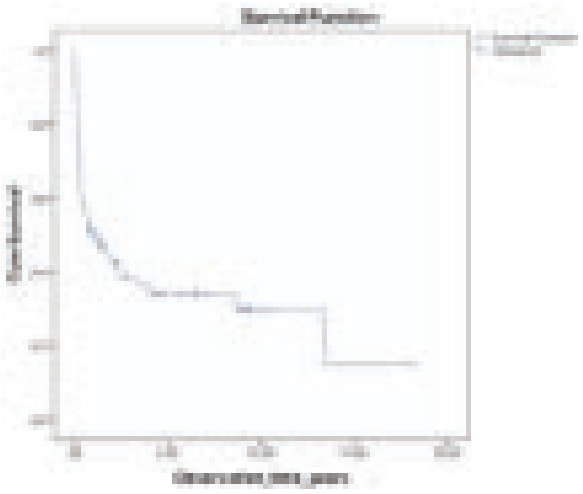

Figure 1.

PB1849

DEPO-ITV: A NEW SCENARIO WITH INTRATHECAL AND INTRAVENOUS DEXAMETASONE TO PREVENT CHEMICAL ARACHNOIDITIS RELATED WITH LIPOSOMAL CYTARABINE

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Background: Intrathecal Liposomal Cytarabine (LC) is FDA/EMA approved for Lymphomatous Meningitis (LM) treatment. Retrospective studies have evaluated LC in LM prophylaxis. Chemical arachnoiditis (headache, nausea, vomiting and high temperature) is the most common related adverse effect (25-40%). Steroids co-administration, intrathecal and/or systemic, is suggested to prevent this adverse event but there is no consensus about the best pattern of use.
Aims: The aim of this study is to evaluate the incidence of chemical arachnoiditis in patients with hematologic malignances and high risk of LM, treated with the new Depo-ITV (LC 50 mg IT + Dexametasone 4 mg IT + Dexametasone 20 mg iv, day 1) as LM prophylaxis.
Methods: We carried out a study on 3 different sites to analyze the toxic profile and the effectiveness of Depo-ITV. Inclusion criteria were as follows: patients≥ 18 at the time of treatment, confirmed diagnosis of hematologic malignance, treated with Depo-ITV as LM prophylaxis in the period 1st January 2010 to 1st January 2014. Exclusion criteria: previous lymphomatous or infectious meningitis, previous neurological toxicity related to systemic chemotherapy. Depo-ITV toxic profile was evaluated as *CTCAE v3.0 of NCI scale*. We also recorded demographic and clinical data, systemic treatment, systemic response, and LM incidence. This study has been approved by local ethics committees.
Results: One hundred twelve administrations of Depo-ITV in 54 pt, median per pt 2 (1-5), were analyzed during the above-mentioned period. Baseline characteristics median age 55 (20-79), M/F 32/22, histologic subtype; DLBCL 25 pt, Lymphoblastic Leukemia 13 pt, Follicular Lymphoma 4 pt, Myeloblastic Leukemia 4 pt, MCL 2 pt, Richter 2 pt, Burkitt 2 pt, T cell lymphoma 2 pt. HiDAC occurred in 1 case, high dose methotrexate in 4 cases and HiDAC plus high dose systemic methotrexate in 19 cases. Toxicity: There were 3 pt with grade 3 (no grade 4) AE, 4 (3.5%) cases out of 112 administrations, and the cases were

as follows: Case 1: 70 year old female diagnosed with DLBCL treated with 2 Depo-ITV administrations, after 1st administration grade 3 orthostatic headaches occurred, but the orthostatic characteristic the headache was also considered depo-ITV related. A second administration of Depo-ITV (no dose reduction) was performed without AE. Case 2: 41 year old male, Richter, headache grade 3 after 1st Depo-ITV admintration. Treatment was discontinued. Case 3: Lymphoblastic Leukemia, systemic HiDAC and 4 depo-ITV administrations. Headache grade 3 after 2nd and 4th administrations (no dose reduction after 2nd). Sings of intracranial hypertension occurred after 4th Depo-ITV. This toxicity was reversible. Systemic responses were as follows: CR 41, PR4, progression disease 7 and 2 non evaluated patients. With 10.4 (0.5-49) month median follow up, we report no cases of LM, 37 pt are alive and in CR, 13 pt are dead (10 p disease progression), 3 pt are currently in active treatment and 1 case is lost for follow up.
Summary and Conclusions: Depo-ITV reduces the incidence of post LC chemical arachnoiditis and is effective in preventing LM. The Depo-ITV pattern suggested here ensures chemical arachnoiditis prevention in contrast to the multiple day steroids patterns.

PB1850

HIGH-DOSE CYTARABINE AND MITOXANTRONE ±RITUXIMAB FOR COLLECTION OF PERIPHERAL BLOOD STEM CELLS IN HIGH-GRADE NON-HODGKINS´ LYMPHOMA. SINGLE CENTER EXPERIENCE WITH FILGRASTIM BIOSIMILARS.

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Background: For the patients with aggressive non-Hodgkins´ lymphomas (NHL) chemotherapy – high-dose of cytarabine with mitoxantrone ±rituximab (R±HAM) is used for the standard collection of peripheral blood stem cells at our Department as a part of Sequential Protocol chemotherapy (2xPACEBO, IVAM, PACEBO, HAM ±rituximab).
Aims: We describe here the experience with the efficacy and safety together with the comparison of two filgrastim (G-CSF) biosimilars.
Methods: R±HAM was given as follows: rituximab 375 mg/m^2 on day 1; cytarabine 2g/m^2 in 4 doses on day 1 and 2; mitoxantrone 10 mg/m^2 in 2 doses on day 2 and 3. In patients with age over 60 the dose of cytarabine and mitoxantrone was reduced to 0.5 g/m^2 and 7 mg/m^2, respectively. Three different types of G-CSF at the same dosage (10μg/kg of body weight) were used to stimulate peripheral blood stem cells: Neupogen (39 patients), Zarzio (28 patients) and Tevagrastim (11 patients). Retrospective analysis was performed in cohort of 78 patients with NHL in the period January 2009 - September 2013. Median of age was 53 (22-64) years.
Efficacy of the type of G-CSF in terms of the leucopheresis number (collection days) and number of CD34+ cells per kg collected was the first goal of our study. The incidence of infections and other side-effects were recorded in addition. Kruskal-Wallis and Fisher exact tests were used for comparison.
Results: There were no differences among the groups of patients with respect to the gender, age, marrow involvement and previous treatment. There were no statistically significant differences in number of collection procedures among the groups (p=0.084); on average 1.69 procedures for Neupogen, 1.53 procedures for Zarzio and 1.81 procedures for Tevagrastim. Also there were no statistically significant differences in number of CD34+ cells per kg collected (p=0.497); on average 8.29x10^6 CD34+ cells per kg for Neupogen 8.51x10^6 CD34+ cells per kg for Zarzio and 6.66x10^6 CD34+ cells per kg for Tevagrastim. Patients hospitalized and central venous line inserted for the whole period of the priming (42 patients) have a significant higher risk of septicaemia (p=0.002). Patients who proceed to the transplantation engrafted in all cases.
Summary and Conclusions: R±HAM is well tolerated and highly efficient priming chemotherapy (average number of CD34+ cells collected was 7,82x10^6 per kg, 78 patients). Different types of G-CSF show no statistically significant differences in the stimulation efficacy. Prolonged hospitalization and central venous catheterization is associated with higher risk of septic complications. More patients should be analysed in order to further increase the validity of results.
Supported by the grants MSM-6198959205 and IGA UP LF-2013-004.

PB1851

ELEVATED BONE MARROW PROLYMPHOCYTES PREDICT SUPERIOR OUTCOME IN DIFFUSE LARGE B CELL LYMPHOMA

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Background: Elevated prolymphocytes with normal cytomorphology can be seen in bone marrow (BM) of patients with diffuse large B-cell lymphoma (DLBCL) from time to time. Whether elevated BM prolymphocytes, serving as a surrogate of host immunity, can predict prognosis of DLBCL patients remains unknown.
Aims: To explore whether elevated BM prolymphocytes serving as a surrogate of host immunity, could predict prognosis of DLBCL patients.
Methods: Two hundred and ten de novo DLBCL patients from 2001 to 2012 were retrospectively analyzed in the present study.
Results: Elevated BM prolymphocytes with normal cytomorphology (>1.5%) could be found in 18 DLBCL patients (8.6%). There was no significant difference in gender, age, B symptoms, performance status, LDH, stage and IPI score between patients with elevated BM prolymphocytes *versus* all the remaining cases (p>0.05). Patients with elevated BM prolymphocytes showed significantly superior OS and PFS compared with all the remaining patients (p=0.031 and p=0.043, respectively), especially in those high-risk patients (p=0.041and p=0.027, respectively). Of 40 patients with BM involvement (BMI), 20 had concordant BMI and 20 had discordant BMI. Concordant BMI had significantly shorter OS and PFS than uninvolved BM patients (p<0.001and p=0.005, respectively), whereas discordant BMI did not (p=0.390 and p=0.918, respectively). Multivariate analysis revealed elevated BM prolymphocytes remained a favorable factor for PFS (RR, 0.130; 95% CI, 0.018-0.950, p=0.044), but not for OS. However, concordant BMI was an unfavorable predictor for OS (RR, 2.474; 95% CI, 1.138-5.377, p=0.022, respectively), but not for PFS.
Summary and Conclusions: Elevated BM prolymphocytes at diagnosis may imply a good prognosis, allowing the identification of a superior outcome subgroup in high-risk DLBCL patients. Concordant BMI has a negative impact on survival in DLBCL patients.

PB1852

PROGNOSIS FACTORS IN ELDERLY PATIENTS WITH PRIMARY CNS LYMPHOMA TREATED WITH NONRADIATION, INTERMEDIATE-DOSE METHOTREXATE-CONTAINING REGIMEN

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Background: Nearly half of all patients with primary central nervous system lymphoma (PCNSL) are known to be aged over 60 years. However, clinical factors affecting treatment outcomes in elderly patients are understudied.
Aims: In this study,we intended to identify prognosis factors in elderly patients with PCNSL treated with nonradiation, intermediate-dose methotrexate (MTX)-containing regimen.
Methods: We analyzed 38 patients with PCNSL older than 60 years. All patients were treated with nonradiation, intermediate-dose MTX-containing protocol comprising an induction therapy and maintenance therapy between March 2005 and May 2013 at the University of Tsukuba Hospital. As an induction therapy, 1 g/m^2 intravenous (i.v.) MTX was given on days 1, 10, and 20; 40 mg/m^2 i.v. ranimustine, on day 1; 60 mg/m^2 oral procarbazine, from days 1 to 7; and i.v. or oral methylprednisolone, at a dose of 120 mg/m^2 every other day from days 1 to 20 and then at a dose of 60 mg/m^2 from days 21 to 45. Fifteen milligrams of MTX and 40 mg of cytarabine were injected intrathecally (i.t.) on days 1, 5, 10, and 15 with leucovorin rescue. As a maintenance chemotherapy, 1 g/m^2 i.v. MTX was given on day 1; 40 mg/ m^2 i.v ranimustine, on day 1; and 60 mg/m^2 oral procarbazine, from days 1 to 7; and 15 mg i.t. MTX and 40 mg i.t. cytarabine on day 1. Patients who achieved a complete remission or partial remission after 1 course of induction chemotherapy proceeded to further therapy with 5 courses of maintenance chemotherapy every 6 weeks. When progressive disease was documented, the protocol was stopped. Overall survival (OS) was calculated from the first date of the induction chemotherapy to the date of any cause of death. Progression free survival (PFS) was defined as the period from the first date of the induction chemotherapy to the first date of the documentation of disease progression or death as a result of PCNSL. The OS and PFS were estimated with the Kaplan-Meier method. Univariate analysis to see impact of clinical factors on OS or PFS was performed with the log-rank test. Independent predictive factors for OS were analyzed by Cox proportional hazards regression model.
Results: Twenty-four patients were able to finish the full protocol. Three-year OS and PFS rates were 56.2% [95% confidence interval (CI): 36.2%>76.2%] and 29.8% (95% CI: 9%>50.6%), respectively, with a median follow-up of 36.5 months. We found age > 75 years, Karnofsky performance score<70, altered mentation, and creatinine clearance (CrCl) > 90 ml min^{-1} were significant (p<0.05) factors associated with a worse survival by univariate analysis. The only parameter significantly associated with PFS was altered mentation with univariate analysis (p=0.024). Although patients with CrCl > 90 ml min^{-1} showed tendency toward worse PFS, it was statistically insignificant (p=0.118). Multivariate analysis revealed CrCl [p<0.05; hazard ratio (HR) = 3.39; 95% CI, 1.08–10.68] and altered mentation (p<0.05; HR = 6.27; 95%CI, 1.37–28.83) were independently significantly associated factors with OS. The most frequent adverse effect was myelosuppression, with grade 3 to 4 hematologic toxicities in 28 patients. Grade 3 to 4 hepatic toxicity was observed in 6 patients. Renal toxicity was documented in 13 patients, 5 of whom required dose reduction or modification of the protocol. No delayed neurotoxicities were observed.

Summary and Conclusions: More aggressive therapy may be introduced in selected patients with poor prognosis factors to improve outcomes.

PB1853

ROLE OF FDG-PET SCANS IN THE MANAGEMENT OF PEDIATRIC NON-HODGKIN'S LYMPHOMA. CCHE EXPERIENCE

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Background: Malignant lymphomas are the third most common malignancy among children and adolescents, with a propensity for widespread dissemination. 18-F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) is a noninvasive, 3-dimensional imaging modality that has become widely used in the management of patients with malignant lymphomas. PET is the most important recent advance in noninvasive lymphoma assessment. Current applications of PET in lymphoma may be divided into pretreatment staging, restaging, therapy monitoring and post therapy surveillance. Evaluation of residual masses and the recommendation to document them histologically before intensifying treatment in cases of incomplete remission is one of the difficulties during patients' treatment. Usefulness of PET scanning to assess viability of a residual mass is under evaluation in childhood lymphoma with the hope that it might replace histologic documentation, but this evaluation is less advanced than in adult.
Aims: To evaluate the sensitivity, specificity and predictive values of PET scan during management of pediatric Non-Hodgkin's lymphoma. Correlation of PET results with conventional CT, the patients' clinical outcome, and its impact on the treating physician decision was done.
Methods: A retrospective study enrolled on pediatric patients with NHL at Children Cancer Hospital of Egypt (CCHE) during the period from July 2007 till end of June 2013 was done. Inclusion criteria included the diagnosis of mature B cell NHL, for whom PET - in addition to conventional CT scan- was done at any stage of the treatment. Blind revision of all PET and CT scans was specifically done for this study.
Results: For 115 pediatric NHL patients, 152 PET and 152 CT scan were done. Median age was 5.7 years (range 1-18 years). They were 85 males (74%) and 30 females (26%). One hundred twenty six scans (82.9%) were done for 100 Burkitt lymphoma patients, while 26 scan (17.1%) done for 15 DLBC. Nineteen examination (12.5%) were done before starting chemotherapy, 107 (70.3%) at time of evaluation while 26 (17.1%) during Follow up. For all patients, sensitivity was 91.6% for PET, while was 70.0% for conventional CT (p=0.02). Specificity was 84.1% for PET and 58.9% for CT (p< 0.001). PPV for PET was 50%, while was 22% for CT scan (p<0.001). NPV for PET was 98%, while was 92% for CT (p=0.01). In burkitt lymphoma; sensitivity of PET was 91.6%, while was 66.6% for CT (p=0.08). Specificity was 82.4% and 57.8% for PET and CT respectively (p=<0.001), while PPV and NPV were 35.4%, 14.2, 98.9%, 94.2% for PET and conventional CT (p= 0.0005) (p=0.05) respectively. In DLBCL patients; sensitivity was 85.7% and71.4% for PET and CT respectively (p value=0.31). Specificity was 89.4%, 57.8% for PET and CT (p =0.05). PPV and NPV were 75%, 38.4%, 94.4%, 84.6% for PET and CT (p =0.02) (p =0.18) respectively.
Summary and Conclusions: PET scan is significantly more sensitive than conventional CT in the management of aggressive mature B cell pediatric NHL.

PB1854

EFFICACY AND SAFETY OF DARBEPOETIN-ALFA IN POOR PROGNOSIS DIFFUSE LARGE B LYMPHOMA CELLS PATIENTS TREATED WHIT TWO-WEEKLY DA-EDOCH14

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Background: Erythropoiesis-stimulating agents (ESAs) raised safety concerns about decreased overall survival (when used to maintain Hb>12 gr/dl) and increased venous thromboembolic (VTE) events in cancer patients. The impact of these agents has not been studied in aggressive lymphomas treated with dose-dense immunochemotherapy regimens, a strategy that triplicate the risk of anaemia and transfusion. (Leuk Lymphoma 2011;52:996).
Aims: The aim of the present study was to evaluate the efficacy and safety of darbepoetin alpha (DPA) in a prospective phase 2 study, single centre, of poor prognosis (aaIPI 2-3) diffuse large B-cell lymphoma (DLBCL) treated with the modern infusional regimen DA-EDOCH14-R. (Br J Haematol. 2013 Feb;160(4):510-4).
Methods: From 2008 to 2013, 26 patients (median age 56 years; range 18-70) newly diagnosed poor prognosis DLBCL were treated with 6 cycles of two-weekly DA-EDOCH14-R. These patients were treated with DPA s.c and blood transfusions according to usual clinical practice. Initial DPA dose: 150mcg/week. Increase dose DPA to 300 mcg/week if no response is seen in 6 weeks' time. We stopped DPA when response is seen, used to be 4 weeks after QT. We analyze these risk factors: HBP, DM, DL, smoke, CVC, steroids, cancer, obesity and age >60.
Results: We administered DPA at 25 patients (96%) for anaemia: 14(56%) Hb>11gr/dl, 10 (40%) Hb 9-11gr/dl and 1(4%) Hb<9. Median Hb when we initiated treatment of DPA: 11.4gr/dL. 18 patients (72%) started DPA within the first two months of treatment. Duration treatment median: 3 months. The dose was increased in 5 patients (20%). High incidence of DVP (24% patients) was distributed as follows: 2 VTE, 1 DVP left axillar, 1 DVP femoral popliteal, 1 mammary and jugular left vein, 1 DVP basilica left vein. No relationship was observed between VTE and high Hb level (median Hb: 10.1gr/dl (patients with VTE) *vs* 10gr/dl (patients without VTE), or with risk factors (median: 5(with TEV) *vs* 4(without TEV)). At a median follow up of 40.5 months, 3-year progression-free was estimated and overall survival was 85%.
Summary and Conclusions: Our data suggest that the use of DPA is associated with a low rate of transfusion, but a high incidence of VTE. ESAs don't seem to worse progression-free survival in patients treated with 14DA.EDOCH. ESAs recommendations should be followed in order to minimize thrombotic disease.

PB1855

A REVIEW OF HIGH GRADE NON-HODGKIN LYMPHOMA CARE IN A UNIVERSITY HOSPITAL GROUP IN IRELAND

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Background: High-grade non-Hodgkin lymphoma (NHL) is an aggressive malignancy that requires urgent, intensive management. It is estimated that 60% of patients are cured (Cancer Research UK) so rapid diagnosis and commencement of chemotherapy are crucial to ensure the best possible outcomes. Clinical practice guidelines are systematically developed statements designed to assist practitioners and patients in making decisions about appropriate healthcare for specific clinical circumstances. However, there remains a significant variation in the utility of recommended treatments in Lymphoma (Loberiza et al Leuk Lymphoma 2014).
Aims: In Ireland, data is lacking on how closely non-Hodgkin lymphoma management follows guidelines. This study is the first to address this issue.
Methods: The study was carried out in two university affiliated hospitals in Ireland. 125 consecutive high-grade non-Hodgkin lymphoma patients were identified from the National Cancer Registry of Ireland. Appropriate quality markers were chosen (Wennekes et al, J Clin Oncol 2011) and, in keeping with previous research, 90% adherence was set as the target. Data was obtained through a systematic retrospective chart analysis of paper and electronic records.
Results: Analysis focused on three areas – diagnosis and staging, treatment, and organization of care. Diagnosis and staging markers were generally well adhered to. 92% of patients were diagnosed with an appropriate biopsy, and 98% of patients had appropriate blood tests performed. However, adherence rates for treatment and organization of care markers were much lower. 85% of patients received a DA-CHOP-R or DA-EPOCH-R regimen, and 75% of patients received a histological diagnosis within the 21 day target period. Chemotherapy was commenced within 28 days of diagnosis in 83% of patients. Adherence rates for the recording of standardized information were particularly low. 54% of patients had an International Prognostic Index (IPI) score recorded. Use of the Cheson criteria to report treatment response was minimal.
Summary and Conclusions: These results are in keeping with previously published studies. Management guidelines are being met in 8 of 21 areas. Although guideline adherence rates are similar to international data, more needs to be done to achieve optimal standards of care. Follow-up investigations post-chemotherapy need to be carried out in a higher proportion of patients. Standardised information such as IPI and stage needs to be documented so that patients can be appropriately risk-stratified and treated according to the most up to date evidence. It is also important to document the severity of disease being treated in an institution. Given that some criteria are rarely applied, there is a question about their practicality in clinical practice.

PB1856

DIAGNOSIS AND CLINICAL OUTCOME OF 9 CASES OF NEUROLYMPHOMATOSIS; SINGLE INSTITUTIONAL EXPERIENCE OVER THE 7 YEARS

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Background: Neurolymphomatosis (NL) is an extremely rare neurologic manifestation of no-Hodgkin lymphoma (NHL) in which peripheral nerve infiltration of lymphoma cell is a dominant feature in both clinically and pathologically. We previously reported a high frequency of NL as a relapse disease of intravascular large B cell lymphoma (IVL), although NL could be presented as an initial disease as well as relapsed disease in other types of NHL. In addition, the clinical features and treatment outcomes of NL is largely unknown. However, recent enthusiastic use of PET/CT in lymphoma has facilitated the diagnosis of NL.
Aims: In this paper, we updated our experience on NL at our hospital over the period of January 2007 to February 2014.
Methods: We reviewed the clinical records at the Hematology/Oncology Department of Kameda Medical Center. The diagnosis of NL required the 1) clinical symptoms and neurological examination findings referable to the cranial or spinal nerves, and 2) histological confirmation of malignant lymphoma cells within the peripheral nerve, nerve root/plexus, or cranial nerve or 3) CT/MRI demonstration of nerve enhancement and/or enlargement of peripheral nerve(s) or nerve root that were also demonstrated by the accumulation of FDG by FDG-PET/CT. Patients with stomach limited mucosa associated lymphoid tissue (MALT) lymphoma and leukemic infiltration of peripheral nerve due to acute leukemias were excluded from the study.
Results: Over the past 7 years, there were 581 patients diagnosed as a NHL except for mucosa associated lymphoid tissue (MALT) lymphoma of stomach. Among them, we identified 9 cases (1.55%) diagnosed as having NL. The patients consisted of 2 men and 7 women with median age of 72 years (range; 63-83 years). NL occurred as a part of presenting disease in 2 patients and as a relapse disease in remaining 7. Four patients were presented NL as a relapse disease of intravascular large B-cell lymphoma (IVL), 3 patients were as a concomitant extranodal lymphoma (stomach, ileum, and uterus), and 2 were as a relapse disease of nodal DLBCL. IVL patients showed significantly higher frequency in the development of NL compare to non-IVL patients (4/14 *vs* 5/567, p=0.004). CD5 was positive in 8 cases. Diagnosis of NL was made by neurological findings, enlargement and enhancement of affected cranial or peripheral nerves by MRI, and FDG-uptake of affected nerve demonstrated by PET/CT in all of the patients. Autopsy also confirmed the lymphoma infiltration in one patient. Affected nerve included lumbosacral nerve (4 cases), peroneal nerve (1 case), cranial nerves (2 cases). Cerebrospinal fluid cytology was positive in 4 cases (44%). Six patients developed CNS involvement subsequently. Eight patients received high dose methotrexate (MTX) containing treatment in addition to systemic chemotherapy, and 4 patients additionally received but only 2 patients responded. Four patients additionally received involved nerve irradiation and all of them responded transciently. Five patients died due to NL with median of 5.1 months after NL development and 3 patients still receiving chemotherapy and irradiation.
Summary and Conclusions: NL is a challenging disease with respect to both diagnosis and treatment, but careful neurologic examination and contemporary imaging technique especially PET/CT often detect relevant neural involvement. Although most effective regimen remained undetermined, high dose MTX combined with systemic chemotherapy might benefit in a subset patients.

PB1857

THE SIGNIFICANCE AND OUTCOME OF THE FINDING OF PERSISTENT LOW GRADE UPTAKE ON FUNCTIONAL IMAGING USING 18F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) IN LYMPHOMA PATIENTS

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Background: Functional imaging using FDG-PET is increasingly used to assess the response to therapy of FDG-avid lymphomas. Clinicians generally refer to scans as either 'positive' or 'negative' however nuclear medicine physicians recognise a continuum of uptake, with persistent disease more likely at higher levels of activity. Importantly, the glucose analogue used in this technique is not specific for lymphoma. Recently published studies that used a program of biopsy or serial scanning and patient follow-up suggest poor positive predictive value of FDG-PET scans in some lymphoma subtypes.
Aims: We have analysed the clinical outcome of patients with initially FDG-avid lymphomas treated with curative intent who following completion of treatment had positive yet indeterminate findings at multi-disciplinary team (MDT) meetings in two London teaching hospitals. Our aim was to determine how patients with such findings are managed and to determine the clinical outcome for this cohort of patients.
Methods: We retrospectively reviewed all FDG-PET-CT end-of-treatment scan reports of patients with curable lymphoma subtypes over a seven year period. Cases were selected if the scan reported persistent low grade uptake of uncertain significance. The reports were not centrally reviewed. The patients' clinical course was interrogated for the presence of disease recurrence, results of subsequent FDG-PET scans, whether a biopsy of the area was performed as well as results of any ancillary investigations or review by other specialists.
Results: There were 42 patients (M:F 1.3:1) with a median age of 51 (range 20-76). The scans were performed at end of treatment and at varying time points thereafter. 23% of PET-CT performed were equivocal (80 scans from the 347 end of lymphoma treatment PET-CT scans performed at NPH). Histologies included Hodgkin lymphoma (15), diffuse large B-cell lymphoma (15), Burkitt lymphoma (5), and primary mediastinal B-cell lymphoma (2). After a median follow up of 21 months, 34 of 42 patients with persistent uptake at the end of treatment were alive and disease free. Five patients had died, only one from lymphoma. Seven patients had relapsed (five confirmed by biopsy) and all seven received salvage chemotherapy. Fifteen of 42 patients in this group underwent biopsy- five had confirmed disease. Other biopsy results included inflammatory or necrotic tissue, solid organ malignancy, tuberculosis and a mycetoma. Of the patients who did not undergo a biopsy, serial FDG-PET scanning (median 1 further scan, range 1-5) or additional studies such as MRI or high resolution CT-scanning suggested the following diagnoses: post-treatment inflammatory change (21 patients), thymic rebound (3) and pulmonary infection (3).
Summary and Conclusions: The finding of persistent low-grade uptake on end-of treatment FDG-PET is not an uncommon finding in our MDT meetings. In our series of patients, treated with curative intent, the majority of patients with positive but indeterminate findings did not have a clinical course consistent with persistent disease. The non-specific nature of FDG underlines the importance of careful review of patients with persistent low-grade uptake at the end of treatment, especially where decisions about further toxic therapies are made. We recommend a biopsy prior to further therapy wherever possible. For patients unable to undergo biopsy we suggest a team based approach that considers alternative causes coupled with follow-up scanning. Our study is limited by the fact that a range of lymphoma subtypes and different disease stages have been included.
**FA and SZ contributed equally to the work.*

PB1858

FIRST-IN-MAN STUDY OF 4SC-202, A NOVEL ORAL HDAC INHIBITOR IN PATIENTS WITH ADVANCED HEMATOLOGICAL MALIGNANCIES; (TOPAS STUDY)

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Background: 4SC-202 is a specific inhibitor of protein deacetylases HDAC1, 2 and 3 and lysine specific demethylase LSD1 (KDM1A). Enzymatic inhibition leads to transcriptional repression of Wnt and Hedgehog signaling. 4SC-202 provokes the inhibition of stemness-related properties of cancer cells and affects their viability.
Aims: Patients with advanced hematological malignancies including AML, ALL, CLL, MM, MDS or lymphoma were orally dosed either once daily (QD) or twice daily (BID) from days 1 to 14, or BID continuously, in a 21-day cycle. Dose escalation followed a 3+3 design. Study objectives include safety, tolerability and pharmacokinetics (PK), determination of MTD and DLT as well as optimization of dosing. Biomarker program includes measuring of HDAC inhibition, total lysine acetylation and gene expression profiling. Additionally, the anti-tumor effect of 4SC-202 was evaluated. Dose escalation part is completed and data allow for reliable evaluation. Further patients will be enrolled to establish the MTD at prior dose levels.
Results: 24 patients were treated at 7 dose levels of 25/50/100/200/400mg QD and 200mg BID (N=3, each), and 200mg BID continuous dosing (N=6). Treatment was very well tolerated by heavily pre-treated patients up to the highest dose group. Possibly drug-related AE were less frequent, *e.g.* low-grade GI complaints such as nausea. Higher-grade hematological toxicity *e.g.* leading to treatment changes was not observed. Elevation of liver enzymes was observed at 200mg BID during continuous dosing. Two objective responses (PR & CR, unconfirmed) were observed. 75% of patients went into follow-up treatment due to stable disease. Median time on treatment was 98 days with 3 patients treated > 400 days . Plasma exposure reached one digit µM concentrations over 24h. Modulation of Wnt pathway gene-signature was observed in blood samples, as well as HDAC inhibition and lysine acetylation.
Summary and Conclusions: 4SC-202 could be safely administered up to 200mg BID. Anti-cancer activity could be demonstrated by two patients achieving PR and CR, respectively, and long term stabilization of several patients. The mode of action of 4SC-202 is demonstrated by biomarker response.

PB1859

MANAGEMENT OF RELAPSED/REFRACTORY LYMPHOMA WITH BRENTUXIMAB VEDOTIN : OUR EXPERIENCE IN THE TREATMENT OF YOUNG PATIENTS

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Background: Brentuximab vedotin is a CD30-directed antibody-drug conjugate (ADC), currently approved for treatment of relapsed Hodgkin lymphoma and relapsed anaplastic large-cell lymphoma after front-line chemotherapy.
Aims: In this work, we analyze a successful experience with brentuximab vedotin in the management of relapsed and refractory lymphoma.
Methods: Case 1 : N.A., 21 y.o. male patient, with diagnosis in November 2011 of Systemic anaplastic large-cell lymphoma (ALCL), Stage IIIB, non B non T, ALK-positive, CD30+. In November 2011 he underwent to frontline treatment CHOP/14 x 6 with CR. Then, he underwent to IEV protocol, but, after 2 courses, the patient referred to our Institution for abdominal pain : a PET/CT was performed documenting a relapse. So, he switched to DHAP, with an apparent improvement, but, one month after the first course there was a worsening of clinical conditions. Then, for progressive disease, there was a switch to Brentuximab treatment. After first course, the patient went to Emergency Medicine for respiratory problems, and, after the solution, we continued the protocol. The treatment was well tolerated, and, after 6 courses, the patient is in CR and he undergoes, in November 2013, to Allogenic BMT. Now, after 4 months, the patient has no signs of disease. Case 2 : T.A., 35 y.o. female patient, with diagnosis in February 2011 of classic nodular sclerosis Hodgkin's Lymphoma, Stage IIB, with mediastinal bulky disease. Frontline treatment was ABVD, 12 courses, with the result of a PR. After, Radiotherapy was started on mediastinal mass, remaining in SD. After 3 months, there were new localizations documented from PET/CT and IGEV protocol was started, total of 3 courses, after which she underwent to AutoBMT in September 2012. PET/CT performed in February 2013 documented a progressive disease : Brentuximab was started and even if after 3 courses the patient was in SD, after 6 courses there was a PR, which became CR after 12 courses. The treatment was well tolerated, and the only documented side effect was alopecia. Case 3 : G.R., a female 30 y.o., with diagnosis in April 2004 of classic nodular sclerosis Hodgkin's Lymphoma, Stage IIB, with splenic localization. Frontline treatment was VEBEP, 11 courses, with the result of a PR. Then, Radiotherapy was started but a relapse of disease was documented in May 2005. Then, programming a AutoBMT procedure, IGEV treatment was started, 3 courses and then she underwent to AutoBMT with a CR. In September 2006 PET/CT documented the second relapse : MOPP protocol was started (12 courses), until April 2007, when a PR was documented. In October 2007 a third relapse of disease was documented, treated with Gemcitabine, with a SD. The patient underwent to DHAP-R treatment, 4 courses, with the result of a PR. So, in October 2009, the patient started Rituximab maintenance, until July 2012, when it was interrupted for progression of disaese. In that moment the general condition were bad and the patient was not anymore able to walk. In that moment she switched to Brentuximab treatment and after 3 courses there was a nCR and after 6 courses she was in CR. The only side effect was a Grade 1 Neuropathy, which was documented only in the first three courses.
Results: Brentuximab Vedotin has been proved to be effective either in relapsed/refractory Hodgkin's and Non-Hodgkin's Lymphoma as single agent, at the dose of 1.8 mg/kg.
Summary and Conclusions: In our experience, Brentuximab Vedotin can be considered an effective option for advanced patients, relapsed and refractory to almost all available therapeutic resources, and moreover it could be considered as a bridge-treatment to AlloBMT.

Stem cell transplantation - Experimental

PB1860

STIMULATION OF TOLL-LIKE RECEPTOR 2 IN ALLOREACTIVE T CELLS PROVIDES RESISTANCE AGAINST CALCINEURIN INHIBITOR

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Background: Toll-like receptors (TLRs) play a fundamental role in innate immunity through their capacity to recognize pathogen-associated molecular patterns. Also, TLRs have been shown to play an important role in T cells as costimulatory receptors. However, the significance of TLR expression in allogeneic T cells remains unclear.
Aims: we investigated the role of TLR2 signal in allogeneic T cell responses and its function to resist against calcinurine inhibitors (CNIs).
Methods: Mixed lymphocyte reaction (MLR) cultures were set up with CFSE-labelled naïve Balb/c T-cell responder and B6 TLR2 KO splenic stimulator per well in the presence or absence of TLR2 agonist Pam_3CSK_4 (PAM). The MLR cultures were treated with various concentrations of CNIs (CsA and FK506) and determined the resistance through proliferation and cytokine levels. Purely isolated naïve T cells were stimulated with soluble anti-CD3 in the presence or absence of PAM and determined intracellular signaling pathway.
Results: Stimulation with TLR2 agonist promoted alloreactive T cell proliferation and survival. IL-2 and IFN-g production were also significantly increased by TLR2 ligation. In the condition of high dose CNI treatment, the activation of allogeneic T cells was prevented in either TLR2 stimulation or not. However, as the lower dose of CNI treatment, TLR2 stimulation led proliferation of allogeneic T cells but not without TLR2 stimulation. We found that TLR2 stimulation enhanced TCR-mediated IL-2 production through PI3K/AKT signaling which brought CNI-resistance.
Summary and Conclusions: Our results demonstrate that TLR2 stimulation directly enhances allogeneic T cell responses and provides the resistance against CNIs. Our study suggests that TLR2 signal might be responsible for meek efficacy of CNIs on GVHD prophylaxis.

PB1861

THE SPECIAL PHENOTYPIC CHARACTERISTICS OF IMMUNE RECONSTITUTION AFTER CORD BLOOD TRANSPLANTATION

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Background: Cord blood transplantation (CBT) is being increasingly used for treatment of hematological malignancies because it's efficacy in the treatment of adult patients and showed many advantages. The immaturity of T cell contained in the graft and the T cell reconstruction is later than bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT), but the relapse rate after CBT to be comparable to that BMT or PBSCT from HLA matched sibling donors. Our clinical analysis revealed the outcome of CBT for high risk acute leukemia relapse after HLA-mismatched CBT was lower than that BMT or PBSCT, but the incidence of graft-*versus* host disease (GVHD) was similar. These clinical findings suggest that immunocompetent cells other than T cells may mediate the GVL effect after CBT. Natural killer (NK) cells play a major role in the development of GVL effect after an HLA-mismatched stem cell transplantation. The GVL effect by NK cells depends on the presence of cell recovery and this coincident with NK cells' characteristics of reconstruction. Because the recovery of NK cells promptly after CBT, so it is probably that NK cells may be more likely to contribute to the development of GVL effect.
Aims: Our purpose is to explore the characteristics of NK cells' reconstruction after CBT by detecting NK cells and their related functional phenotype on their subsets in this research.
Methods: Twenty-six CBT patients and eighteen BMT were studied in this research. We detected the expression of CD11b, CD27 and CD57 on $CD3^-CD16^+CD56^+$NK and their subset by multicolor flow cytometry at the different time after transplantation. Data were summarized as mean±SD. Student t test was used to determine whether there was a statistically significant difference between samples, with two-tailed P values less than 0.05 indicating a significant difference.
Results: 1) In general, the frequency of $CD3^-CD56^+CD16^+$NK cells in lymphocytes was below in CBT receptors compared with BMT, but the absolute number was below only on 1 , 6, 9 month. 2) The proportion of $CD3^-CD56^{bri}CD16^+$NK in CBT group was higher than that of BMT group. 3) The expression of CD11b on $CD3^-CD56^{dim}CD16^+$NK subset in CBT group was

higher than the BMT group only in the early 2 months, the expression of CD27 was higher than the BMT in 4 to 9 months, and the CD57 expression was lower than that of BMT group in 1 to 9 months. 4) The $CD3^-CD56^-CD16^+$NK in CBT group were higher than BMT group after 3 months, and the absolute number exceeds BMT group starting from the 2nd months. Compared with $CD3^-CD56^{dim}CD16^+$, $CD3^-CD56^-CD16^+$ was slightly lower in CD11b higher in CD27 and lower in CD57. The expression of CD11b and CD27 on $CD3^-CD56^-CD16^+$NK subset in CBT group had no significant difference with BMT group while the expression of CD57 was lower than BMT group in 2 to 9 months.

Summary and Conclusions: The frequency of $CD3^-CD56^+CD16^+$NK cells in lymphocytes was below in CBT recipients in the early stage after transplantation compared with BMT and there had no difference after two years, but the absolute number of $CD3^-CD56^+CD16^+$NK cells was not coincidence fully. The expression of CD11b on $CD3^-CD56^{dim}CD16^+$NK subset were higher in the early phase (<2M) after CBT suggest an increase in their strong ability of killing. The expression of CD27 or 57 on $CD3^-CD56^{dim}CD16^+$NK in CBT group showed they were secreting type and more immature in the early phase. This group of cells maybe have lower cytotoxicity but secrete plenty of cytokines. A marked increase in $CD3^-CD56^-CD16^+$NK cells in the peripheral blood was observed in the receptors after CBT. This NK subset showed more immature but was similar to $CD3^-CD56^{dim}CD16^+$NK cells in cytotoxicity or cytokine phenotype. Take together, our results suggest the special characteristics of NK cells in their unique subset and phenotype may contribute to enhance their GVL effect after CBT.

PB1862

COMPARATIVE ANALYSIS BETWEEN CORD BLOOD FROM MAIN BAG AND FROM ATTACHED SEGMENT AFTER THAWING: ATTACHED SEGMENT HAS HIGHER CD34+ CELLS AND CFU-GM THAN THE MAIN BAG AFTER THAWING

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Background: A contiguous segment attached to the main bag of cryopreserved cord blood (CB) unit is used in release test for verifying HLA types, cell viability, and, possibly, hematopoietic potency before hematopoietic stem cell transplantation (HSCT), because CB of attached segment is considered to be representative of CB of main bag used in HSCT. However, little is known regarding the characteristics of contiguous segments in comparison with main bag of CB units, due to the difficulty in accessing a large number of cryopreserved CB units.

Aims: We evaluated whether CB from segment is representative of CB from main bag by comparing various CB parameters measured after thawing of a large number of cryopreserved CB units.

Methods: We used the 245 CB units rejected from the Seoul Metropolitan Government Public Cord Blood Bank inventory due to inappropriate test results after conventional processing. After thawing of the cryopreserved CB units, the numbers of total nucleated cells (TNC) and CD34+ cells, cell viability, apoptotic cells, colony-forming unit-granulocyte/macrophage (CFU-GM), and CFU-granulocyte/erythrocyte/macrophage/megakaryocyte (CFU-GEMM) were examined in both CB from main bag and CB from segment, respectively. We conducted the comparative and correlation analysis for identifying the statistically significant differences and correlation in the results between the CB from main bag and the CB from segment.

Results: The number of TNC in CB from main bags was significantly higher than that in CB from segments ($P<0.001$); in contrast, the number of CD34+ cells was significantly higher in CB from segments ($P<0.001$). In addition, the viability of TNC (determined by trypan blue and 7-AAD) and the viability of CD34+ cells (determined by 7-AAD) were also significantly higher in CB from segments ($P<0.001$, $P<0.001$, and $p=0.005$, respectively). While the proportion of apoptotic TNC was significantly higher in CB from segments ($P<0.001$), there was no significant difference in the proportion of apoptotic CD34+ cells ($p=0.105$). There was no significant difference in the number of CFU-GEMM between CB from main bags and segments ($p=0.990$); however, the numbers of both total CFUs and GM-CFUs were significantly higher in CB from segments ($p=0.017$ and $p=0.005$, respectively). All of the numbers of TNC, CD34+ cells, total CFU, CFU-GEMM, and CFU-GM in CB from segments showed significant correlations with the respective parameters in CB from main bags. While a significant correlation was observed between the viability of TNCs in the two parts of the CB units, no correlation was observed between the viability of CD34+ cells in the two parts of the CB units. There were significant correlations between the proportion of apoptotic TNC and CD34+ cells in the two parts of the CB units.

Summary and Conclusions: CB of segment has higher numbers of CD34+ cells and CFU-GM and lower number of TNC than CB of main bag, although the main bag and segment of cryopreserved CB units are highly correlated with each other. Therefore, it is concluded that CB from segment could serve as a source for quality evaluation of the cryopreserved CB units before HSCT. We expect that the results of our study are useful not only for interpreting the release tests of CB units but also for assisting the selection of high-quality CB units suitable for HSCT.

Stem cell transplantation - Clinical

PB1863

ANTIFUNGAL PROPHYLAXIS WITH FLUCONAZOLE IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Invasive fungal infection (IFI) is a potentially life-threatening complication of patients undergoing hematopoietic stem cell transplantation (HSCT). In several published papers fluconazole (FCN) proved to provide an excellent yeast-active prophylaxis in autologous and low-risk allogeneic HSCT recipients.
Aims: In order to decide if a systematic change to one of the new agents was necessary, we retrospectively analyzed the tolerability, safety and efficiency of primary prophylaxis with FCN in the last HSCT recipients in our center.
Methods: During the period January'08-November'13 we have performed 422 HSCT in 382 pts (217 male, 165 female) with a median age of 50 years (1-73). The patients' baseline diseases were: monoclonal gammapathy (41%), lymphoproliferative disorders (33%), acute myelogenous leukemia/ myelodysplastic syndrome (12%), acute lymphoblastic leukemia (6.5%), chronic myelogenous leukemia/myeloproliferative syndrome (1.5%), and others (6%). Distribution according to type of transplantation was: 278 auto-HSCT cases and 144 allo-HSCT (53% from unrelated donors and 47% from family donors,). A variety of conditionings were employed, 21.5% of which were RIC regimens. All patients with adequate liver function tests (defined as transaminases lower than three times the normal values) received FCN at 400 mg/day (or equivalent doses from children) from the beginning of the conditioning until day +30 (in the auto-HSCT setting) and until day+75 (in allo-HSCTs without prolonged neutropenia [> 2 weeks] or steroid therapy for graft *versus* host disease). The route of administration of FCN was oral or intravenous, depending on availability in each specific moment. In cases with history of proven or probable IFI, antifungal prophylaxis was started with a mould-active drug.
Results: 18 patients (4.2%) with a history of proven or probable IFI received secondary prophylaxis from the begging of the conditioning. In the rest of the cases (404; 95.8%) FCN was administered from the admission as scheduled. Of those, 334 (82.7%) tolerated FCN at 400 mg/day (or equivalent doses from children) during the scheduled period of time. Fifty-five cases (13.6%) presented with mild liver function tests alterations, which were easily overcome by reducing FCN dose to 200 mg/day and/or by discontinuing several doses of the drug. In only 15 cases (3.7%) FCN had to be definitively discontinued due to abnormal liver function tests (attributable or not to the azol), while antifungal prophylaxis was performed with an alternative drug. Peri-transplant mortality occurred in 14 cases (3.3%); causes of death included infections (60%), cardio-vascular complications (26%), and sinusoidal occlusive syndrome (1%), among others. No cases (0%) of invasive yeast infection occurred in our series.
Summary and Conclusions: Our study shows that administration of fluconazole at 400 mg/day (or equivalent doses from children) is a well tolerated, safe, and cost-effective approach for antifungal prophylaxis in autologous and low risk allogeneic and hematopoietic stem cell transplantation.

PB1864

DIFFUSE LARGE B-CELL LYMPHOMA WITH BONE MARROW INVOLVEMENT: RESULT'S OF HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELLS TRANSPLANTATION

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Background: Bone marrow involvement occurs in 10-20% of cases of diffuse large B-cell lymphoma (DLBCL). DLBCL with bone marrow involvement (DLBCL BM) is characterized by aggressive course and poor response to standard chemotherapy (CT). The worst results were obtained for the group of patients with concordant bone marrow involvement (CBM) 5-year overall survival (OS) rate of less than 10%. Application of the modified NHL-BFM-90 program in treatment of DLBCL BM patients (pts) didn't improve results. In the majority of cases we observed long-term persistence of the tumor clone in the bone marrow according to the study of B-cell clonality in complete regression of the rest of the tumor mass. Data of the efficacy of high-dose sequential chemotherapy (sHDT) with autologous stem cells transplantation (ASCT) to eradicate residual tumor in the bone marrow is limited and contradictory.
Aims: To evaluate the efficacy of the mNHL-BFM-90 program with sHDT and ASCT in the treatment for patients with DLBCL BM.
Methods: Since Feb 2005 18 untreated DLBCL BM pts (the median age was 44 yrs (23-58), the M/F ratio 2,6. IPI: IH 39%, H 61%; bone marrow involvement: concordant 44%, discordant 56%) were enrolled in the mNHL-BFM-90 with sHDT with/without ASCT treatment program. All the patients underwent 6-8 courses of polychemotherapy (PCT): 6 blocks of mNHL-BFM-90 chemotherapy program or 4 blocks of mNHL-BFM-90 chemotherapy program plus sHDT. sHDT was carried out, if complete remission (CR) wasn't achieved after 4 blocks. sHDT scheme included 1 DHAP course and 3 high-dose courses with the hematopoietic stem cells (HSC) mobilization and collection: cyclofosfamide 4 g/m^2 day 1, methotrexate 8 g/m^2 day 15 and vincristine 2 mg day 15, etoposide 500 mg/m^{2}q 12 hrs days 31–34. In cases of sufficient quantity of collected CD34+ cells (≥ 2x10^6/kg), the BEAM conditioning and the ASCT were applied. Due to unsufficient HSC collection, patients completed the therapy after the sHDT or one additional Dexa-BEAM course was carried out. The rates of overall survival (OS) and relapse free survival (RFS) were estimated by using the Kaplan-Meier method. Statistical analysis was done using JMP ver. 10.0 (SAS, Cary, NC).
Results: CR was achieved in all patients except one, who died after 3 PCT courses from infectious complications. Mortality associated with toxicity was 6%. In 7 cases of sufficient quantity of collected CD34+ cells, the BEAM conditioning and the ASCT were applied. The average amount of harvested HSC was 3,7x10^6/kg (range 2-7,2x10^6/kg). There were 2 pts in this group with relapse after 6 and 68 months of remission, with CBM both. Furthermore there was 1 course of Dexa-BEAM was applied in 4 of the 10 patients who did not have the sufficient amount of collected HSC after induction therapy (relapse occurred in 1 patient with DBM after 7 months of remission). In 6 of the 10 patients, where a sufficient number of CD 34 + cells was not collected , the therapy was accomplished after 6-8 courses PCT (recurrence in this group is not). At the median of 49 months (range 5–109) disease free survival was 88%±8%, and the overall survival was 79%±11% (Figure 1). In the group with concordant bone marrow involvement 6 out of 8 patients remission retained, meanwhile the observation time points were from 11 to 77 months.

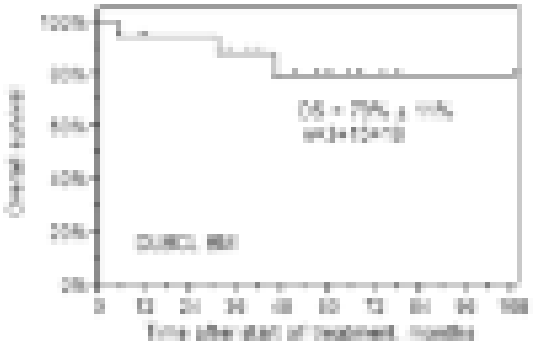

Figure 1. Time after start of treatment, months.

Summary and Conclusions: Bone marrow involvement in patients with DLBCL is an indication for high-dose chemotherapy. Long-term persistence of the tumor clone in the bone marrow necessitates prolonged treatment time. Application of high-dose PCT limited the mobilization and collection of HSC. sHDT with consolidation (ASCT or Dexa-BEAM course) is highly effective in achieving eradication of residual tumor in the bone marrow, after conducting an intensive induction program mNHL-BFM-90. Encouraging results of treatment obtained in the most unfavorable group of patients with DLBCL BM dictate continued research, increasing the number of patients and time of observation.

PB1865

BENDAMUSTINE IN THE TREATMENT OF HEAVILY PRETREATED PATIENTS WITH LYMPHOID MALIGNANCIES RELAPSING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Allogeneic stem cell transplantation (alloSCT) is potentially curative therapy in advanced lymphoid malignancies. Relapsed disease is the major cause of treatment failure. Treatment options are limited and prognosis is poor with short survival after relapse. Bendamustine is an effective therapy in various lymphoid malignancies, however, there is no data on the safety and expected outcomes in the treatment of relapsed disease after alloSCT.

Aims: To evaluate the feasibility, safety and outcome following bendamustine in the treatment of relapse of lymphoid malignancies after allogeneic SCT.
Methods: The outcomes of all patients (pts) relapsing after alloSCT and given bendamustine were analyzed retrospectively.
Results: Fourteen pts with lymphoid malignancies were given bendamustine for relapse after alloSCT. The median age at treatment was 52 years (21-69), 7 female, 7 male. Diagnoses included aggressive lymphoma (n=5), follicular lymphoma (n=1), CLL (n=1), mantle cell lymphoma (n=1), Hodgkin lymphoma (n=3), multiple myeloma (n=3). The donor was a matched sibling (n=8), matched unrelated (n=4), haploidentical (n=1) or cord blood (n=1). The conditioning regimen was reduced-intensity in all pts. At the time of alloSCT pts had a median of 3 lines of prior chemotherapy (2-6) including an autologous SCT in 11 pts. Pts relapsed in a median of 13 months (1-48) after SCT. Pts were given a median of 1 line of chemotherapy (0-4) and 5 pts were also given donor lymphocyte infusion (DLI) prior to bendamustine. In all, bendamustine was the median 7th line of therapy (range, 5th-10th). Eight pts had chronic GVHD before starting bendamustine. Bendamustine was started a median of 4 months after relapse (1-51). A median of 2 courses of therapy were given (1-6) at a dose of 50-100 mg/m^{2}x 2 per course either as a single agent (n=6) or combined with rituximab (n=4), bortesomib (n=2) or thalidomide (n=2). The overall response rate was 50%, CR (n=2), PR (n=5). Two of 3 pts converted from PR to CR with DLI given adjacent to response. The median duration of response in the 7 responders was 18 months (5-19). Only 2 of the responders subsequently progressed, 5 and 8 months after treatment, both did not achieve CR with bendamustine alone or following DLI. Responses were seen across all histologies including 3 of 5 pts with aggressive lymphoma, 1/1 in follicular lymphoma, 1/1 in CLL, 1/3 in Hodgkin lymphoma and 1/3 in myeloma. Treatment was relatively well tolerated. Four pts had ≥grade 3 neutropenia and 4 pts had ≥grade 3 thrombocytopenia. One pt died of sepsis and multiorgan failure during neutropenia after the first course. There was no significant effect on GVHD in most pts although one pt had worsening of chronic GVHD during treatment. With a median follow-up of 18 months (2-47), 7 pts are alive and 7 died, 4 of progressive disease, 1 of sepsis and 2 of late unrelated causes more than 1 year after stopping treatment while in CR. The median survival after bendamustine was 18 months and the 2 year overall survival (OS) was 37% (95%CI, 6-67). Median survival was 19 months and 6 months in pts responding and not responding to bendamustine, respectively (p=0.07).
Summary and Conclusions: Bendamustine is relatively well tolerated when given for relapse of lymphoid malignancies after alloSCT in a group of very heavily pre-treated pts. It is associated with a relatively high response rate of 50% and some of the responses are durable. Bendamustine treatment may serve as a bridge to immune therapy with DLI, and possibly may be synergistic with this treatment. These observations merit further study in larger prospective comparative studies.

PB1866

INCREASED PREVALENCE OF METABOLIC SYNDROME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Hematopoietic stem cell transplantation (HSCT) is widely used in the treatment of malignant and nonmalignant diseases. An increasing number of long-term survivors recover from their primary disease, but they are at risk of adverse late effects, including metabolic syndrome (MS), which seems to be common in long-term survivors of HSCT.
Aims: Our purpose was to determine the prevalence of metabolic syndrome, a clustering of risk factors associated with cardiovascular disease, among adult survivors with a history of allogeneic HSCT.
Methods: We analyzed our data on 74 patients with a median age at transplant of 35 years (12-63 years), who had been followed for a median of 5 years (range 2-23 years) after allogeneic HSCT. Metabolic syndrome was defined according to the National Cholesterol Education Program's Adult Treatment Panel III criteria by the presence of at least three of its five defining characteristics. Body mass index, waist circumference, arterial pressure, and triglycerides, HDL-cholesterol, and glucose levels were recorded at the time of study entry. The Framingham Heart Study's General Cardiovascular Disease (10-year risk) calculator for predicting the risk of cardiovascular disease was used.
Results: The prevalence of metabolic syndrome was 40,5% (95% CI, 27,3% - 57,8%) among HSCT recipients, a 2,02-fold (95% CI, 1,5–2,7, P <0.01) increase compared to the general Slovak population. Metabolic syndrome was more common in men (48,7% *vs* 30%), hormone replacement therapy in females may have influenced their cardiovascular risk. The most common MS features were abdominal obesity (96%), hypertriglyceridemia (70%), and a hypertension (76%). The prevalence of metabolic syndrome was 8,8% in the 20–29 years age group; 26,6% in the 30-39 years age group; 36,8% in the 40-49 years age group; 55,5% in the 50-59 years ager group and 88,8% in the 60-69 years age group. The 10-year cumulative incidence of MS was 32,5%. The most significant risk factor for MS was total body irradiation (P =0.03), positive family history (P=0,04) and age > 40 years at HSCT (P <0,01). Chronic graft-*versus*-host disease, corticosteroid therapy and smoking were not statistically significant. Three patients developed cardiovascular complications (coronary artery disease in 2 cases, myocardial infarction in 1 case). The median (95% confidence interval) 10-year general cardiovascular risk score for males was found to be 9,91% (7,82% - 21,3%) and 4,16% (2,73% - 7,28%) for females. Combination of four-five components were present in 41% of patients with metabolic syndrome.
Summary and Conclusions: We report a high prevalence of metabolic syndrome (40,5%) among adult allogeneic HSCT survivors compared to the general Slovak population. Male survivors of allogeneic HSCT have a long term persisting risk of cardiovascular events. We recommend prolonged follow-up for transplant recipients and appropriate treatment of cardiovascular risk factors such as diabetes, hypertension, and dyslipidemia for all HSCT recipients.

PB1867

POST-TRANSPLANT CYTOMEGALOVIRUS INFECTION IN HEMATOLOGICAL PATIENTS RECEIVING AUTOLOGOUS STEM CELL TRANSPLANTATION: IMPACT OF NOVEL THERAPIES

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Background: Routine monitoring of CMV in haematological patients (HP) undergoing autologous stem cell transplantation (ASCT) is not recommended because the low incidence of clinically relevant CMV infection in this setting. The recent use of novel agents such as Bortezomib and Rituximab in pre-transplant treatment of Multiple Myeloma (MM) and non-Hodgkin lymphoma (NHL) has led to a significantly increased incidence of viral infections. However, few studies have examined the relationship between CMV reactivation and development of complications after ASCT in the era of newer agents.
Aims: The aim of this study was to determine the incidence and clinical impact of post-engraftment CMV reactivations in HP after ASCT at a single institution.
Methods: From 2008 to 2014, 55 consecutive patients underwent ASCT were retrospectively analyzed: 27 MM (all of them received a pre-transplant bortezomib-based regimen), 23 NHL (13 received a pre-transplant Rituximab-based regimen), and 5 acute myeloid leukaemia (AML). The majority (91%) of patients were CMV-positive before ASCT. Routine DNAemia surveillance was performed weekly by PCR assay for at least the first 100 days after transplant. According to local policy, we started oral valganciclovir as pre-emptive therapy in all cases of CMV infection.
Results: Overall, 10 patients (18.2%) developed CMV infection at a median of 27.8 days (range 3 to 44) post-transplant. Patients with MM had a slightly higher CMV infection rate (6 cases; 22.2%) after ASCT than patients with NHL (4 cases; 17.4%) and AML (0%) (Table 1). All 6 patients with MM and CMV infection remained asymptomatic with moderate levels of DNAemia after pre-emptive therapy using oral valganciclovir. Interestingly, among the 4 patients with NHL and CMV infection, 2 patients (both of them had received pre-transplant Rituximab) developed graft failures. Both cases were rescue with hematopoietic growth factors and a second hematopoietic stem cell infusion, respectively.

Table 1. Patient characteristics and incidence of post-ASCT CMV infection.

Summary and Conclusions: 1) Overall, our CMV infection incidence was lower to that reported in the literature (50-60%); 2) but even so, MM patients treated with bortezomib-based regimens are at higher risk of CMV infection after ASCT as compared with other types of treatment (1%); 3) CMV-related graft failure can occur in the rituximab-treated patients; and 4) CMV surveillance strategy should be considered, especially in patients with pre-transplant Rituximab treatment.

PB1868

DEVELOPMENT AND APPLICATION OF A DOSE INDIVIDUALIZATION PLATFORM FOR INTRAVENOUS BUSULFAN IN GREEK HOSPITALS

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Background: Busulfan (BU) is widely used as an alternative to total body irradiation (TBI), in the preparative regimens before hematopoietic stem cell transplantation (HSCT). Since the introduction of the i.v. formulation, two dosing schemes have been developed the 0.8 mg/kg every 6 hours for 16 doses that was safer [4] than per os administration and the 3.2 mg/kg once daily for four consecutive days that was the safest. However, both i.v. and oral BU administration present a narrow therapeutic range (TR) and are associated with veno-occlusive disease (VNO) or graft failure. As a consequence they require dose individualization.
Aims: The aim of this study is to develop a BU dose individualization platform applied in two Bone Marrow Transplant Centers (BMTC) as a pilot. The ultimate plan is to form a nationwide network for BU individualization that will include five BMTCs in Greece and a central Pharmacokinetic Center (PKC). The platform consists of a Standard Operating Procedure (SOP) for collecting and handling blood samples at the Hospital site and, a low-cost validated HPLC-UV bio-analytical method for BU measurement and a procedure for dose modification for the PKC.
Methods: A new low-cost HPLC-UV bio-analytical method for BU quantification in small plasma volume was developed and validated. The method was applied to measure BU plasma levels in blood samples taken from 15 patients. Nine were administrated a 3 h i.v infusion of a once daily dose of 3.2 mg/kg of BU (BMCT, Rio General Hospital, University of Patras) and six were administrated a 2 h i.v infusion of a 0.8 mg/kg dose four times daily (BMCT, "ATTIKON" Hospital, University of Athens). Three blood samples were collected from each patient according to a SOP for blood sampling and handling at the hospital site developed within the framework of the proposed BU individualization platform. BU dose was individualized after BU plasma level quantification using AUC (Area under the Plasma Concentration-Time curve) as the PK parameter of choice as indicated by the manufacturer in the SPC of the BU marketed product Busilvex, assuming linear pharmacokinetics and applying the trapezoidal rule.
Results: The HPLC-UV bio-analytical procedure of Bu was developed for small plasma volume. The intra-day accuracy and precision ranged between 90.3-90.6% and 3.9-7.25% respectively, while the inter-day accuracy and precision ranged between 98.2-99.2%, and 1.9-4.0% respectively. The limit of quantification is 40.7±4.00% ng/mL. The SOP procedure describes in detail the blood sampling scheme, sample preparation, storage and the shipment from BMCT to PK center and was deemed necessary to ensure the smooth collaboration of the sites. Two out of the six patients who were administrated 0.8 mg/kg were found above the TR, while out of the nine who were administrated 3.2 mg/kg, 2 were found below the TR and one above the TR. Dose was corrected for all patients found outside the TR and BU plasma levels were measured again after dose correction. All individualized patients were measured within the TR after dose correction
Summary and Conclusions: A BU individualization procedure was developed and applied successfully in two BMTCs. The present study constitutes a pilot study for further application to all five Greek BMTCs one of which is for children. Thus a nationwide network for the individualization of BU will be formed. Further plans are to expand application to oral BU using a Bayesian method with population pharmacokinetic model.

PB1869

SECOND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION DUE TO GRAFT FAILURE OR HAEMATOLOGICAL MALIGNANCY RELAPSE FOLLOWING THE FIRST STEM CELL TRANSPLANTATION

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Background: Hematological malignancies relapsing after allogeneic hematopoietic stem cell transplant (HSCT) have a poor prognosis. A 2ndHSCT offers an opportunity for cure, but with a high rate of treatment failure and transplant related mortality (TRM). Graft failure is uncommon and can be overcome by additional HSCT, however lack of prospective studies makes difficult to establish basic principles.
Aims: The aim of this study is to analyse the outcome of these patients to identify factor predicting survival.
Methods: Between 1996 and 2013, 595 patients have received an allo-HSCT in our center. 32 of them underwent a 2nd allo-HSCT due to hematological disease relapse or graft failure. Statistical analysis was performed with SPSS v.20.
Results: Median age at 2nd HSCT was 39 years (range 14–65). Transplant was performed due to disease relapse in 28 cases (47% AML/MDS, 29%ALL, 3%MCL, 3%CLL, 3% NHL and 3%MM), and graft failure in 4 (3% ALL, 3% AML and 6% aplastic anaemia). Median time between 1st and 2ndHSCT was 13 months (1-72). In the group transplanted due to relapse, 92% patients received chemotherapy (alone or plus radiotherapy and/or donor lymphocytic infusion). Disease status includes complete remission (CR) in 41% of patients (22%: positive residual minimal disease –RMD-), active disease in 30% and with aplastic bone marrow in 26%. Overall, 65% patients had active disease at the 2ndHSCT (morphological evidence or positive RMD). Same donor was employed in 44% (72% sibling donor). Conditioning regimen was myeloablative in 37%. 97% received peripheral blood progenitor stem cells. Graf-*versus*-host disease prophylaxis in 2ndHSCT was cyclosporine alone (13%) or plus MMF (6%) or methotrexate (32%); or tacrolimus (plus rapamicine 16% or methotrexate 6% or MMF 3%). Incidence of acute and chronic graft-*versus*-host disease (aGVHD/cGVHD) was 59% (18% III-IV) and 47% (6% severe). 44% of cases had cytomegalovirus reactivation (CMV), 28% fungal infection (FI), 6% microangiopathy (TMA) and 13% veno-occlusive disease (VOD). With a median follow-up of 53 months (1-111), estimated overall survival (OS) and progression free survival (PFS) at 6 months, 1 year and 5-10 years was 52%, 32% and 21%, and 33%, 23% and 18%. All except 1 alive patients remain in CR. Estimated TRM is 20%, and TRM at day +100 is 6%. The main cause of death was progression disease (n=18). Factors significantly associated with a better OS were: graft failure as cause of 2ndHSCT (not reached *vs* 6 months; p=0.00), not active disease (23 *vs* 5 months; p=0.03), no development of FI (11 *vs* 4 months; p=0.00) or TAM (7*vs* 2 months; p=0.05), development of cGVHD (28 *vs* 7 months; p=0.043) and achieving CR as best response (8 *vs* <1month; p=0.00). No development of aGVHD in the 1stHSCT was the only factor associated with present cGVHD at the 2ndHSCT. A better PFS was associated with: not active disease (16 *vs* 4 months; p=0,015), no FI (6 *vs* 2 months; p=0.09) and achieve CR (5 *vs* <1 month; p=0.00). Considering only patients transplanted due to relapse, they had better OS and PFS when the interval higher than 1 year from 1st to 2nd HSCT (7 *vs* 2; and 5 *vs* <1 month p=0.00).
Summary and Conclusions: Patients with graft failure should be considered for a 2nd HSCT. In relapsing diseases, although TRM is acceptable, most of the patients will die due to disease. New agents improving quality of remission before transplant and perhaps maintenance after the transplant are needed.

PB1870

LIVER IRON CONCENTRATION AND STIFFNESS AND ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION OUTCOMES

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Background: Elevated ferritin levels were reported associated to adverse outcomes following hematopoietic cell transplantation (HCT). It is not clear if elevated ferritin level is cause of iron overload or infectious or inflammatory concomitant diseases.
Aims: To better clarify the role of iron overload in the outcome of the HSC we designed a study where we measured Liver Iron Concentration (LIC) and Liver Stiffness (LS) by non invasive methods, in patients underwent allogeneic HCT
Methods: We evaluated the LIC and LS in 52 patients who underwent an HCT, because of haematologic malignancies.The patients (pts) had a serum ferritin (SF) level > 500ng/ml or received transfusion therapy with more than 20 U of RBC. LIC was assessed by MRI-T2* or Magnetic Iron Detector (MID). LS was assessed by Fibroscan® and converted in Metavir hepatic fibrosis score. Patients were followed for 3 years after transplantation.
Results: Median age was 40 yrs (range:14-71), 1 pts resulted not iron overloaded (LIC <2.5 mg/g). Among patients with iron overload, median LIC was 7 mg/g (interquartile range, IQR: 4.8-11.0), 37 patients (73%) had mild LIC (2.5-12mg/g), 14 patients (27%) had severe LIC (>12mg/g). Metavir was assessed in 42 pts and score resulted F0 in 13 pts, F1 in 20 pts, F2 in 2 pts, F3 in 4 pts, F4 in 3 pts. Median ferritin level was 2209 ng/ml (IQR: 1159-4114). No statistical significant associations were found between the level of LIC and survival, probability of organ failure, acute or chronic GVHD. A positive statistical significant relationship between LS and the presence/degree of GVHD was found, both using the Metavir categorical score (p=0.002) and the liver stiffness continuous measurement (p=0.03); this association was confirmed in a multivariate logistic model, adjusting for age and sex. The levels of LIC, LS and ferritin were not correlated (pairwise Spearman's rho p≥0.3).
Summary and Conclusions: Level of Iron overload does not play a role in the outcome of HCT. Degree of Stiffness is associated with the presence and degree of GVHD

PB1871

A LONG-TERM FOLLOW-UP STUDY ON HEPATITIS B SURFACE ANTIGEN POSITIVE PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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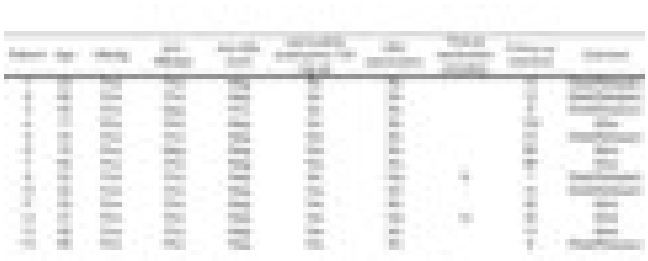
Background: Hepatitis B virus (HBV) infection is one of the most common viral infections worldwide. Reactivation or exacerbation of HBV infection is a serious and potentially fatal complication of immunosuppression. Amongst hematology patients with past exposure to HBV, patients who have received high dose chemotherapy or immunosuppressive therapy can develop HBV reactivation during and after having applied the therapy. Various retrospective studies have identified that patients receiving hematopoietic stem cell transplantation are at a particularly high risk of reactivation.
Aims: The aim of this study is to evaluate the hepatic events and HBV reactivation among HBsAg positive patients undergoing stem cell transplantation.
Methods: Over a twenty year period time (1993-2013), total number of patients who received hematopoietic stem cell transplantation is 398, at Istanbul University Cerrahpasa Medical Faculty. Of those 398, 42 were allogenic and 356 were autologous. The median age at transplantation was 38 years (ranging between 15 and 65). Twenty out of 398 transplant recipients showed active Hepatitis B infection at baseline (HBsAg positivity). 6 out of 20 received allogenic and remaining 14 underwent autologous stem cell transplantation.
Results: The prevalance of HBsAg positivity was 5.02% of total number of our patient population which is 20. HBV reactivation was detected in 3 patients.
Among allogenic transplant recipients, 4 of them received lamivudine propylaxis during and after transplantation process. One patient had HBV reactivation, six months after the transplantation. This patient did not receive lamivudine and died soon due to the fulminant liver failure. 9 out of 14 HBsAg positive autologous transplant recipients, lamivudine propylaxis has been given. HBV reactivated in 2 of those receipients; at 6 and 12 months following the transplantation. One of them died one month later due to septic shock, other one is still alive even 36 months after transplantation. Remaining allogenic and autologous transplant recipients neither experienced major hepatic event nor HBV reactivation (Tables 1 and 2).

Tablel 1. Clinical details of hepatitis B surface antigene postive autologus transplant recipients.



Tablel 2. Clinical details of hepatitis B surface antigene postive allogenic transplant recipients.

Summary and Conclusions: The risk of fatal HBV liver disease among patients who are persistently HBsAg positive after transplantation is approximately 12% according to the literature. While transplantation is frequently avoided or delayed in candidates with abnormal serum aminotransferases, a finding of positive Hepatitis B surface antigen (HBsAg) alone is not considered a contraindication and does not confer an increased risk for VOD. Even in patients with very low levels of viral replication before transplantation and relatively normal liver function and histology, the impaired cellular immunity seen in the first 3 to 6 months after transplantation can result in HBV reactivation. Our retrospective analysis present an experience from a hepatitis prevalence high country. However, definitive conclusions cannot be drawn from these results beyond recommendations to monitor all hepatitis carrier status hematopoietic transplant recipients closely until full immune reconstitution has been established.

PB1872

IS USEFUL TO DETERMINE PRE-TRANSPLANT SERUM FERRITIN LEVELS IN HSCT?

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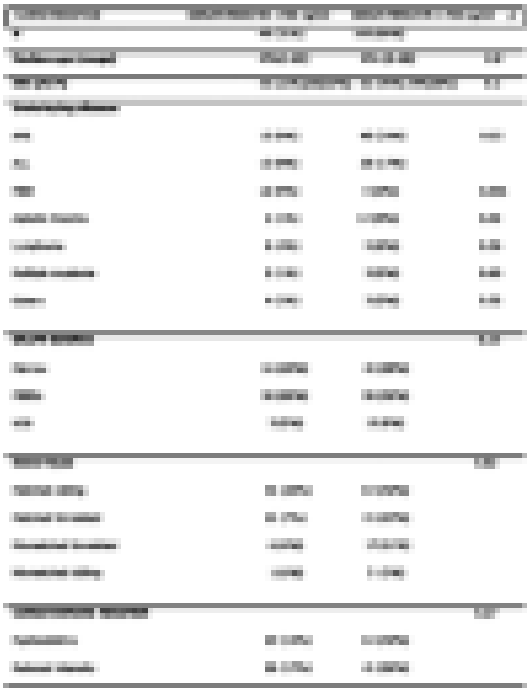
Background: Serum ferritin is an iron overload marker and an acute phase reactant. Several studies have reported associations between elevated ferritin levels and adverse outcomes following allogeneic Hematopoietic Stem Cell Transplantation (HSCT). It is unclear whether the association of elevated ferritin with adverse survival is primarily due to iron overload or to the presence of other infectious or inflamatory conditions in which ferritin might act as an indicator.
Aims: The aim of our study was to analyze retrospectively the pre-transplant serum ferritin levels in patients undergone allogeneic HSCT in our Hospital from 2005 to 2013. Patients were clasificated according to serum ferritin (< and >700 ng/ml) and they were compared to: overall survival, acute and chronic GVHD, infections (bacterial, fungal and cytomegalovirus), mucositis, interstitial pneumonitis, hepatic veno-oclussive disease, relapse, TRM-100 days and TRM-1 year.

Table 1. Patient demographics and transplant characteristics.



Methods: We have retrospectively analyzed the pre-transplant serum ferritin levels in 154 consecutive patients undergone allogeneic HSCT in our Hospital from January 2005 to October 2013. Patients were classified according to serum ferritin (< and >700 ng/ml) and we have analyzed: overall survival, acute and chronic GVHD, infections (bacterial, fungal and cytomegalovirus), mucositis, interstitial pneumonitis, hepatic veno-oclussive disease, relapse, TRM-100 days and TRM-1 year. Statistical analysis was performed using the SPSS 17.1 program.
Results: The baseline characteristics of these patients are shown in Table 1. In our series 154 patients were undergoing allogeneic HSCT (44% Reduced Intensity Conditioning regimen). The median follow up was 30 months (0-86), and the overall survival was 61±4% at 7 years (<700 ng/ml: 65±7% and >700 ng/ml 60±5%). TRM-100 days was 5% (4 patients in the >700 ng/ml group and 4 patients in the <700 ng/ml group. Mean pretransplant ferritin in the global series was 1114 ng/ml (range: 6-13080). Among patients with ferritin >700 ng/ml (105 patients) the mean ferritin was 1554 (704-13080) and <700 ng/ml (49 In patients with pre-HSCT ferritin levels >700 ng/ml we observed more incidence of infections (gram+ bacteremia: n=82 *vs* n=25 (p=0.001) and fungal infections: n=15 (10%) *Vs* n=0 (0%) (p=0.002). Serum ferritin levels >700 ng/ml were

associated with higher incidence of aGVHD N=33 *Vs* n=4 (p=0.001), mainly intestinal aGHVD grade 3-4 13% (n=20) *Vs* 1% (n=2), p=0.001 and higher incidence of chronic GVHD moderate-severe 16% (n=25) *Vs* 4% (n=6) (p=0.035). TRM-1 year was 8% (13 patients) and 4% (6 patients) in the > and<700 ng/ml group respectively. In the group with ferritin >700 ng/ml the TRM-1 year was due to infection (4.5%), GVHD (3%) and others (1.2%). In the group with ferritin <700 ng/ml the TRM-1 year was due to GVHD (2%), infection (1.2%), and others (0.6%). There were no differences in the 1-year probability of overall survival, higher TRM-1year, probability of relapse, Cytomegalovirus infection, mucositis, interstitial pneumonitis and hepatic veno-oclussive disease between goups with serum ferritin levels > or <700 ng/ml. In multivariate analyses, the serum ferritin did not impact risk of overall mortality (p=0.2).

Summary and Conclusions: The cut-off of serum ferritin > 700 ng/ml pre-HSCT is correlated with higher incidence of aGVHD grade III-IV, chronic GVHD moderate-severe and some types of infections, however we found no association between high ferritin levels and allogeneic HSCT outcomes. Future studies should use liver iron content to define iron overload instead of ferritin in this patients.

PB1873

DONOR'S CMV SEROPOSITIVITE, OLDER AGE, AND FEMALE GENDER FOR MALE PATIENT BUT NOT ABO GROUP NEGATIVELY INFLUENCED ON SURVIVAL OF ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS

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Background: Allogeneic hematopoietic stem cell transplant (allo-HSCT) is a potentially curative therapeutic approach for a number of malignant and non malignant disorders. When several comparably HLA-matched family or unrelated candidates are available, various criteria can be used to select a donor.

Aims: The aim of our study was to determine the influence of some of the donor characteristics (gender, age, CMV serologic status, ABO group) on the short and long term outcome of our patients undergoing allo-HSCT.

Methods: We retrospectively analyzed data from 212 patients who consecutively underwent allo-HSCT in our Unit. One hundred and twenty-nine patients were male (60.8%) and 83 female (39.2%). Median age of patients was 46 years old (range: 2-65). 62.3% of patients were CMV sero-positive and 37.7% were sero-negative. Baseline disease was: acute myelogenous leukemia/myelodysplastic syndrome (36.8%), lympho-proliferative disorder (18.4%), monoclonal gammapathy (16.5%), acute lymphoblastic leukemia (15.6%), aplastic anemia (5.7%), chronic myelogenous leukemia (4,2%), and others (2.8%). 64.2% of the allo-HSCTs were from a family donor and 35.8% from an unrelated donor. The SC source was PBSC in 50%, BM in 44.3%, and UCB in 5.7%. A variety of conditioning regimens were employed. Gender of donors was: 117 male (55.2%) and 95 female (44.8%). Median age of donors was 40 years old (range: 6-79). 47.6% of donors were CMV sero-positive and 52.4% were sero-negative. Patient-donor ABO group were major mismatched in 27.4%, minor mismatched in 15.6%, and matched in 57.0%. We investigated the impact of characteristics of the donor on transplant (HSCT-OS), day +100 (d+100-OS) and 1 year overall survival (1y-OS).

Results: CMV- patient/CMV+ donor, male patient/female donor, and older donor were adverse factors for the patients outcome, as shown in the attached Table 1. On the other hand, ABO group mismatched did not affect patients OS (major ABO incompatibility *vs* rest: HSCT-OS= 89.7% *vs* 90.9%; d+100-OS= 87.9% *vs* 84.4%, and 1 y-OS= 70.7% *vs* 63.0%; p=ns).

Table 1.

Summary and Conclusions: Our study shows that, when several comparably HLA-matched donors are available, the selection of CMV negative donors for CMV negative patients, male donors for male patients, and younger donors is an important decision for the patients' short and long term survival. Contrarily, the ABO incompatibility does not seem to be a major factor with regard to the patients outcome.

PB1874

T-LARGE GRANULAR LYMPHOCYTE PROLIFERATIONS IN PATIENTS WITH RITUXIMAB-RELATED LATE-ONSET NEUTROPENIA AFTER AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION FOR LYMPHOMA: POSSIBLE PATHOGENETIC MECHANISMS

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Background: Late-onset neutropenia (LON), an increasingly recognized adverse event of immunotherapy with the anti-CD20 monoclonal antibody Rituximab (R), is currently considered to be an immune-mediated process. Studies from our group have linked R-LON to the outgrowth of cytotoxic T-cell expansions, in particular with a phenotype of a T-large granular lymphocytes (LGL) *i.e.* $CD3^+CD8^+CD57^+$. R-LON develops frequently in patients with non-Hodgkin's lymphoma (NHL) after autologous hematopoietic cell transplantation (AHCT).

Aims: We report 7 patients with NHL presenting with R-LON after auto-HCT in a context of $CD3^+CD8^+CD57^+$ T-LGL lymphoproliferation and explore the possible implication of auto-immune myelosupression by Rituximab.

Methods: These patients concerned 4 males and 3 females, aged 36-58 years, suffering from mantle-cell lymphoma (n=4), follicular lymphoma (n=2) and diffuse large B-cell lymphoma (n=1) who received 5-10 cycles of Rituximab (median 7)±chemotherapy and underwent auto-HCT for either consolidation (n=4) or salvage for relapsed/refractory lymphoma (n=3).

Results: LON grade II-III (median ANC: $0.8x10^9$/l, 0.44-1.05) was observed at a median of 82 days (range, 34-370) after the last Rituximab administration. Two patients received Rituximab pre-HCT and R-LON was documented after engraftment; 5/7 patients developed neutropenia after maintenance with Rituximab for relapsed disease. Neutropenia developed at a median of 266 days (24-1112) after AHCT. R-LON persisted uncomplicated for median 185 days (42-560). Lymphocyte subpopulation analysis during neutropenia revealed (i) T4/T8 ratio<0.7, ii) increased (>1000/μl) T8 lymphocytes [5/7 patients, (mean: 1352/μl, median: 1177/μl)], (iii) increased (>20%) +CD3+CD8+CD57 lymhocytes. T-LGL hyperplasia was present before AHCT in two patients and persisted over 6 months after neutropenia resolution in 4/4 patients. In all patients, T-LGL proliferation was documented long after immune recovery (median 557 days, range: 189-1612). Bone marrow biopsy examination in 6/7 patients revealed: (i) small-to-moderate $CD20^-CD79a^-CD3^+CD45RO^+CD43^+$ (CD3>CD45RO) small lymphocytic infiltration in 3/6 patients; (ii) hypo- or hyperplasia with shift-to-the-left of the granulocytic series in 3/6 patients; and, (iii) pronounced hyperplasia of the erythroid and megakaryocytic series with prominent dyserythropoiesis and dysmegakaryopoiesis, respectively, including abnormal paratrabecular localization, suggestive of myelodysplasia (MDS), however, always with <2% CD34+ cells.

Summary and Conclusions: In conclusion, Rituximab administration in NHL patients undergoing AHCT can be accompanied by lymphocyte imbalances and T-LGL cytotoxic proliferations with variable impact on the hematopoietic marrow. In such cases, neutropenia probably represents the end of a spectrum of T-LGL-mediated autoimmune myelopathy. Further studies are required in order to explore possible implications for the recovery of T-cell compartment in R-LON after AHCT.

PB1875

MAJOR AND BIDIRECTIONAL ABO INCOMPATIBILITY POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: PURE RED CELL APLASIA AND TRANSPLANT OUTCOME

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Background: Approximately 50% of allogeneic hematopoietic cell transplantations (alloHCT) are performed across the ABO system barrier and delayed red blood cells (RBC) engraftment and pure red cell aplasia (PRCA) complicates some, not all of the recipients (6-30%) with major and bidirectional ABO-incompatible transplants. The correlation with the transplant outcome concerning overall survival (OS), graft-*versus*-host disease (GVHD), treatment-related mortality (TRM) and relapse rate (RR) is still a subject of debate.

Aims: The aim of the study was to evaluate the incidence and outcome of delayed RBC engraftment and PRCA, correlation with possible risk factors and the transplant outcome of the pts.

Methods: We retrospectively analyzed the data of all consecutive patients (pts) who underwent an alloHCT during 2001-2011 from major and bidirectional ABO-incompatible donors.

Seventy four alloHCTs were performed in 72 pts suffering from myeloid (34), lymphoid (36) malignancies and aplastic anemia (2). The pts received grafts from major (57) and bidirectional (17) ABO incompatible donors, siblings in 45, not related (MUD) in 27 and double cord blood units in 2 pts, following a myeloablative (MA, 57) and non-myeloablative (NMA, 17) conditioning regimen.
Results: A total of 17 (22.9%) pts were diagnosed with either delayed RBC engraftment (4/17) or PRCA (13/17) with a median of 2 (0-13)% of erythroid precursors in bone marrow after a median time of 31 (20-95) days post alloHCT. The complication was not correlated with granulocyte (p=0.6) or platelet (p=0.1) engraftment delay, the type of donor (sibling *vs* MUD, p= 0.3) or the intensity of the conditioning regimen (MA *vs* NMA, p=0.5). All pts were initially treated with erythropoietin and 10 of them responded. The rest of them (7) were given additionally steroids but this was successful in only one pt. Finally, 6 pts with refractory PRCA were treated with plasmapheresis and all of them responded post 6 (5-11) sessions. None of them experienced a PRCA relapse until last follow-up. With a median follow-up of 33 (1-117) months, the transplant outcome was not found to be significantly different between the delayed red cell engraftment/PRCA pts group and the pts not experiencing red blood cell line delay reconstitution (control group). The primary disease RR was 23% for the PRCA pts group *vs.* 33% for the control group (p=0.4), OS was 64.3% *vs.* 40.4% (p=0.28) and TRM was 23.5% *vs.* 32.7% (p=0.18) respectively. Moreover, the incidence and severity of acute and chronic GVHD was not different in the two study groups (47.16% *vs.* 44.4%; p=0.85 and 70.6% *vs.* 72.7%; p= 0,545, respectively.
Summary and Conclusions: Conclusively in our study, delayed red cell engraftment and PRCA, accounted for 22.9% of ABO major and bidirectional incompatible alloHCT, was successfully treated with erythropoietin and plasmapheresis in refractory cases. It seems that occurrence is not related to the type of transplantation and is not affecting overall survival, TRM, RR or the incidence and severity of GVHD.

PB1876

OUTCOMES OF ESHAP (+/-RITUXIMAB) AS SALVAGE CHEMOTHERAPY FOR RELAPSED OR REFRACTORY NON-HODGKIN'S LYMPHOMA FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: High dose chemotherapy followed by autologous stem cell transplantation (ASCT) is the gold standard treatment for patients with relapsed/refractory Non-Hodgkin's Lymphoma (NHL). Remission status at time of transplant is a significant prognostic factor because patients who do not achieve chemosensitivity after salvage therapy have a poor outcome after ASCT. An optimal salvage therapy should combine therapeutic activity without compromising stem cell mobilization potential and currently there is no proven standard for salvage chemotherapy regimen.
Aims: To analyse the outcomes of ESHAP (+/-Rituximab) as salvage chemotherapy for relapsed or refractory Non-Hodgkin's Lymphoma followed by autologous stem cell transplantation.
Methods: We have retrospectively analyzed the outcomes of 59 patients with relapsed/refractory NHL treated with ESHAP (Etoposide, methylprednisolone, cytarabine and cisplatin) as salvage chemotherapy with intent of receiving an ASCT, admitted to our department from 2002 to 2012.
Results: Median age was 54 (14-69) years. The majority (64.4%; n=38) of the patients had diffuse B cell lymphoma and 45 (84.9%) patients presented stage III-IV disease at time of diagnosis. Overall response rate (ORR) to ESHAP therapy was 72.9%, with 19 (32.2%) patients achieving complete remission (CR) and 24 (40.7%) partial remission (PR) before ASCT. Fifty-three patients (89.8%) have mobilized enough peripheral blood stem cells (PBPC's) for at least one ASCT. Median yield of CD34 collected was 3.12 x 10^6/Kg (0.7 – 15.9). Eight patients (13.6%) died during ESHAP therapy, 7 of progressive disease and one of cerebral haemorrhage. Thirty-four (57.6%) patients have received ESHAP plus Rituximab (R-ESHAP), while 11 (18.6%) did not received Rituximab at any time of the disease. Moreover, 6 patients (10.1%) who have not been exposed to Rituximab with first line chemotherapy received R-ESHAP and 14 (23.7%) patients who have received Rituximab upfront did ESHAP without Rituximab as salvage therapy. Overall response rate for R-ESHAP *vs.* ESHAP was 88.8% *vs.* 52%, respectively (p=.002). Median follow-up post ASCT was 30 months (1-120), the 3-year overall survival (OS) for the all cohort was 76.3% (+/-6.3%) and 3-year progression free survival was 63.3% (+/-7.2%). On univariate analysis, patients with chemosensitive response to ESHAP (+/-R) (3-year OS of chemosensitive *vs.* refractory disease was 86.3% *vs.* 25%, respectively, p<.0001) and patients who have received R-ESHAP (3-year OS R-ESHAP *vs.* ESHAP was 84.7% *vs.* 62.3%, respectively, p=.025) had superior overall survival. Age at transplant, type of NHL, stage at diagnosis and CR *vs.* PR to ESHAP (+/-R) did not had impact on OS.
Summary and Conclusions: Overall, ESHAP (+/-R) as salvage chemotherapy was well tolerated and had an adequate ORR without compromising PBPC's collection. Remission status at time of transplant had a significant impact on survival and it seems that the use of R-ESHAP as opposed to ESHAP increases the probability of having any form of response to salvage chemotherapy which subsequently increases the OS post autologous stem cell transplant.

PB1877

TREOSULFAN-FLUDARABINE-THIOTEPA COMBINATION AS PREPARATIVE REGIMEN FOR ALLOGENEIC HEMOPOIETIC STEM CELL TRANSPLANTATION IN HIGH RISK LYMPHOPROLIFERATIVE DISEASE PATIENTS

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Background: Reduced-intensity conditioning regimens for allogeneic HSCT are accepted treatments for high risk patients non fitting criteria for an ablative approach. Many drugs have been tested to find less toxic but effective preparations. The association Treosulfan-Fludarabine demonstrated to be well tolerated and myeloablative but partially effective due to high relapse risk. To increase the anti-tumor activity we added Thiotepa to the Treosulfan-Fludarabine regimen in a subset of patients with advanced lymphoproliferative disease.
Aims: Primary objective was to evaluate regimen safety and feasibility, secondary objective was evaluation of OS, DFS, TRM and relapse rate.
Methods: Since November 2006 to February 2014, 28 consecutive patients (18 males,10 females) entered the study. Median age was 43 years (range 19-60). Underlying diseases were: NHL(15), HL (8) , Acute Biphenotypic Leukemia (1), Acute Ph+ Lymphoblastic Leukemia (1),CLL (2), Burkitt Lymphoma (1) . All but one patients were heavily pre-treated (19 had received previous autologous transplant). Four (4) had presented severe clinical problems in their medical history: 1 pulmonary Aspergillosis, 1 brain tumour, 1 HCV hepatitis , 1 renal failure with dialysis during the induction therapy. Only 1 patient was in 1st complete remission; 15 were in 2nd or subsequent CR, 2 in PR , and 10 in resistant or progressive disease .Mean HCT-CI was 1 according to the Seattle criteria. Conditioning consisted of Treosulfan 14 gr/m^2 for 3 days, Fludarabine 30 mg /m^2 for 5 days and Thiotepa 10 mg /Kg single day. CSA+short MTX were used as GVHD prophylaxis and anti-Lymphocyte globulins (Thymoglobulin® or ATG Fresenius®) were used in case of MUD transplants. Fifteen (15) patients received HSC from HLA identical siblings and 13 from match unrelated donors. Source of stem cells was bone marrow in 8 patients and peripheral blood stem cells in 20 patients.
Results: Twenty-six (26) patients (93%) regularly engrafted. In two patients engraftment was not evaluable because of early (before day 20) septic death. Five (5) patients experienced grade 3-4 GI toxicity (4 mucositis , 1 GI bleeding), 1 patient had grade 1 renal toxicity, 1 patient presented grade 4 CNS toxicity , 2 patients had grade 2 skin toxicity. Thirteen (13) patients presented grade 1-2 acute GvHD , 1 grade 4. CrGvHD occurred in 8/22 evaluable patients (extended in 1). Eighteen (18) patients are alive (64%), 17 in complete remission, with a median follow-up of 30 months (range 2-86 months). Ten (10) patients died (36%): 4 by recurrent disease and 6 by TRM.
Summary and Conclusions: This single Centre report, even if with the limited number of patients, underlines that the association Treosulfan-Fludarabine-Thiotepa can be safely used for allogeneic conditioning regimen in very heavily pre-treated patients with advanced lymphoproliferative disease. Engraftment is regular and sustained. Post-transplant toxicity is acceptable. The role of Thiotepa seems important in increasing the anti-tumour effect in this reduced toxicity regimen. Further prospective, randomized, larger studies are warranted.

PB1878

PRIOR MD-ARAC CHEMOTHERAPY AFFECTING PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL MOBILIZATION IN AML

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Background: Prior chemotherapy had been reported to affect the efficiency of peripheral blood stem cell (PBSC) mobilization. As one of the major chemotherapeutic drugs for AML, the impact of cytarabine on mobilization remains unknown.Herein, weretrospectively ananlyzed AML patients performed PBSCs apheresis to explore the relation between prior medium-dose cytarabine(MD- AraC) chemotherapy and mobilization.
Aims: To explore the relation between prior MD-AraC chemotherapy and PBSC mobilization.
Methods: We retrospectively analyzed 90 patients with de novo AML, underwent mobilization inthe haematology department of Nanfang hospital from August 1999 and November 2012. Median age at mobilizaton was 38 years(range, 12-60 years). Before mobiliazation, all patients had received various chemotherapeutic regimens, including induction and consolidation or intensive chemotherapies. According to the course of MD-AraC chemotherapy,

patients were divided into group I, II,III. With a complete response, patients received the same mobilization regimen, EA (cytarabine 1.0g in IV, every 12 hours × 3 ~5days; etoposide 0.1~0.2g in IV, every 12 hours × 3~5 days) chemotherapy combined granulocyte-colony stimulating factor(G-CSF)5~10ug/kg/d. G-CSF starting after completion of chemotherapy 4 to 5 days when WBC down to the bottom until the end of thecollection. Apheresis was scheduled to start when the WBC count recovered ≥4.0 × 109/L or the CD34 cells ≥ 0.01% WBC of PB.After24~48h following high-dose conditioning regimens (mostly busulfan/cyclophosphamide) ,grafts were infused. The efficacy ofPBSC mobilization, hematopoietic reconstitution, survival rates were assessed for each AraC group.

Results: The median doses of CD34 cell in those three AraC groups were 4.7×106/Kg, 2.8×106/Kg, 2.2×106/Kg, respectively(P=0.006). In addition, patients collected ≥2.0×106/kg numbers of CD34 cells in groups I need the lowest leukapheresis, total bloodvolume processed, G-CSF total dose and days(P<0.05). A significantly greater proportion of good mobilization (≥2.0×106 CD34cells/kg with at most 3 leukapheresis procedures) in the group I (39/46, 84.8%) compared with the group II (13/22, 59.1%) and groupIII (10/19, 52.6%) ($\chi 2$=8.918, P=0.012). The sex, age, cytogenetic risk, the pior chemotherapy courses, the prior courses of the variouschemotherapeutic agent drugs except MD-AraC did not correlate with mobilization response. Multivariate analysis revealed thecourse of prior MD-AraC chemotherapy was an independent predictive factor for HSC mobilization[OR 0.627, 95% CI 0.421-0.935,P=0.022]. However, the MD-AraC chemotherapy has no effect on hematopoietic reconstitution and survival in AML patients treated with auto-HSCT(Figure1).

Figure 1. OS and RFS. A) Predicted 3-year OS was 72.3% for the group I, 82.9% for the group II, and 82.3% for the group III (P=0.803). B) Predicted 3-year RFS as 60.1% for the group I, 72.1% for the group II, and 64.8% for the group III (P=0.743).

Summary and Conclusions: Exposuring to the bone marrow toxic drugs is the major factor negatively effected mobilization, there mitoxantrone,fludarabine, lenalidomide, platinum, alkylating agent, carmustine, nucleoside analogue, melphalan were reported. As in previousstudies, prior exposure to MD-AraC also was an independent negative predictor for mobilization and without survival benefit. Theconventional chemotherapy not accurately presented a significant influence in the mobilization in AML. In preparation for Auto-HSCT, MD-AraC must be taken into account.

PB1879

AUTOLOGOUS STEM CELL TRANSPLANTATION UPFRONT IN THE TREATMENT OF PATIENTS WITH AGGRESSIVE B CELL LYMPHOMA: ANALYSIS OF PROGNOSTIC FACTOR FROM A SINGLE INSTITUTION IN JAPAN

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Background: The efficacy of autologous stem cell transplantation (auto-SCT) during the first remission in patients with aggressive B cell lymphoma remains to be elucidated. A previous large study found that auto-SCT improved progression free survival (PFS) in aggressive non-Hodgkin's lymphoma patients, but not overall survival (OS). (P.J.Stiff et al, NEJM 2013). Then we need to identify patients who will gain the maximum benefit from auto-SCT in the upfront setting. Also, it is important to establish new prognostic factors to identify the eligible patients. We focused on Glasgow Prognostic Score (GPS) which has been proposed as a powerful prognostic tool for patients with various types of malignant tumors as well as hematologic malignancy. In this study, we evaluated the efficacy regarding auto-SCT for high risk aggressive B cell lymphoma and identified the prognostic factors including GPS for auto-SCT.

Aims: To evaluate significance of auto-SCT for high risk aggressive B cell lymphoma and identify its prognostic factors.

Methods: We retrospectively analyzed 39 patients with high risk aggressive B cell lymphoma who underwent auto-SCT between 2006 and 2012 in Kansai Medical University Hospital. High risk patients were defined as high-intermediate and high risk groups stratified by international prognostic index (IPI). 17 patients received auto-SCT as upfront and 22 patients did as second-line therapy. All patients were treated by rituximab as primary therapy and evaluated by PET-CT scan. The primary endpoints were 3y-OS and PFS.

Results: 3y-OS and 3y-PFS of all patients were 66.7% and 71.8%, respectively. 3y-OS and PFS in upfront group were 94.1% (p=*0.001*) and 88.1% (p=*0.09*). In second-line group, they were 45.5% (p=*0.001*) and 59.1% (p=*0.009*). In multivariate analysis, age (60y/o<) (HR 3.6, p=*0.047*), disease status at auto-SCT (HR 35.3 p=*0.006*), and GPS (HR 8.8, p=*0.047*) were significant predictors for worse 3y-OS and PFS, whereas bone marrow involvement, IPI, and number of prior chemotherapies were not.

Summary and Conclusions: Upfront auto-SCT for high risk aggressive B cell lymphoma appears to be beneficial. Age, disease status, and GPS can predict outcome for auto-SCT. It may be useful to identify the eligible patients using these prognostic factors in undergoing auto-SCT in Rituximab-era.

PB1880

IN SITU INJECTION OF CIK CELLS FOR EXTRAMEDULLARY RELAPSE OF LEUKEMIA AFTER TRANSPLANTATION: A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Extramedullary leukemia relapse after allogeneic hematopoietic stem cell transplantation (HSCT) remains a formidable obstacle. Most of the patients suffered poor prognosis and eventually developed into bone marrow relapse. Current treatments including reducing and/or withdrawal of immunosuppressants, radiotherapy and chemotherapy did not permit any guarantee of promising effect.

Aims: We here report a patient with acute lymphoblastic leukemia (ALL) who had extramedullary relapse in manner of leukemia cutis 76 days after allogeneic HSCT.

Methods: We gave him chemotherapy with cytarabine 2.0 Q12h, d1-d3; idarubicin 10mg / day, d4-d5 from +85d to +89d. After chemotherapy the subcutaneous masses had transient narrowing but increased in two weeks after withdrawing chemotherapy (+103 d). The needle aspiration biopsy found immature leukemia cells. Then each mass had injection of cytarabine (20mg), but there was no significant improvement, subcutaneous masses still gradually increased. No leukemia cells was observed at 123 days in peripheral blood smears so peripheral blood mononuclear cells began to be collected for cytokine-induced killer cells (CIK) amplification *in vitro* after the informed consent was obtained. On day 137 after HSCT, we gave *in situ* subcutaneous injection into each mass with CIK cells (1ml, cell number was 1.56×10^5 / site).

Results: 4 days after CIK injection, the masses were flattening and gradually disappeared. During treatment the patient was well tolerated and did not re-appear the performance of extramedullary relapse afterward. This is the first case of giving adoptive cell therapy with CIK cells in the way of *in situ* injection.

Summary and Conclusions: Our case preliminarily demonstrates that *in situ* injection of CIK cells provides a new treatment option to therapy of extramedullary relapse after allogeneic HSCT and help to overcome the problem of inadequate targeting of extramedullary lesion during homing process of adoptive immune cells *in vivo*.

PB1881

A COMPARATIVE ANALYSIS OF EFFECTIVENESS OF TWO GRANULOCYTE COLONY-STIMULATING FACTORS (GCSF), AN ORIGINAL DRUG NEUPOGEN AND A BIOSIMILAR NIVESTIM, IN MOBILISATION OF PERIPHERAL BLOOD STEM CELLS

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Background: Original biological drug Neupogen (Amgen, Roche) was used for peripheral blood stem cells mobilisation in Clinical University Hospital Centre Zagreb until April 2012. When the drug stopped being available, biosimilar Nivestim (Hospira) was used for the same purpose.

Aims: We evaluated the effectiveness of two granulocyte colony-stimulating factors in mobilisation of peripheral blood stem cells in patients treated for hematological malignancies.

Methods: A retrospective analysis was performed in patients stratified

according to GCSF used for mobilisation (Neupogen *vs* Nivestim) and the hematological malignancy they were treated for (Acute myeloid leukemia, Multiple myeloma, non-Hodgkin`s lymphoma, Hodgkin`s lymphoma). 186 patients who were administered 10 mcg/kg of GCSF were analysed. The following parameters of stem cell mobilisation were surveyed: number of days GCSF was administered, total number of CD34 positive cells per kilogram collected, total number of colony forming units of the granulocyte macrophage order per kilogram collected. A matched pairs analysis was performed on a subset of patients treated for multiple myeloma. Mann-Whitney-U test was used for statistical analysis.

Results: When analysing the subset of patients treated for acute myeloid leukemia , the median number of days GCSF was administered before mobilisation was 11.5 for the Neupogen group, and 14 for the Nivestim group (p 0.892). Median value of CD34 positive cells collected was 7.6x10E6/kilogram for the Neupogen group and 7.0x10E6/kilogram for the Nivestim group (p 0.854). Median value of total number of colony forming units of the granulocyte macrophage order per kilogram collected (CFU-GM) was 58x10E4/kilogram for Neupogen group and 60x10E4/kilogram for Nivestim group (p 0.574). When analysing patient treated for multiple myeloma, we found that the median number of days GCSF was administered before mobilisation was 7 for the Neupogen group, and 8 for the Nivestim group (p 0.064). Median value of CD34 positive cells collected was 11.6x10E6/kilogram for the Neupogen group and 11.9x10E6/kilogram for the Nivestim group (p 0.459). Median value of total number of colony forming units of the granulocyte macrophage order per kilogram collected (CFU-GM) was 70x10E4/kilogram for Neupogen group and 74x10E4/kilogram for Nivestim group (p 0.574). When a matched pairs analysis was performed on this subset of patients no statistically significant difference was observed in the aforementioned categories. When analysing the subset of patients treated for non-Hodgkin's lymphoma, the median number of days GCSF was administered before mobilisation was 10 for both groups (p 0.750). Median value of total CD34 positive cells collected was 8.15x10E6/kilogram for the Neupogen group and 9.02x10E6/kilogram for the Nivestim group (p 0.728). When analysing the subset of patients treated for Hodgkin`s lymphoma, the median number of days GCSF was administered before mobilisation was 9 for both groups (p 0.755). Median value of CD34 positive cells collected was 10.9x10E6/kilogram for the Neupogen group and 13.37x10E6/kilogram for the Nivestim group (p 0.854). Median value of total number of colony forming units of the granulocyte macrophage order per kilogram collected (CFU-GM) was 58x10E4/kilogram for Neupogen group and 33x10E4/kilogram for Nivestim group (p 0.574).

Summary and Conclusions: Since no statistically significant difference was observed in the tested parameters we conclude that biosimilar Nivestim was not inferior when compared to the original biological drug Neupogen in peripheral blood stem cell mobilisation.

PB1882

STEM CELL MOBILIZATION IN ELDERLY MULTIPLE MYELOMA PATIENTS: SINGLE CENTER EXPERIENCE

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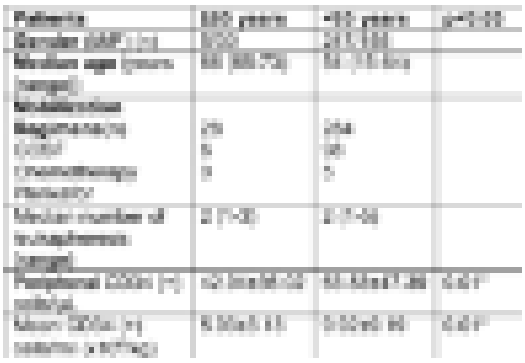
Background: Multiple myeloma (MM) has considerable impact in the older patient population. As the median age at diagnosis is 70 years for MM, more than half of newly diagnosed cases of these malignancies were made in patients' ≥60 years of age.

Aims: We retrospectively compared myeloma patients below the age of 65 with patients above 65 years of age, analyzing CD34 mobilization into peripheral blood and the number of leukapheresis needed to collect at least one single stem cell graft.

Methods: In our clinic; between 1993 and 2013, a total of 437 patients with MM who received autologous stem cell transplantation enrolled this study. Our thereshold for leukapheresis was 10 CD34 positive cells/μL whole blood. Only in patients achieving >10 CD34 positive cells/μL blood was a stem cell collection initiated. Thirty five of 437 patients were above 65 years of age (median age 66, range 65-73) and 402 patients were below the age of 65 (median age 54, range 15-64). Patients' characteristics are summarized in Table 1. Mobilization regimens for the younger patient population were cyclophosphamide based (n: 95), etoposide based (n: 3), G-CSF only (n: 284) and plerixafor+ G-CSF (n: 5). Mobilization in the older population was with cyclophosphamide based (n: 6) and G-CSF only (n: 28).

Results: There were no significant statistical differences in time from diagnosis to mobilization, number of prior therapies, disease status, and type and frequency of comorbidities between both patient groups. The number of CD34-positive circulating cells before scheduled leukapheresis was mean 62.11 cells/μL (median 48 cells/ μL, range 10-197; SEM±46.875) in all patients. The results are summarized in Table 1.

Table 1.



Summary and Conclusions: Our data support the observation that after a standard mobilization regimen with anti-myeloma chemotherapy and once-daily growth factor support, patients above 65 years of age show an impaired CD34 mobilization into peripheral blood compared to a younger population. This can be overcome by an increased number of leukaphereses.

PB1883

ASSOCIATION OF GRAFT-VERSUS-HOST-DISEASE SEVERITY WITH LEUKEMIA RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN

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Background: The graft-*versus*-leukemia (GVL) effect plays a major role in reducing the risk of relapse after allogeneic hematopoietic stem cell transplantation (HSCT). Because the GVL effect is extremely similar to that underlying graft-*versus*-host-disease (GVHD), severity of GVHD is believed to correlate with the GVL effect. GVHD may be associated with a reduced incidence of leukemic relapse; however, it is the leading cause of treatment deaths and increases treatment-related mortality.

Aims: The aim of this study was to investigate whether clinical outcomes of allogeneic HSCT in children are affected by GVHD severity.

Methods: We retrospectively analyzed 191 pediatric patients who received allogeneic HSCT at a single institution. All 191 patients received 217 allogeneic HSCTs from January 1, 1985, to March 31, 2013; these included 116 patients with acute lymphoblastic leukemia (ALL) and 75 patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Acute GVHD (aGVHD) was evaluated according to the International Bone Marrow Transplant Registry grading system and chronic GVHD (cGVHD) was categorized as either limited or extensive.

Results: In a comparison of grade 0-I *versus* grade II-IV aGVHD, overall survival (53.4% *vs.* 51.9%, p=0.958), leukemia-free survival (50.4% *vs.* 42.7%, p=0.586), cumulative incidence of relapse (41.2% *vs.* 42.6%, p=0.395), and treatment-related mortality (8.4% *vs.* 14.7%, p=0.856) did not significantly differ. For the absence *versus* the development of cGVHD, overall survival (52.5% *vs.* 56.5%, p=0.401), leukemia-free survival (47.3 *vs.* 51.6%. p=0.418), cumulative incidence of relapse (45.2% *vs.* 31.7%, p=0.137), and treatment-related mortality (7.5% *vs.* 16.7%, p=0.136) did not significantly differ. The group of patients who developed cGVHD had a tendency of lower cumulative incidence of relapse and higher treatment-related mortality than those who did not develop cGVHD. Compared with the entire cohort, patients with ALL or AML had similar survival probabilities.

Summary and Conclusions: The severity of aGVHD and cGVHD does not affect the clinical outcome of allogeneic HSCT in children. However, the clinical outcome of patients who develop cGVHD may improve as treatments for cGVHD advance and decrease treatment-related mortality.

PB1884

THE ASSESMENT OF THE STEM CELL MOBILIZATION IN LYMPHOMA PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: High-dose chemotherapy in conjunction with auto-SCT is widely recognized as the preferred modality of treatment for patients with relapsed or refractory Hodgkin disease or non-Hodgkin lymphoma at the time of chemo-

sensitive first relapse. Successful transplant requires the infusion of a sufficient number of hematopoietic stem cells. Previous studies in patients with lymphoma varying definitions of 'mobilization failure', show that 10-25% of patients fail to mobilize a sufficient number of HSC.

Aims: The purpose of this retrospective analysis is to identify the factors causing the mobilization failure with lymphoid malignancies.

Methods: A total of 90 patients with HL or NHL, referred for the Autologous SCT to the Bone Marrow Transplantation Unit in Ankara Numune Training and Research Hospital between 2004-2013, evaluated in this retrospective study.Mobilization failure were defined as patients failed to collect at least 2x106 CD34 cells/kg of body weight in ≤4 leukapheresis procedure.

Results:

Through the first line mobilization regimen, 70 of 90 patients (78%) have been successfully mobilized except for 20 (22%) patients. The mean age of the successfully mobilized patients is 36.4 whereas the mean age in unsuccessful ones 43.6 (p=0.023). 15 of 20 patients with mobilization failure have the diagnosis of NHL and 5 of 20, HL (p=0.008, OR= 4.24). 10 of 20 patients (50%) have bone marrow involvement at the time of the diagnosis (p=0.044 OR = 2.68).No relationship has been found among the mobilization failure and gender (p=0.24), disease stage (p=0.39), B symptoms(p=0,18) splenic involvement (p=0.47) disease status at the time of the mobilization (p=0.74), the number of the chemo regimens (p=0.121), the mean number of total chemo cycles (p=0.895) and Rituximab Therapy for NHL group (p=0.18). Among the salvage regimen, no relationship has been detected between the mobilization failure and the salvage regime used. When the patients have been evaluated according to their mobilization regimens: Cyclophosphamide+Etoposide+G-CSF with 100% success (p=0.018), Cyclophosphamide+G-CSF with 72.2% success (p=0.301), G-CSF with 70% success (p=0.369), ICE+G-CSF with 66.7% success (p=0.454), DHAP+G-CSF with 100% success (p=0.331). The mean CD 34 yield in Cyclophosphamide+Etoposide+G-CSF group is 5,38 x 106 cells/kg while in G-CSF group the mean is 3,68 106 cells/kg , in Cyclophosphamide +G-CSF group the mean is 3,88x106 cells/kg. (p=0,001 and p=0,004). In multivariate analysis, the older age (p=0,029), bone marrow involvement at diagnosis (p=0,047), the number of prior chemo regimens (p=0,044) play a role in mobilization failure (Table 1).

Table 1.

Age		38(16-63)
Gender (Male/Female)	Male 55 (61%)	n=90
	Female 35 (39%)	
Diagnosis	Non-Hodgkin Lymphoma	44 (47%)
	Hodgkin Lymphoma	46 (53%)
Bulky disease		7 (7,8%)
Splenic involvement		12(14.4%)
Bone marrow involvement		29 (32.3%)
Radiotherapy		33 (36%)
B symptoms		76 (84%)
Rituximab for NHL		22 (50%)

Summary and Conclusions: Diagnosis of NHL, older age, bone marrow involvement at the time of diagnosis, the number of prior chemo regimens, and the used mobilization regimen are the factors affecting the mobilization in our group. Randomized and controlled multi-center prospected studies are to describe the definite risk factors for the mobilization failure in lymphoma patients. In this context, "the high risk group" suggesting the use of newer agents like plerixafor and the use of etoposide addition to the cyclophosphamide in the first line is available.

PB1885

TRANSFORMING GROWTH FACTOR BETA GENE POLYMORPHISM AS A RISK FACTOR FOR THE DEVELOPMEMT OF GVHD AFTER STEM CELL TRANSPLANTATION FROM AN IDENTICAL SIBLING

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Background: Stem cell transplantation (SCT) is a standard therapeutic modality for many benign and malignant hematological disorders. Graft *versus* host disease (GVHD) is among its serious complications. The role of some cytokine gene polymorphisms has been documented. Few studies are available about the role of TGFβ and the impact of polymorphisms at codon 10 or codon 25 on the development of aGVHD and cGVHD with controversial results.

Aims: We wanted to evaluate the impact of both codon 10 and codon 25 TGFβ polymorphisms on the development of acute and chronic GVHD as well as infectious complications.

Methods: The work was performed according to Helsinki declaration, the protocol was approved by the IRB of the NCI, Cairo University and an informed consent was obtained from all subjects. The study cohort included 97 patient/donor pairs and 8 more patients (total 105 patients) who received SCT from an identical sibling at Nasser Institute, MOH, Egypt in the period from December 2010 –February 2013. We determined the genotype of TGFβ, by SSP typing using Cytokine Genotyping Tray REF CYTGEN (one Lambda, Inc, 21001 Kittridge st., Canoga Park, CA 91303 USA) classifying cases as high, intermediate and low producers. Patients were followed up for at least one year; development of GVHD and infectious complications was recorded.

Results: Of the 107 cases, 29 developed acute GVHD, 7 developed chronic GVHD on top and another 12 developed de-novo chronic GVHD. The genotypes with the corresponding phenotype are presented in Table 1. TGFβ was of the low producer genotype in 27/29 (93.1%) patients who developed acute and in 18/19 (94.7%) patients who developed chronic GVHD. The corresponding figure for those who did not develop GVHD was 55/64 (85.9%). Among donors of patients who developed GVHD, low producer genotype was encountered in 27/28 (96.4%) for acute and 16/17 (94.6%) for chronic GVHD; the corresponding figure for donors of patients with no GVHD was 51/57 (89.5%). Pulmonary infectious complications developed in 14 patients, 13 (92.9%) of which were low producers as compared to 80/91 (87.9%) in patients with no infectious complications. Though the differences did not achieve statistical significance, a clear trend of association of low TGFβ producer genotype with GVHD and pulmonary infection is evident. Lack of statistical significance may be partly attributed to the predominance of the low producer genotype among our population presented in both patients and donors which might be an ethnic characteristic.

Table 1. TGFB1 Genotypes and corresponding phenotypes in patient and donors in relation to graft *vs.* host disease after allogeneic stem cell transplantation.

Summary and Conclusions: TGFβ of high or intermediate producing genotypes are uncommon in patient/donor pairs but has a trend to be protective from GVHD and pulmonary infections in patients receiving allogeneic stem cell transplantation; yet statistical significance was not achieved.

PB1886

STANDARDIZATION OF MOBILIZATION PROTOCOL IS ASSOCIATED WITH SUCCESSFUL COLLECTION OF PERIPHERAL BLOOD STEM CELLS FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH ONCOHEMATOLOGICAL DISEASES

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Background: Autologous stem cell transplantation (AutoSCT) is a very important stage of oncohematological patients' therapy as there is the possibility to treat the patients with myeloablative regimen to reduce the volume of minimal residual disease and to improve relapse-free or overall survival.

The main condition of successful AutoSCT is harvesting of $2x10^6$/kg CD34+ cells or more. There are different methods to increase the quantity of CD34+ cell in autotransplant and we supposed that one of them is to standardize regimen of mobilization regimen.

Aims: The aim of the study was to compare the characteristics of autotransplants in different periods when different mobilization regimens were used.

Methods: Retrospective analyses of autotransplants which were harvesting during 1995-2011 and 2012-2013 periods have been done. During 1995-2011 period (Group 1) next mobilization regimens were used: filgrastim alone in dose 10 mkg/kg s.c. 1-5 days or cyclophosphomide (Cph) 1-5.0 g/m^2 in combination with filgrastim or lenograstim 5 mkg/kg s.c. beginning from day 3 after mobilization regimen. During 2012-2013 period (Group 2) the scheme of mobilization regimen was unique for all patients: Cph 3.0 g/m^2 plus G-CSF: filgrastim (10%), lenograstim (85%) or pegfilgrastim (5%). Injections of G-CSF were started on day 5 after mobilization regimen. The everyday dose of G-CSF was 10 mkg/kg. The treatment was begun after the signing of the informed consent. The level of CD34+ cells in peripheral blood more than 10/mL was chosen to start aphaeresis.

Results: In Group 1 there were 105 patients with Me age 45 y. (16-65). The patients were diagnosed with multiple myeloma (MM) (45%), acute myeloid

leukemia (AML) (19%), non-Hodgkin lymphoma (NHL) (16%), acute lymphoid leukemia (10%) and Hodgkin disease (HD) (8%). Me number of aphaeresis was 2 (1-3). Me number of collected CD34+ cells was $2.8x10^6$/kg (0.05-12.2). Poor mobilization with the number of CD34+ cells less than $2x10^6$/kg was verified in 37/105 (36%) patients. In Group 2 there were 21 patients with Me age 48 y. (24-67). The patients were diagnosed with MM (71%), AML (14%), NHL (10%), and HD (5%). Me number of aphaeresis was the same like in Group 1. Me number of collected CD34+ cells was $6.7x10^6$/kg (0.2-29.4). It was significantly higher than in Group 1; p=*0.010*. Poor mobilization was diagnosed in 4/21 patients (19%) and it was significantly lower than in Group 1; p=*0.000*. It is necessary to point that there was no increased rate of organ toxicity and no any case with disease progression in Group 2.
Summary and Conclusions: We conclude that standardization of mobilization regimen with increased dose of Cph and G-CSF is accompanied with improved results of autotransplant collection in oncohematological patients irrespective of diagnosis. This strategy is not more toxic than previously used regimens. Nevertheless there were some patients with poor stem cells collection who are the potential candidates to treat with plerixafor during mobilization regimen.

PB1887

PERIPHERAL BLOOD CELL MOBILIZATION AND COLLECTION PROCEDURES IN PLERIXAFOR ERA: A MONOCENTRIC RETROSPECTIVE EXPERIENCE

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Background: High dose chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT) represents a potentially curative treatment in hematological malignancies. Granulocyte colony stimulating factor (G-CSF), alone or in combination with chemotherapy are usually used to mobilize stem cells in peripheral blood which are then collected through one or more apheresis procedures. A suboptimal collection has been associated to delayed or engrafment failure, as well as to a reduced PFS and OS and increased transplant related toxicity. Unfortunately a considerable proportion of patients, ranging from 11% to 40%, is unable to collect this target and is therefore considered ''poor mobilizer''.
Aims: By this retrospective analysis we wanted to report our monocentric experience in the process of stem cells mobilization and collection, during the era of novel reversible inhibitor of CXCR4, Plerixafor.
Methods: We retrospectively analyzed 66 patients: 2 Hodgkin Lymphoma (HL), 25 non-Hodgkin Lymphoma (NHL), 27 Multiple Myeloma (MM), and 12 Acute Myeloid Leukemia (AML), who underwent a mobilization procedure at our institution from January 2011 to January 2014. Our group was constituted by 43 males and 23 females, whit a median age of 56 years (range 18-75).
Results: In the triennium of analysis we performed 73 mobilization procedures (6 second attempt) and 61 autologous PBSCT (n=24 MM, n=21 lymphomas, n=8 AML) including 8 second procedures. Median time from diagnosis to mobilization was 8.3 months in MM, 12.5 in lymphoma, 2.6 in AML. The mean number of prior lines of therapy was 1 for MM patients, 2 for NHL, 2 for HL and 1 for AL. 14 patients (n=8 MM and n=6 NHL) received radiotherapy prior to mobilization. At mobilization, 37% (n=10) MM patients were in Complete Remission (CR), 18.5% (n=5) achieved Very Good Partial Remission (VGPR) and 44.5% (n=12) was in Partial Remission (PR). In Lymphoma (HL and NHL) group, 26% (n=7) were in CR, 67% (N=18) in PR and 7% (n=2) in PD. All AML patients (n=12) achieved morphologic CR before stem cells collection. In line with published data, we report 22% mobilization failure (n=16, 10 in first and 6 in second attempt). The group failing first mobilization, was constituted by 7 females and 3 males, 1 MM, 5 NHL and 4 AML, the median age was 52 (range 32-70). The median number of previous therapy was significantly higher in patients failing than in patients succeeding first mobilization, both in MM (6 *vs* 1.27) (p=*0.0003*) and in Lymphoma (2.4 *vs* 1.8) (p=*0.005*). Of the seven patients who underwent a second mobilization procedure, 86% failed. Thirty (16 MM, 14 NHL) of the 54 MM and lymphoma patients, received plerixafor at any time of their mobilization history, and in 90% (n=27) of cases, a dynamic approach based on the CD34 monitoring, guided us in plerixafor use "on demand". The median number of plerixafor injection was 1.6 (range 1-3). The median interval from mobilization to collection was respectively 11 days for MM and 15 days for Lymphoma patients mobilized without and 13 days for MM and 14 days for Lymphoma mobilized with plerixafor. Considering CD34+ cells/µl baseline count at the expected day of collection and after plerixafor injection, we found a 5.7 and 4.1 fold increment, respectively in MM and lymphoma patients. The median CD34+ x 10^6/kg harvest was 4.16 in MM and 3.7 in lymphoma patients receiving the new mobilizing agent and 5.3 for MM and 11.67 for lymphoma patients group who did not receive plerixafor *(p<0.001)*. Seven patients (n=3 MM, n=4 Lymphoma) mobilized without chemotherapy, 3 in first and 4 in second attempt. The median CD34+ x 10^6/kg yield of this group was 0.64 (range 0.39-1). Considering the number of apheresis, we reported a significant difference in Lymphoma patients mobilized with and without plerixafor (1.2 *vs* 1.8) *(p=0.001)*, but not for MM patients (1.68 *vs* 1.7) *(p=0.08)*.
Summary and Conclusions: This retrospective analysis underlines the importance of a dynamic approach in plerixafor use, to change the course of a "poor mobilizer" first mobilization procedure into a chance to go through an high-dose treatment.

PB1888

ALLOGENEIC UNRELATED DONOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN HAEMOGLOBINOPATHIES – A PAEDIATRIC BONE MARROW TRANSPLANTATION UNIT EXPERIENCE

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Background: Supportive and preventive therapeutic interventions, such as regular transfusions and chelation treatment, remain the standard of care for patients with congenital haemoglobinopathies. Allogeneic bone marrow transplantation is the only approach available with curative intent. More than 90% of transplanted patients with matched sibling donors demonstrate reconstitution of normal haemopoiesis, with a success rate of nearly 100% when the transplantation is performed in a young age. The implementation of conditioning regimens that eliminate the risk of rejection, as well as the refinement of histocompatibility testing have allowed the performing of non related HSCT. In literature however, the use of unrelated donors (VUD) has been complicated by poor engraftment, excessive regimen related toxicity and graft *versus* host disease (GVHD).
Aims: The principal objective of this study is to present our results in survival and the complications associated with allogeneic VUD HSCT in children with haemoglobinopathies. Correspondingly, to assess and examine the risks against the potential long term gain of HSCT.
Methods: From January 2004 until February 2014, 13 haemoglobinopathy patients (8 male), with a median age of 3.4 years (range 1.2-14.8 years) were transplanted from unrelated donors in our unit. 9 patients had β-Thalassaemia while 4 of the patients suffered from Sickle Cell Disease (SCD). 9 out of 13 patients were given a fully matched graft while the rest 4 patients had 1 HLA antigen mismatched donors. 11 patients had bone marrow and 2 patients had peripheral blood as the source of HSCs. 4 grafts were manipulated accordingly in order to not exceed more than $5x10^7$/kg $CD3^+$ cells. The conditioning protocols used were Busulfan based with the addition of a combination of Cyclophosphamide, Fludarabine and ATG. GvHD prophylaxis consisted of Cyclosporin A and Methotrexate. The median numbers of $CD34^+$ and $CD3^+$ cells were $7x10^6$/kg and $4{,}25x10^7$/kg respectively.
Results: Engraftment was achieved in 11/13 patients. Autologous back up HSCs were given to the remainder 2 patients, with autologous haematopoietic recovery. Neutrophil and platelet engraftment occurred at a median of 19 (range 14-28 days) and 22 days (range 18-30 days) respectively. Subsequent donor lymphocyte infusions (DLI) were administered in 2 patients with mixed chimerism and restored engraftment in one of them, whereas the second one experienced an eventual late graft rejection. Acute GvHD (stage II-IV) was recorded in 7 patients, in one of whom post the administration of DLI. Chronic GvHD has been evident in 2 patients. With a median follow up of 58,3 months (range 1.1-111.0), all patients are alive and well with 10 of them having an entirely normal haemopoiesis with complete chimerism, nonetheless one of them developed an episode of autoimmune haemolytic anaemia later in the post transplant period and was successfully managed accordingly. 3 out of the 13 patients remain transfusion dependent.
Summary and Conclusions: Even though there is an overall survival of 100%, VUD allogeneic HSCT in children with haemoglobinopathies, is likely to be related with an increased risk of complications. Evaluation in larger patient cohorts, is necessary to further assess the long term benefits against the risks and perhaps to attain some insight into the mechanism of graft failure. As the development of GvHD and older age at transplant appear to be important impairing factors, providing there is a 10/10 HLA allele matched donor available, HSCT should be performed after thoughtful contemplation of the individual parameters and risk factors, also taking into account the survival expectancy conferred by conservative therapy.

PB1889

ALLOGENEIC TRANSPLANT OUTCOMES IN MPN BLAST PHASE

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Background: The Philadelphia (Ph) negative myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET), and

primary myelofibrosis (PMF) are clonal disorders of the hematopoietic system associated with an approximate 5%>10% risk of leukemic transformation. Allogeneic stem cell transplant is the only potentially curative therapy.
Aims: We retrospectively reviewed 10 consecutive patients with MPN blast phase that underwent allogeneic stem cell transplant (ASCT) and report our centers experience.
Methods: Consecutive patients were identified at Mayo Clinic Arizona who underwent ASCT between 2006-2013. All patients had a previous diagnosis of a Ph negative MPN (ET, PV, PMF) and underwent leukemic transformation to MPN blast phase. Both myeloablative and reduced intensity conditioning regimens were employed, all transplants were performed utilizing peripheral blood stem cells, and outcomes were retrospectively analyzed.
Results: MPN Blast Phase Presentation and Management: 10 patients with post-PV/ET fibrosis or PMF MPN blast phase were identified. Prior MPNS were PV in 5/10 (50%), ET in 3/10 (30%), and PMF in 2/10 (20%). Jak V617F mutation occurred in 70%. Cytogenetic analysis revealed poor risk karyotype (3 complex, 1 del (7)) in 4/10 (40%) and intermediate risk (3 normal, 2 trisomy 8, 1 t(16;21)) in 6/10 (60%). Median performance status was EGOG 1. Induction chemotherapy included Ara-C, daunorubicin, and etoposide (ADE) in 5/10 (50%) and cytarabine and daunorubicin (7+3) in 5/10 (50%) patients. High dose cytarabine (HiDAC) consolidation was utilized in 3/10 (30%). Salvage mitoxantrone based chemotherapy was employed in 30%. A CR was obtained in 7/10 (70%) of patients however, 2 patients (20%) had <5% residual marrow blasts and 1 patient (10%) had >5% marrow blasts. Allogeneic Stem Cell Transplant: At time of transplant, the median age was 61 years. Conditioning regimens used were myeloablative fludarabine+busulfan in 2 patients, and reduced intensity in 8 patients, including 5 patients who received fludarabine/BCNU/Melphalan and 3 patients who received fludarabine+busulfan. Donors were matched related donor (MRD) in 50% and 10/10 matched unrelated donor (MUD) in 50% peripheral blood stem cells. GVHD prophylaxis regimens included ATG in 7/10 (70%), and tacrolimus was utilized in all regimens with either the addition of mycophenolate (60%) or methotrexate (40%). Grade 3-4 GVHD (GI) developed in 1 patient. Chronic GVHD was seen in 4 patients (2 skin, 1 GI, and 1 skin and GI). Long Term Outcomes: At median followup of 44 months, 5 patients (50%) are alive in CR, 4 patients (40%) have expired, and 1 patient was lost to follow up. The median survival after transplant was 31 months. Transplant related mortality occurred in one patient. Causes of death included relapsed disease in two, and one patient with gram-negative sepsis two years after transplant. Of those with persistent disease after induction chemotherapy (3/10), two expired of relapsed disease, occurring at 8 and 2 months post transplant.
Summary and Conclusions: Historically, the median survival for MPN blast phase is approximately 6 months or less. Allogeneic stem cell transplant may offer improved survival for MPN blast phase patients who are candidates for this intervention. Adequate leukemic clearance prior to ASCT is associated with improved outcomes. Conversely, those with poor risk karyotype have worse outcomes. More studies are needed to evaluate long-term survival as well as optimal induction and conditioning regimens in this patient population.

PB1890

ALTERATION OF HEPATITIS B SEROSTATUS AMONG ALLOGENEIC BLOOD STEM CELL TRANSPLANT RECIPIENTS AT INHA UNIVERSITY HOSPITAL

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Background: Chronic hepatis B is prevalent in many countries in Asia. Reactivation of hepatitis B in recipients or infection of hepatitis B from donors has been reported in the setting of allogeneic blood stem cell transplant (alloBSCT) to be uncommon although worrisome.
Aims: We have investigated the alteration of hepatitis B serostatus in 72 recipients of alloBSCT performed at Inha University Hospital, to estimate the true prevalence of hepatitis B serostatus change during the process of alloBSCT in South Korea, where hepatitis B virus (HBV) infection is still endemic.
Methods: Medical records of a total of 113 pairs of recipients and donors for alloBSCT were reviewed from which data regarding hepatitis B serology were retrieved. Hepatitis B surface antigen (HBsAg)/antibody (HBsAb) assay was done by chemiluminescence immunoassay, using the Architect i2000 system (Abbott Laboratories, Abbott Park, IL).
Results: Post transplant serology of the recipient was unavailable in 41 cases (death, omission of the test, or lost to follow up) and data of 72 pairs were available. There were no HBV carriers among donors; 23 were seronegative and 48 had antibody against HBV. There were six HBV carriers among recipients; 20 were seronegative and 46 seropositive for HBV antibody. Two HBV carriers among recipients showed seroconversion with loss of HBsAg and gain of HBsAb, one from seronegative donor and the other from HBsAb positive donor. One HBV carrier lost HBsAg without seroconversion after receiving alloBSCT from seropositive donor. Three HBV carrier remained persistent carrier after receiving alloBSCT from two seropositive and one seonegative donors. Twelve seronegative recipient gained HBsAb, 8 from HBsAb positive donors and 4 from seronegative donors. Seven seropositive patients lost HBsAb, 3 recipients from HBsAb positive donors and 4 from HBsAb negative donors. Reverse seroconversion occurred in one patient who was transplanted from HBsAb positive donor.
Summary and Conclusions: Twenty three of 72 allogeneic blood stem cell transplant recipients (32%) showed alteration in their serostatus against HBV. Adoptive seroconversion was noted in 2 of 6 HBV carriers (33%). Seroreversion occurred in one of 46 seropositive recipients (2%). For further refined study, prospective study design and serial quantitative titration of HBsAb are needed.

PB1891

MAINTENANCE THERAPY AND EVENT-FREE SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA IN POSTTRANSPLANT PERIOD.

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Background: Autologous stem cell transplantation (AutoSCT) is the important stage of treatment of patients with multiple myeloma (MM) younger than 65 y. The role of maintenance therapy (MT) is controversial and there are some publications that MT is the method to improve event-free survival (EFS) of MM patients who were treated with AutoSCT.
Aims: The aim of the study was to evaluate the possibility of MT to improve RFS of MM patients after AutoSCT.
Methods: The data of 48 patients with MM were analyzed retrospectively. The data from 1995 till 2012 was collected. There were 23 men and 25 women. Median age of patients was 49 y. (25-64). According to the Durie-Salmon scale distribution of patients was next: 17 patients with II stage and 31 patients with III stage. According to immunochemistry variant distribution of patients was next: 27 patients with IgG, 9 patients with IgA, 10 patients with BJ and 2 patients with nonsecreted MM. The number of treatment lines before AutoSCT was 1-3. Complete response was diagnosed in 17 patients and partial response in 31 patients. Tandem AutoSCT was done in 27 patients and interval between transplantation was 4-9 months. 22 patients were treated with MT: 16 patients with CR and 6 patients with CR. Patients were treated with immunomodulators or proteosome inhibitors. To evaluate the effectiveness of MT criteria of EBMT were used. Relapse, progression, serious adverse events of death of patients were considered as events.
Results: Relapse and progression were diagnosed in 25 patients (52,1%). Relapse were diagnosed in 14 patients after tandem AutoSCT and in 11 patients after single AutoSCT. EFS in these groups of patients were 23 and 24 months respectively. 8 patients died according to MM progression or chemoresistance. The reason of death of 2 patient was not related to MM.
In the group of patients with CR who were treated with MT EFS was 31.6 months. In the group of patients with CR who was not treated with MT EFS was 8.3 months; p=*0.000*. The difference in EFS between groups of patients with PR was not significant.
Summary and Conclusions: We conclude that MT after AutioSCT is effective method to improve the time to relapse or to progression in MM patients.

PB1892

UNRELATED CORD BLOOD TRANSPLANTATION FOR PATIENTS WITH SEVERE ACQUIRED APLASTIC ANEMIA□RESULT OF A SINGLE CENTER

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Background: In recent years, umbilical cord blood transplantation(CBT) has become a promising treatment for patients with hematologic diseases with its advantages of rapid availability and lower rate of graft *versus* host disease despite broader HLA disparity.
Aims: The aim of this work is to analyse the outcome of patients with SAA underwent unrelated CBT.
Methods: Nine patients were included in this study and median age was 23 years(range 13-34 years). The patients received a conditioning regimen composed of cyclophosphamide (total dose 75mg/kg), rabbit antithymocyte globulin (ATG, total dose 15 mg/kg).Cyclosporin and mycophenolate mofetil were used as GVHD prophylaxis. For Cord blood, total nucleated cells (TNCs) had to exceed 3×10^7/kg and HLA(A,B,DR-B1) at least four of six loci matched. Patients had monthly chimerism check in the first three months after CBT by analysis of STR polymorphisms then repeated according to the patients' condition.

Results: All cases received one cord unit transfusion and HLA loci 5/6 matched for six, 4/6 matched for three.The median TNCs of cord units was 3.9×10^7cells/kg(range 3.1-6.4) and the $CD34^+$ cells was 2.5×10^5cells/kg (range 0.8-5.36).One case wasn't evaluated because of early death on day 14 due to intracranial hemorrhage. All of the remaining eight patients acquired autologous myeloid recovery and the median time to neutrophil count of 0.5×10^9/L was 37 days (range 14-45 days). The median time to 20×10^9/L platelets was 79 days (range31-120days). Only 1 case had engraftment with complete donor chimerism on day 30 and the time for neutrophil cell>0.5×10^9/L and platelet>20×10^9/L was respectively day 14 and day 31 after CBT. But this patient's chimerism became mixed of donor and recipient two months after CBT and turned back to recipient three months after CBT with neutrophil cell and platelet transiently decreased leastly to 0.9×10^9/L and 35×10^9/L,then increased gradually to normality.
Summary and Conclusions: Althoug with low engraftment, CBT for SAA using CTX and ATG-based conditioning may quicken autologous recovery and improve survival with low risk of transplant-related mortality.

PB1893

THE ROLE OF AUTOLOGOUS HAEMATOPOIETIC CELL TRANSPLANTATION IN SYSTEMIC SCLEROSIS: REGRESSION OF DERMAL FIBROSIS AND STABILIZATION OF VISCERAL DISEASE

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Background: Autologous haematopoietic cell transplantation (AHCT) is considered a treatment option for selected patients (pts) with severe progressive systemic sclerosis (SS) and may induce regression of dermal fibrosis, sustained improvement functional status, stabilization of visceral involvement and lower mortality due to disease progression.
Aims: The aim of the study was to evaluate the outcome of the pts who underwent AHCT for severe and progressive SS in our centre.
Methods: We retrospectively analyzed the outcome of 8 pts (6 women, 2 men), 35(30-48) years old, who underwent AHCT for SS in our unit. The pts were transplanted for severe progressive disease post 3(2-7) lines of immunosuppressive treatment, including cyclophosphamide (Cy) pulses in 7/8 pts [total dose 6(2.5-12) g] and at a median (Δm) time of 4(2-13) years post disease diagnosis. The Δm modified Rodnan skin score was 32(2-49) out of 51. All pts presented joint, lung and gastrointestinal involvement. Peripheral hematopoietic stem cells were mobilized with Cy 4g/m2 and GCSF without major complications. The conditioning regimen used was Cy 200 mg/kg plus ATG 7.5 mg/kg and pts received 6(3.9-8.65) x 10^6/kg CD34+ cells.
Results: Engraftment was prompt and sustained in all pts, at a Δm of 10(7-14) days for neutrophils, 9(5-13) for platelets and 7/8 pts were discharged on day +14(13-38). A female pt with severe and rapidly progressive disease, post 7 lines of immunosuppressive treatment, died on day +87 from sepsis and multiorgan failure. In the rest of the pts the complications observed were: pneumothorax during central line catheter placement (1), neutropenic fever (6), mild pulmonary infection (1), BK polyoma virus hemorrhagic cystitis (1), herpes zoster (1), CMV (2) and EBV (4) reactivation. Modified Rodnan skin score was improved post-transplant and was 15(2-41) at +3 months (m) and significantly lower 7(2-18) post 32 (14-87) m in 5/7 pts who could proceed to our unit for further follow-up. With a Δm follow-up 20 (4-87) m, all pts referred improvement or complete remission of dysphagia symptoms and significant improvement of performance status. Respiratory evaluation revealed stable FEV1 and DLCO in 3, improved in 2, asymptomatic mild progression in 1 and clinically symptomatic progression (15m post-transplant) in another pt. Until now 5/7 pts are off further treatment. One female pt was diagnosed with sarcoidosis at +27m and is under corticosteroid treatment.
Summary and Conclusions: With the limitations of a retrospective study and the small number of pts, AHCT seems to be a valuable treatment in selected pts with SS offering them a better quality of life.

PB1894

WASHING OF HEMATOPOIETIC PROGENITORS FOR DMSO REMOVAL BEFORE TRANSPLANT. COMPARISON OF TWO SOLUTIONS

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Background: Cryopreservation with liquid nitrogen is necessary in the autologous hematopoietic stem cells transplant. Cryoprotectant agents are needed to preserve the viability of the cells during freezing. DMSO is the most commonly used cryoprotectant agent. However, DMSO is known to produce toxicity in the patients during the infusion of the thawed product. To avoid this toxicity, our group tested the efficacy of washing the cells using a closed automated system (Sepax) and normal saline with 2.5% albumin (NSA) (Sanchez-Salinas et al, 2012). An excellent CD34+ cells recovery and cell viability after washing was demonstrated, as well as absence of infusion-related events and no negative impact on engraftment dynamics. We also collaborated in the validation of a simpler washing solution without human derived products consisting in Voluven 6% (Saccardi *et al.* ASH 2012), obtaining good results.
Aims: To compare the efficacy of NSA and Voluven as washing solutions in terms of total nucleated cells (TNC) and CD34+ cells recovery, as well as cell viability.
Methods: 102 transplant procedures matched for age, sex, weight, and diagnosis, performed between December 2009 and September 2013 were selected. NSA was used in 51 procedures and Voluven in the remaining 51. T-Student test for paired data was used to study if each of the solutions was able to adequately recover the TNC and CD34+ cells and keep their viability. T-Student test for independent data was used to inquire if there was any difference between both solutions.
Results: The 290 washing procedures (142 with NSA and 148 with Voluven) needed to perform the 102 transplant procedures were analyzed. Confirming our previous studies, there were no significant differences in CD34+ cells numbers or cell viability before and after thawing using either NSA 1 (p 0,237 and p 0,306, respectively) or Voluven (p 0,760 and p 0,799, respectively). However, a significant number of CNT were lost with both solutions. When comparing both solutions, there were no significant differences in CD34+ cells recovery + (p 0,454) or cell viability (p 0,608). NSA presented an increased CNT loss (mean -0,809+/-1,303) than Voluven (mean: -0,588+/-0,535) with marginal significance (p 0,059).
Summary and Conclusions: Both NSA and Voluven are equally effective for washing cryopreserved hematopoietic stem cells with Sepax.

PB1895

THE INFLUENCE OF HAPLOTYPE HLA-A3/A11 IN EVOLUTION AFTER HSCT

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Background: It is known that some patients haplotypes are ligands for inhibitories and activatories donors KIR allele in HSCT.The same haplotype can be protective again postHSCT complication,like ligand or not.
Aims: Haplotype HLA-A3/A11 is described in literature like ,,good,, haplotype.In this study,we try to demonstrate the influence of this haplotype at patients with acute leukemia after HSCT.
Methods: Eighteen pairs patients-donors are evaluated :patients with acute leukemia,lymphoblastic and non-lymphoblastic and their genoidentics donors.Fifteen patients have HLA-A3/A11 haplotype.Following the impact of inhibitory KIR3DL2 and activatory KIR2DS4 on survival and complication development,we proved the protective efect of HLA-A3/A11 haplotype.The source of HSCT was PBSC.The method used was PCR-SSP (Innotrain DIAGNOSTIK GMBH,Dynal BIOTECH PEL-FREEZE)The complications like graft *versus* host disease acute and chronic,relapse,TMA and the recovery with leucocytes and thrombocytes are followed
Results: HLA-A3/A11 haplotype is protective for both types of leukemia,the patients survival is much better(56 month ,comparative with 10 month)with statistical significance(sig<0,05).Is protective against relapse,TMA ,cGVHD and leucocytes recovery,with statistical significance.Like ligand for KIR2DS4 improve survival.Like ligand for KIR3DL2,also improve survival,so,,missing ligand,, theory in this case is not confirmed.
Summary and Conclusions: Like ligand or not,HLA-A3/A11 improve survival and protect against most complication at patients with acute leukemia and related donors with 100% allele match.

PB1896

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR LANGERHANS CELL HISTIOCYTOSIS

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Background: Langerhans cell histiocytosis (LCH) is a reactive clonal proliferation of dendritic cells(1-3) that comprises a wide range of clinical presentations, from localized disease (single system disease) with excellent outcome to disseminated disease involving 2 or more organ or systems (multisystem disease, MS,LCH). Treatment has varied from conservative to intensive combination chemotherapy. When the systems involved are ''risk organs'' and/or the patient is younger than 2 years at diagnosis, MS-LCH has

been considered particularly devastating, and as carrying a potentially fatal prognosis. LCH treatment is not standardized, and based on anecdote. A strategy for the management of this disease needs to be developed. More intensive treatments than the standard prednisolone and vinblastine regimens are required, and the addition of methotrexate , etoposide or the cytarabine based regimens have been explored. Despite the treatment intensification, the mortality rate is approximately 40%. Refractory patients and those with multiple reactivations present a challenge. Cladribine, Cladribine – cytarabine arabinoside combination and clofarabine can be used as salvage therapy.
Aims: We report 3 patients with refractory Langerhans Cell Histiocytosis who were treated with hematopoetic stem cell transplantation.
Results: See Tables 1 and 2.

Table 1. Patients characteristics.

Table 2. Details of hematopoietic stem cell transplantation and outcome.

Summary and Conclusions: We report three resistant langerhans cell hystiocytosis which respond well to hematopoetic stem cell transplantation.

PB1897

SUCCESSFUL MOBILIZATION WITH PLERIXAFOR PLUS GRANULOCYTE COLONY-STIMULATING FACTOR IN PEDIATRIC PATIENTS
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Background: Collection of sufficient hematopoietic stem cells is required for autologous hematopoietic stem-cell transplantation (ASCT) after high-dose chemotherapy.
Aims: This study evaluated the clinical efficacy and tolerability of plerixafor, a CXCR4 receptor antagonist, in pediatric patients.
Methods: We retrospectively reviewed 13 patients (7 males, 6 females) who received plerixafor plus granulocyte colony-stimulating factor (G-CSF) for hematopoietic stem cell mobilization at Seoul National University Children's Hospital.
Results: We used plerixafor plus G-CSF in patients who previously failed peripheral blood stem cell mobilization by chemotherapy and G-CSF. All patients received G-CSF (10 μg/kg) for 4 days, without prior chemotherapy. Then plerixafor (240 ug/kg) and G-CSF (10 ug/kg) were administered subcutaneously, at 10 and 2 hours before each apheresis. All 13 patients were mobilized successfully, and the median number of CD34+ cells were 9.54 (range 3.17-28.97) x 10^6/kg after 1 to 3 cycles of apheresis without serious complications. Twelve ASCTs were performed and 1 patient is planning to have ASCT. Ten patients achieved neutrophil engraftment at median 12 days (10-13 days) after ASCT. Platelet engraftment was achieved at median 18 days (14-231 days) in 9 patients and 1 patient who is now at 22nd day of ASCT is waiting for the platelet engraftment. Two patients showed treatment-related mortality before engraftment.
Summary and Conclusions: Our study suggests that the mobilization with plerixafor and G-CSF could improve the success rate of peripheral stem cell mobilization in pediatric patients.

PB1898

AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS WITH PLASMA CELL MYELOMA OR LYMPHOMA
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Background: The feasibility and efficacy of high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation (auto-SCT) in elderly patients with plasma cell myeloma (PCM) or lymphoma are discussed.
Methods: A total of 392 patients have undergone auto-SCT between July 2010 and January 2014. Of these, 29 (7.4%) were elderly with a median age of 67 years (range 65-73 years). Most of the patients (72%) had PCM. The majority (79%) had received at least 2 (range 1-4) prior regimens of chemotherapy. Twenty-three (79%) patients had active or residual disease at the time of transplantation and 4 (13,8%) patients had received at least one cycle of melphalan or fludarabine or lenalidomide treatment. The median time from diagnosis to transplantation was 10 months (range; 5-135).
Results: Mobilization: 34 mobilizations were performed for 29 patients. In 3 patients, chemomobilization was repeated with another agent because of mobilization failure. Of 34 mobilizations, 21 were done with high-dose (HD) cyclophosphamide + G-CSF, 7 with HD etoposide +CSF, 4 with ICE + G-CSF and 2 with DHAP + G-CSF. Seventy-six percent of patients were able to have successful CD34+ cell collection (>2 x 10^6 /kg CD34+ cells) within a median 3 apheresis (range; 1-8) days. Median total peripheral CD34+ cell count was 9.2 cells/μl (range; 1.2-209). Median total CD34+ cells/kg collected was 8 x 10^6 (range; 0.14-31.3). Twenty (58.8%) of the mobilizations required at least one RBC transfusion and 5 (14.7%) required at least one platelet transfusion. Febrile neutropenia occurred in 7 (20.6%) mobilization. Transplantation: Source of stem cells was the peripheral blood in all patients. A high dose sequential chemotherapy regimen named as BEAM was employed as a conditioning regimen for lymphoma patients and high dose melphalan for PCM patients before auto-SCT. Median total infused stem cells was 5.4 x 10^6 cells/kg (range; 3.2-21.3). Time to neutrophil engraftment was 10 (9-13) days and time to platelet engraftment was 11 (9-17) days. Incidence of bacterial infection was 12.1%. No viral infection was demonstrated. A median admission day at hospital was 21 (13-36) days. Ten patients (34.5%) have shown relapsed or residual disease within a median follow-up 4 (1-33) months. Four PCM patients were undergone second auto-SCT. Of these one died due to intracranial hemorrhage in the first 100 day (1/29, first 100 day mortality 3.4%).Overall survival at one year was 77.5%. Non-relapse mortality at one year was 11.7% (2/17).
Summary and Conclusions: The current study seems to provide evidence for the efficacy of auto-SCT in elderly PCM and lymphoma patients. A prospective study of auto-SCT in elderly patients using strict eligibility criteria is required to evaluate the prolongation of survival in the era of novel agents.

PB1899

AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION (AHSCT) CAN BE A SUCCESSFUL THERAPY FOR PATIENTS WITH ADVANCED FOLLICULAR LYMPHOMA
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Background: Follicular lymphoma (FL) is one of the most frequent non-Hodgkin lymphoma (NHL) with a recurrent disease course. Standard induction treatment includes an anti-CD20 monoclonal antibody combined with chemotherapy. High dose chemotherapy (HDT) followed by autologous haematopoietic stem cell transplantation (AHSCT) is a method of choice for the pts who haven't achieved complete remission (CR) after the first line of treatment or achieved CR after at least the second line of treatment. However high-dose therapy followed by autologous stem cell transplantation (ASCT) is becoming more widely used, especially as a remission consolidation in patients (pts) with advanced disease or as second-line therapy, leading to improved progression-free survival (PFS) rates.
Aims: We here present the treatment's results of 54 pts with FL who underwent AHSCT between March 1993 and April 2013 in our Department.
Methods: There were 28 males and 26 females, with a median age of 45 (range 19-69 yrs). Ann Arbor staging at the diagnosis was as follows: II-9%(n=5), III-35%(n=19), IV-56%(n=30); 52%(n=28) of pts manifested B-symptoms. 83% of pts had FLIPI1 (International Prognostic Index for Follicular Lymphoma) 2(n=26), 3(n=24) or 4 (n=1). The induction treatment consisted of R-CHOP (rituximab, cyclophosphamide, vincristine, adriamycin, prednisone) in 21 pts, CHOP in 15 pts, R-CVP (rituximab, cyclophosphamide, vincristine, prednisone) in 10 pts ,CVP in 4 pts and other schema in 4 cases. Complete remission was achieved in 29 pts (53%), and a partial response in 25 pts (47%).Conditioning regimens preceding AHSCT consisted of CBV (karmustine, cyclophosphamide, etoposide) in 44 cases, BEAM (karmustine, etoposide,

arabinoside cytosine, melphalan) in 4 and Z-BEAM (zevalin, +BEAM) in 6 pts. A median number of transplanted CD34+ cells was 6,5 (1.7 - 27x10^6/kg).
Results: All pts successfully engrafted. Hematopoietic recovery was as following: WBC count > 1,0x10^9/L after median of 14 days (range 8-21 days), ANC> 0,5x10^9/L after median of 14 days (range 8-21 days) and platelet count >50x10^9/L after median of 14 days (range 10-21 days). The major complications after AHSCT were rare and included: bacterial infections of the respiratory tract (n=15), viral infections (n=10), oral mucositis (n=9).
Eight year disease free survival (DSF) was estimated to be 29% with a 9 year overall survival (OS) of 44%.
Summary and Conclusions: HDT followed by AHSCT seems to be a effective and safe procedure for FL pts with high risk at the diagnosis.

PB1900

STUDY OF FEW CYTOKINES GENES POLYMORPHISM AND EVOLUTION AFTER HSCT

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Background: Introduction:Cytokines gene polymorphism is part of transplant immunology with strong implication in evolution and development of early and late complication.This remain an open and acute problem for study and discussion.
Aims: We studied a lot of eighteen pairs ,patients with acute leukemia, lymphoblastic and non-lymphoblastic, and their donors ,for polymorphism genes of few cytokines.
Methods: Methods:All related donors and recipients had 100% HLA alleles match.The source of HSCT was PBSC. The following cytokines are observed:IL-1a pos 889,IL-1RA pos mspa 11100,gIFN pos 874,TGFβ1 codon 10,TNFa pos 308,IL-6 pos 174,IL-10 pos 1082,IL-10 pos 592.The method used was PCR-SSP (Dynal Genotyping SSP Kit)The complications like graft *versus* host disease acute and chronic, relapse,TMA and the recovery with leucocytes and thrombocytes are followed.
Results: Results:IL-1a pos 889 ,absence of CC/TC ,TNF-a pos 308 GA/GA,absence of GG/GG,IL-10 pos 1082 AA/AA are favorable for early recovery with thrombocytes(<17 days) with statistical significance.gIFN pos 874,absence of AT/AT are protective against TMA.IL-10 pos 592 AA/CA are also favorable for thrombocytes recovery.No influence,with statistical significance ,was established in our study.
Summary and Conclusions: Conclusions:A study like this,a small number of pairs,genoidentical,with PBSC like HSCT source,prouve us that some alleleles can influence the complication like TMA, and early thrombocytes recovery .

Hematopoiesis, stem cells and microenvironment

PB1901

PLATELET DERIVED GROWTH-BB INHIBITS PROLIFERATION OF HUMAN BONE MARROW DERIVED MULTIPOTENT MESENCHYMAL STEM CELLS IN PRESENCE OF FETAL CALF SERUM AND RAPIDLY SUPPRESSES PDGF-RB EXPRESSION

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Background: Platelet Derived Growth Factor (PDGF)-BB is a growth factor largely synthesized by platelets, although it is produced by other cells as well. It is a potent mitogen for cells of mesenchymal origin and a major source of growth activity in serum. We have previously found that local PDGF-BB levels in the human bone marrow (BM) endosteal region are twofold lower ($p<0.05$) in comparison to the vascular region. In contrast, the PDGF-Receptor b (PDGFRb or CD140b) was significantly higher expressed by Mesenchymal Stem Cells (MSCs) derived from endosteal than from vascular BM samples.
Aims: Here, we wanted to explore the interactions between PDGF and its receptor in order to unravel their role in the BM hematopoietic microenvironment.
Methods: Granulocyte-Colony Stimulating Factor (G-CSF) treated (n=8) and unstimulated (n=15) healthy donor BM samples obtained from endosteal (superficial) and vascular (central) bone marrow punctures were used to isolate MSCs. MSCs were maintained in complete medium (CM), consisting of 60% DMEM-LG/40% MCDB201, supplemented with 10% heat-inactivated FBS, 1% penicillin/ streptomycin and 2 mM L-Glutamin. MSCs were cultured up to passage 3 (P3) and when confluent MSC/P3 supernatants were collected, filtered 0.45 µm and stored at -20ºC until use. PDGF levels in supernatants from endosteal and vascular region derived MSCs were determined using the PDGF-BB human ELISA kit (Abcam, ab100624). Proliferation indices were assessed using the XCelligence Real Time Cell Analysis system (Roche, Turkey) and FACS analysis was performed for expression of CD140b (PDGFRb) using a FACSARIA (Becton Dickinson, Turkey).
Results: PDGF-BB levels in supernatants of unstimulated healthy donor MSC/P3 derived from the endosteal region (8,79±1,96 pg/mL) were found to be significantly higher ($p=0.01$) than in supernatants from vascular region MSC/P3 (5,25±2,41 pg/mL). G-CSF treatment of donors resulted in a significant increase ($p<0.001$) in PDGF-BB levels by MSC/P3, indicating a lasting effect of G-CSF treatment on bone marrow stromal cells. Stimulation of MSCs with doses of PDGF-BB ranging from 5-20 ng/mL in CM, resulted in a reciprocal effect, with increasing doses of PDGF-BB resulting in increased suppression of proliferation. Incubation with doses of PDGF exceeding 5 ng/mL resulted in irreversible suppression of proliferation. In contrast, doses of 5 ng/mL resulted in delayed proliferation, but after initial suppression, proliferation at day 10 was comparible to cultures maintained in CM only. In comparison to expression levels of CD140b by MSCs maintained in CM only (83.9%), incubation of MSCs in presence of 5 ng/mL PDGF-BB for 24 or 48 hours resulted in a rapid and reproducible initial decrease of CD140b expression to 31.3% at 24 hrs with some recovery of expression of CD140b to 44,6% at 48 hrs. In contrast, doses of PDGF in excess of 5 ng/mL resulted in permanent suppression of the PDGF receptor.
Summary and Conclusions: PDGF-Receptor expression by MSCs was rapidly adjusted in response to exogenously delivered PDGF-BB. Prolonged exposure to high doses (>5 ng/mL) of PDGF-BB resulted in irreversible suppression of PDGFR expression and MSC proliferation. Exposure to moderate doses of PDGF-BB (5 ng/mL) resulted in temporary suppression of PDGFR expression, followed by recovery, resulting in delayed, but overall not decreased proliferation of MSCs. Based on these results, we hypothesize that the low expression of PDGFR by MSCs from the vascular region is caused by locally produced high levels of endogenous PDGF-BB, whereas the increased expression of PDGFR by MSCs from the endosteal region is the result of relatively low levels of PDGF in the endosteal BM microenvironment.

PB1902

ASYMMETRIC ANEUPLOIDY IN ADULT MESENCHYMAL STROMAL CELLS AND IN BONE MARROW WITH HEMATOLOGIC MALIGNANCIES DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION: SUGGESTIONS FOR NORMAL REFERENCE VALUES FOR ST

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Background: *In vitro* culture of stem cells confers clonal heterogeneity with

emergence of cytogenetic aberrations. G-banding assay, most widely used for cytogenetic study, is not sufficient for detection of minor clones and sensitivity of molecular methods is too low. We investigated the frequency of cytogenetic aberrations of mesenchymal stromal cells (MSCs) by G-banding implemented with fluorescence *in situ* hybridization (FISH) and suggest the reference values for aneuploidy in MSCs.
Aims: The aims of this study were to use *in situ* karyotyping and FISH techniques to detect chromosomal abnormalities and aneuploidy in primary MSCs, to determine the most effective method to screen MSCs for medical use and to determine the criteria for the selection of safe MSCs, and to to comparison of the pattern of aneuploidy in stem cells with those of aneuploidy found in bone marrow cells from patients with hematologic malignancies, to have a guidance for assessing the significance of aneuploidy clones.
Methods: Cytogenetic analysis was done on 103 consecutive cultures from 68 kinds of MSCs. We compared the aneuploidy patterns of MSCs with those of 259 patients with hematologic malignancies and 22 patients with benign hematologic diseases.
Results: Interphase FISH showed variable proportions of aneuploid clones (1% to 20%) in 68 kinds of MSCs. The patterns of aneuploidy were asymmetric, and aneuploidy of chromosome 16, 17, 18, and X was most frequent. Clones with polysomy was significantly higher than those with monosomy ($P<0.001$). The cut-off value of maximum polysomy rates (upper 95-percentile value) was 13.0%. By G-banding, 5 among 61 MCSs presented clonal chromosomal aberration. The structural abnormalities frequently involved chromosome 7. When compared the aneuploidy patterns of hematologic diseases, patients with various hematologic malignancies presented heterogeneous and asymmetric patterns of aneuploidies in different chromosomes, while patients without malignant cells showed tetraploidy clones with symmetric pattern.
Summary and Conclusions: We suggest the cut-off value for aneuploidy as 13%, and FISH for aneuploidy of chromosome 16, 17, 18, and X would be informative to evaluate the genetic stability of MSCs. Though it is uncertain whether aneuploid clones might mean the senescent cell population or actually transforming cells, more attention should be paid for the safety of MSCs, and G-banding combined with FISH should be performed.

PB1903

CLEC12A: A NEW AML STEM CELL-ASSOCIATED ANTIGEN
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Background: Acute myeloid leukemias (AML), a heterogeneous and complex group of diseases, is a clonal malignant disorder derived from a small number of leukemic stem cells (LSCs), which are sustained by self-renewing and responsible for the propagation of leukemic blasts (LBs). Monoclonal antibodies have emerged as effective targeted therapies for the treatment of human malignancies and their mechanisms of action are able to deliver the therapeutic effects with minimal toxicity.
Aims: The challenge is the identification of cell surface antigens which could be preferentially expressed on AML LSC compared with normal hematopoietics stem cells and that could be helpful to target therapies.
Methods: On 16 AML patients (pts) at diagnosis (peripheral blood of 12 pts and bone marrow of 4 pts) myeloblast leukemic cells were identified using a FACSCanto flow cytometer (Becton Dickinson), based on low expression of CD45 and low side scatter (SSC) properties (CD45 low/SSC low). On these subpopulation we used an antibody panel against cell surface antigens, for the detection of immature markers and potential leukemia-associated antigens: CD34, CD38, CD90, CLEC12A (C-type lectin domain family 12 member A), CD44, CD99, TIM-3 (T cell immunoglobulin mucin-3), CD32, CD133, CD74, CD47, CD58, CD25, CD22, CD96.
Results: The proportion of LBs was positively correlated with CD45 low/SSC low (median 51,8%). This gated population of LBs were positive for CD34 (median 44,65%), CD38 (median 18,75%), CD90 (median 0,90%), CLEC12A (median 93,7%), CD44 (median 99,9%), CD99 (median 96,6%), TIM-3 (median 84,4%), CD32 (median 14%), CD123 (29,15%), CD133 (median (8,95%), CD58 (median 97,5%), CD47 (median 99,9%), CD74 (median 3,2%), CD25 (median 0,6%), CD96 (median 87%), CD22 (median 0,55%).
Summary and Conclusions: The expression of CD34 and CD38 antigens is heterogeneous in LBs. In particular we found that the 50% of patients were $CD34^+$ and 50% were $CD38^+$. CLEC12A, CD44, CD99, TIM-3, CD58, CD47 and CD96 were highly expresses in LCSs. CD90, CD32, CD123, CD133,CD74, CD25 and CD22 were low. Interestingly we identified that the expression of CLEC12A distinguished two different populations: the $CLEC12A^{high}$ cells correlated with the blast cells $CD45^{low}/SSC^{low}$; on the other hand, $CLEC12A^{low}$ cells could be compared with $CD45^{high}/SSC^{high}$ population, representing normal hematopoietic cells. In conclusion, this marker is a good candidate to target therapies against leukemic stem cells. However further studies with an higher number of patients must be carried out to confirm that CLEC12A is an appropriate antigen for a new monoclonal antibody-based therapy.

PB1904

POTENTIAL HEMATOTOXICITY OF NEW DRUG CANDIDATES MEASURED IN HEMATOPOIETIC PROGENITORS IN BONE MARROW SAMPLES
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Background: Hematotoxicity, the result of Bone Marrow (BM) failure, contributes significantly to morbidity and mortality by inducing severe infections and bleedings. Recently, knowledge of the specific genetic markers responsible for hematological malignancies and their associated signaling pathways has generated many new targets that promise to increase drug efficacy while reducing side effects such as hematotoxicity. However lesser hematotoxicity of drug candidates is not investigated until expensive preclinical studies in dogs. There is a need to estimate human hematotoxicity in early drug development.
Aims: To measure depletion analysis of different subsets of CD34+ progenitors in human healthy bone marrow samples that could reflect the degree of drug's induced hematotoxicity, using our flow cytometry-based automated Exvitech© platform.
Methods: 10 Normal Bone Marrow (NBM) samples at diagnosis from lymphoma patients prior to any therapeutic intervention with confirmed absence of BM infiltration by flow cytometry were included. For a first approach, we have selected two known and related nucleoside cytotoxic drugs (Cytarabine and Clofarabine). The whole sample was plated into 96-well assay plates containing 8 concentrations of each drug and incubated for 48-hours. A multiple staining (CD45v450/Anexin-FITC/CD117-PE/CD34PerCP/CD38-APC/CD19APCya7) was capable to identify and distinguish the most immature population ($CD34^+/CD45^{dim}/CD38^+$ or $CD38^-$), B-precursors ($CD34^+/CD45^{dim}/CD19^+$) from the more mature B-($CD45^+$/ $CD19^+/SSC^{lo}$), or T-($CD45^+$/ $CD19^-/SSC^{lo}$) lymphocytes. Drug response was evaluated as a depletion survival index of each cell population relative to the average of 6 control wells in each plate.
Results: As expected, both drugs induce hematotoxicity in most of the studied person's samples, but not all. Using the same drug concentration for each drug for all the patient samples, results reflect that Cytarabine has similar activity than Clofarabine in terms of efficacy (Emax: 28% *vs* 27%) but with 33-fold less potency (EC_{50}: 7μM *vs* 0.21μM) in the immature population. This reflects a lower hematological toxicity which is consistent with clinical practice. Interestingly, for both drugs there is a large range of interpatient variability inside this population in terms of efficacy (Cytarabine, range Emax: 2%>76% and Clofarabine, range Emax: 12%>42%) and potency (Cytarabine, range EC_{50}: 3μM-14μM and Clofarabine, range EC_{50}: 0.01μM-2μM) suggesting that in a subsets of vulnerable patients, drug doses could be tailored. Interestingly, the figure shows a person's sample where Clofarabine eliminates all precursors, while Cytarabine eliminates only 20% of the progenitors and would thus not be expected to cause hematotoxicity. Figure 1 show these drug's effects on progenitor subtypes CD34+, myeloid and totipotential; their dose responses are similar as expected given their non-selective mechanisms of action.

Figure 1.

Summary and Conclusions: These preliminary results show that Vivia Exvitech© platform is able to measure hematopoietic progenitors depletion in addition to other cell populations for novel drugs or before patient´s treatment that could contribute to a more selective drug development or a better clinical management of patients. Because increasing number of novel drugs with different mechanism of action are coming to the clinic, Vivia Exvitech© platform represent an attractive method to screen potential effects in any of the interested cell subsets, including the more immature ones that are associated with hematologic BM complications.

PB1905

HYDROXYCARBAMIDE DEMONSTRATES NITRIC OXIDE SYNTHASE DEPENDENCE IN PROLIFERATION AND APOPTOSIS
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Background: It has been reported that nitric oxide (NO) inhibition of erythroleukemic cell growth is due to inhibitory interaction with ribonucleotide reductase, similarly to the cytostatic hydroxycarbamide effect.
Aims: The study has been performed to investigate hydroxycarbamide dependence of NO in proliferation and apoptosis of K562 erythroleukemic cells.
Methods: The globin gene expression was examined by real-time PCR in human erythroid progenitor cultures, while quantity of bone marrow burst forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) was determined by colony assays in mice. K562 cell cycle analysis was achieved by flow cytometry with propidium iodide, while proliferation and apoptosis were evaluated by immunocytochemistry using Ki67 antibody and apoptotic index.
Results: To examine the erythroid phenotype during NO treatment, we showed the NO stimulation of γ/β ratio (1.7 fold) in erythroid progenitors, while *in vivo* NO synthase (NOS) inhibition by L-NAME significantly increased the number of BFU-E and CFU-E colonies in mouse model. Moreover, hydroxycarbamide and NO donors demonstrated permanent *versus* temporary inhibition of erythroid cell growth during their differentiation. Treatment of K562 erythroleukemic cells with NO donors (CysNO, DEANO or DETANO) and hydroxycarbamide resulted in a similar dose-dependent and steady inhibition of cell growth up to 40%. Cell cycle analysis revealed that prolonged treatment with NO donors resulted in a higher proportion (58-62%) of cells in G_0/G_1 phase, similarly to hydroxycarbamide (65%). DETANO and hydroxycarbamide also decreased the percentage of K562 cells in G_2/M phase. L-NAME reversed hydroxycarbamide effects, increasing percentage of K562 cells in G_0/G_1 (1.6 fold) and G_2/M (2 fold) phases and reducing a cell cycle arrest in S phase (1.6 fold). More profound inhibition of K562 cell proliferation, up to 50%, was observed with 50 μM hydroxycarbamide after 24 hours. NO donors significantly increased apoptosis both after 24 and 48 hours, whereas hydroxycarbamide preferentially augmented apoptosis after 48 hours of incubation in K562 cells. Hydroxycarbamide stimulation of apoptosis was 33% reduced by L-NAME both after 24 and 48 hours.
Summary and Conclusions: Hydroxycarbamide and NO donors share a long term stimulation of apoptosis and a short term inhibition of proliferation, inducing cell cycle arrest in the G_0/G_1 phase. Obtained results indicate that the effects of hydroxycarbamide on proliferation and apoptosis of erythroleukemic cells are NOS dependent.

PB1906

CYTOGENETIC CHARACTERIZATION OF BONE MARROW MESENCHYMAL AND HEMATOPOIETIC STEM CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND FROM HEALTHY CONTROLS

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Background: Mesenchymal Stem Cells (MSCs) are functional cellular components of the Bone Marrow (BM) microenvironment and their immunomodulatory and hematopoiesis support functions make them an ideal candidate for clinical application. Myelodysplastic Syndromes (MDS) are acquired clonal BM disorders characterized by ineffective hematopoiesis and high risk of evolving into leukemia. The role of BM MSCs in the pathogenesis of MDS remains elusive.
Aims: To confirm our previous results regarding the cytogenetic characteristics of BM MSCs in comparison to Hematopoietic Stem Cells (HCs) from MDS patients and healthy controls by expanding our sample pool and to examine the genetic stability of BM MSCs during passages (P).
Methods: Conventional cytogenetic analysis using GTG-banding was performed on BM cultured HCs and MSCs at P2 in 29 MDS patients and in 25 healthy controls, at P6 in 18 MDS patients and 10 healthy controls and at P10 in 10 MDS patients and 6 healthy controls. Whenever possible, 15 – 20 metaphases were analyzed. When needed, molecular cytogenetic analysis (Fluorescence *in situ* Hybridization - FISH) was performed.
Results: Conventional cytogenetic analysis of BM HCs revealed clonal abnormalities in 9/29 MDS patients (del(5q) n=4, del(5q) and an additional del(7q) subclone n=1, trisomy 8 n=2, loss of Y n=1, trisomy 11 n=1). The chromosomal analysis of the corresponding MSCs showed chromosomal abnormalities in 4/29 MDS patients (trisomy 7 n=1, del(12q) n=1, trisomy 5 n=1, 51,XY,+4,+5,+5,+7,+12 n=1) at P2, in 3/18 MDS patients (trisomy 5 n=2, trisomy 7 n=1) at P6 and in 1/10 MDS patients (trisomy 5 and an additional trisomy 5 and 7 subclone) at P10. Conventional cytogenetic analysis of BM HCs from all healthy controls showed normal karyotype. However, chromosomal analysis of the respective MSCs revealed chromosomal abnormalities in 2/25 healthy controls (trisomy 5) at P2 and in 1/10 healthy controls (trisomy 5) at P6. Using FISH technique, the presence of all abnormalities was verified, 2 more MDS patients and 2 more healthy controls were identified with trisomies 5 and 7.
Summary and Conclusions: The present results indicate that the cytogenetic abnormalities found in BM MSC cultures from MDS patients are different from those found in the corresponding BM HCs at diagnosis and accordingly they do not belong to the abnormal clone. It must be noted that most of the reported aberrations in BM MSCs are non – characteristic of MDS and have also been identified in MDS cases with normal HC karyotype and in MSCs derived from normal controls, implying that they could be attributed to the *in vitro* cell expansion process. MSCs cultures from both MDS patients and healthy controls displayed chromosomal stability during passages, however, they may develop irrelevant chromosomal aberrations with unknown pathophysiologic significance. Larger future studies are needed to examine the chromosomal stability of BM MSCs during passages, since it is of great interest their therapeutic potential in hematologic malignancies.
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PB1907

CHARACTERIZATION OF MICROPARTICLES FROM HEALTHY AND MALIGNANT BLOOD CELLS BY FLOW CYTOMETRY

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Background: Recently, microparticles (MPs) have been described as a new way of intercellular communication. MPs are plasma membrane fragments with sizes ranging from 0.1 to 1μm, containing products of the original cell, such as microRNA, mRNA and proteins that can be delivered to other cells. However, little is known about their phenotypic characterization.
Aims: Here, we would like to investigate the phenotypic characterization of healthy and malignant blood cell-derived MPs by flow cytometry.
Methods: We analyzed blood-derived MPs from platelets, erythrocytes, B-cells, T-cells, NK-cells, monocytes and chronic lymphocytic leukemia (CLL) B-cells. Cells were purified by positive magnetic-separation and cultured during 48h. Cells and MPs (obtained after ultracentrifugation at 20000g) were phenotypically characterized by the following monoclonal antibodies; CD19,20,184,45 for B-cells, CD3,8,5,27 for T-cells, CD16,56 for NK-cells, CD14,11c for monocytes, CD235a for erythrocytes and CD41,61 for platelets. All analyses were performed on a Navios cytometer, calibrated by Megamix Beads PLUS (Biocytex). Isolated MPs were stained with Annexin-V-FITC and gated between 300nm and 900nm. Then latex bead technique for easy detection of MPs (increasing element's size and antigen's detection) was also performed.
Results: For all samples, MPs defined as positive event for Annexin-V and included in gate of 300-900 nm of size were detected. MPs production was confirmed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In principle, MPs are characterized by antigen expression from mother cells. Our results showed that characteristic antigens of platelets (CD41 and CD61) and of erythrocytes (CD235a) were found on MPs. However, for other cell type-derived MPs, we were not able to detect any antigen present on the original cells (T, B, NK, monocytes or CLL-cells) while these antigens were clearly expressed on the cells. Using latex bead technique, we confirmed detection of CD41, CD61 on platelet-derived MPs and CD235a on erythrocyte-derived MPs. However, for all other antigens, results found turned out to be false positives, proved by the use of other type of negative controls (same labeling on MPs from different origins).
Summary and Conclusions: We observed that mother cell antigens were not always detected on corresponding MPs by direct flow cytometry or latex bead flow cytometry. Our results demonstrated that the characterization of the MPs is a difficult field requiring the use of several negative controls.

PB1908

SUCCESSFUL TREATMENT OF ADULT LANGERHANS CELL HISTIOCYTOSIS. A CASE REPORT

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Background: Langerhans Cell Histiocytosis (LCH) is a very rare and heterogenous disease that may affect various organs of the body. There is no standard first line therapy for LCH in adults, in contrast with the established therapeutic protocols in children. Therapy may be urgent, especially if vital organs are involved or if specific sites are affected, such as the facial bones that are associated with intracranial tumor extension.

Aims: To present the diagnostic difficulties and the selection of treatment during the eight-years clinical course of a young adult with LCH.
Methods: A 26 yrs-old male patient presented in 2009 at a periodontologist with tooth ache, gum tenderness and aggressive periodontitis not responding to treatment with antibiotics during the last three years. He had a history of cigarette smoking during the last ten years and suffered from a persistent cough during the last year. The diagnosis of LCH was based on a superior maxilla bone biopsy, which revealed characteristic morphological and immunological findings of histiocytosis from Langerhans cells (Cd1a+). The patient was referenced to our hematology clinic for further assessment. Screening tests revealed a simultaneous involvement of the zygomatic bones and the lungs according to a bone scan and a chest High Resolution Computerized Scan (HR CT), respectively. Therapy was based on the protocol of the first international study for adult LCH (LCH-A1). Initial treatment comprised Prednisone (60 mg/day), as a 4-week course (tapering over a period of 2 weeks), and vinblastine (8 mg/week) until day 36. The patient responded to initial treatment and continued with maintenance treatment with 6-mercaptopurine (50 mg/day), prednisone (60 mg, day 1-5, every 3 weeks) and vinblastine (8 mg day 1, every 3 weeks), administered for 12 months.
Results: The treatment with vinblastin and steroid was very well tolerated with no relevant toxicity. At the end of therapy, in 2010, there was no evidence of residual disease at the maxilla and zygomatic bones, and there was a clear improvement of the lesions in the lungs, according to HR-CT. Unfortunately, the patient restarted smoking and, six months later, HR-CT documented disease progression in the lungs. Then the patient stopped smoking but refused initiation of any therapy. In 2011, six months after cigarette smoke withdrawal, a new HR-CT showed no new lesions and even a slight improvement compared to the previous evaluation. On the contrary, both clinical and imaging findings from the facial bones were significant worse. We decided to retreat him with the same chemotherapy protocol. The patient tolerated the complete twelve-month therapy without any complications and significant improvement of the initial bone lesions was documented. Two years later he is still in follow-up, in a very good condition (Figure 1).

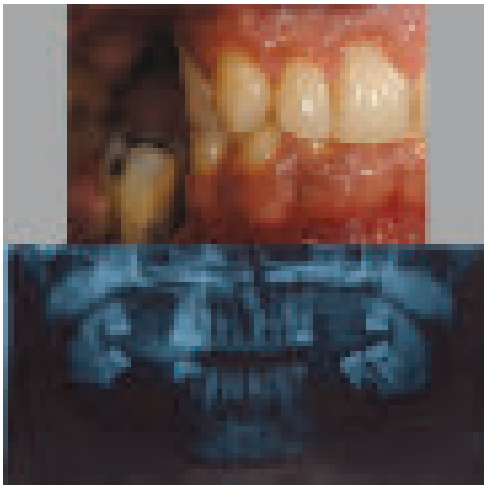

Figure 1.

Summary and Conclusions: Multidisciplinary approach is instrumental in diagnosis and treatment of LCH. Although the trial LCH-A1 had to be stopped due to insufficient accrual, treatment with vinblastin and steroid (even when repeated for a second full 12-month course) turned out to be safe and effective in this patient. The relationship with cigarette smoking in patients with pulmonary disease is well known and its role as a triggering agent is confirmed also in this case.

PB1909

IMPACT OF VITAMINS B12, B6 AND FOLATE SUPPLEMENTATION ON THE HOMOCYSTEINE METABOLISM OF AN ELDERLY COMMUNITY OF SHARPEVILLE, SOUTH AFRICA

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Background: In a vulnerable low income group, like the elderly in the day-care centre, with a confirmed high risk of cardiovascular disease an acute intervention is needed in order to improve their health profile. The effect of vitamin B12, B6 and folate supplementation on the inflammatory response, thrombotic risk, lipid profile, hypertension, risk for metabolic syndrome and the homocysteine metabolism in an elderly, black South African population has never been reported.
Aims: The aim of this interventional study was to assess the effect of vitamins B12, B6 and folate supplementation at 200% RDA for six months on the homocysteine metabolism of an elderly semi-urbanised black South African community.
Methods: A homogenous group of respondents were included into the study. All subjects were equivalent in age (>60 years), race (black), unemployed / pensioner (socio-demographic) and 60 years and older attending a day care centre in Sharpeville, situated in the Vaal region, Gauteng, SA. Informed consent for inclusion in this study was given by the participants. The distinctiveness of this study was the evaluation the red cell parameters, serum homocysteine levels in correlation with the increased nutritional intake of vitamin B6, B12 and Folate.
Results: A very high incidence (66.36%) of hyperhomocysteinaemia is present in the sample. The mean serum homocysteine level in group A decreased statistical significantly (25.30±8.00 µmol/l to 18.76±12.00 µmol/l), whereas the mean homocysteine level in group B increased (12.04±3.40 µmol/l to 13.64±6.00 µmol/l). Serum vitamin B12 and folate levels in both groups fell within the normal reference range. Although a slight but statistical significant decrease of these two parameters occurs after intervention in both groups, the mean levels were still within the normal reference range. Very low vitamin B6 levels were observed in both groups and improved statistical significantly after the intervention. In group A the serum vitamin B6 increased from 1.35±0.69 µg/ml (baseline) to 5.03±0.69 µg/ml (follow-up), in group B the serum vitamin B6 increased from 1.40±0.80 µg/ml (baseline) to 5.72±6.15 µg/ml (follow-up). The prevalence of macrocytosis decreased significantly. In group A the respondents with macrocystosis decreased from 31% (baseline) to 7% (follow-up) and in group B the percentage decreased from 23% to 5%.
Summary and Conclusions: It is concluded that a vitamins B12, B6 and folate supplementation at 200% RDA for six months had a homocysteine lowering effect in hyperhomocysteinaemic individuals but not for normohomocystaemic individuals. The supplementation was beneficial to all the individuals (independently of their homocysteine status) vitamin B6 serum levels and on their haemopoeiesis (decrease macrocytosis).

PB1910

CYTOGENETIC HETEROGENEITY AND DYNAMIC CHANGES DURING CANCEROUS TRANSFORMATION OF MESENCHYMAL STEM CELLS

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Background: G-banding analysis is widely used to detect the cytogenetic aberrations of mesenchymal stem cells (MSCs). However, G-banding reflects the chromosomal status of metaphase cells only, which compose less than 0.01% of the tested cells.
Aims: The aim of this study is to investigate whether the minority cells within a population have a minimal impact on the biology of population and are ultimately suppressed during passaging. Using interphase FISH combined with G-banding analysis, we tracked the quantitative changes of the cells with chromosomal aberrations during passaging.
Methods: In MSCs that acquire cytogenetic aberrations during cell culture, we tracked the quantitative changes of each clone in a population of heterogeneous clones during passaging, using G-banding and fluorescent *in situ* hybridization (FISH). Using FISH, we counted the number of chromosomes using enumeration probes (chromosome 7 and 9) and we also used locus specific probes and bacterial artificial chromosome probes to confirm structural anomalies. In each passage, telomere length was measured using interphase FISH.
Results: . In adipose MSCs and umbilical cord MSCs, 4 types and 2 types of chromosomal aberrations, respectively, appeared during passaging. Abnormal clones could be detected earlier using interphase FISH than when using G-banding analysis. Through all passages, the quantitative G-banding results were inconsistent with those of the interphase FISH analysis. Telomere length gradually decreased until a cytogenetic aberration appeared and then increased again.
Summary and Conclusions: The cytogenetic profile of late passage cells was similar to that of transformed cancer cells; however, G-banding did not detect abnormal clones in earlier passages. We observed that MSCs with only 1 aberrant cell, which does not fit into definition of clone, become predominant at late passages. FISH revealed hidden aberrations in stem cells that had normal karyotypes by G-banding analysis. It is critical to test chromosomal aberrations by both G-banding and FISH to ensure the safety of human stem cells that are manufactured *in vitro* for clinical application.

PB1911

COMPARATIVE ANALYSIS OF INTRACELLULAR NEUROTRPHIC FACTORS IN MONONUCLEAR CELLS OF CORD BLOOD AND G-CSF MOBILIZED PERIPHERAL BLOOD

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Background: Among a series of stem cell sources used to repair neurological diseases, intravenous administration of autologous cord blood (CB) has been used to try to counteract neurological injuries and impairments. We suggest that mobilized peripheral blood mononuclear cells (mPBMC) could potentially be used for treating neurological impairments, because stem cells from CB and mPBMC are comparable in long-term culture-initiating cells. While previous studies have investigated the cytokines and/or neurotrophic factors of mesenchymal stem cells derived from CB or mPBMC, to our knowledge there have been no comparative studies between mononuclear cells of CB and mobilized peripheral blood.
Aims: To investigate a possible use for cell therapy in the field of neurological disorders using mPBMCs as well as CB, we compared the expression of inflammatory cytokines and neurotrophic factors in PBMCs and mPBMCs from children with cerebral palsy (CP) to those from healthy adult donors and to CB donated from healthy newborns.
Methods: We evaluated the intracellular expression of neurotrophic factors and inflammatory cytokines in PBMCs and mPBMCs from 14 CP children and 14 healthy adult volunteer donors as well as CB mononuclear cells (CB-MNCs) donated from healthy newborns. Both PBMC collected prior to G-CSF administration and apheresed mPBMC were cryopreserved. We performed flow cytometric analysis with intracellular staining for various cytokines after thawing PBMCs and mPBMCs as well as CBs.
Results: In cells from CP children, the expression of IL-6 was significantly increased in mPBMC as compared to PBMC, and IL-3 was significantly decreased in mPBMC as compared to PBMC. In healthy adults, the expression of both IL-1β and IL-6 were significantly increased in mPBMC as compared to PBMC. The expression of BDNF in mPBMC from CP children was significantly higher than in CB or mPBMC from healthy adults. The expression of G-CSF in mPBMC from CP children was comparable to that in CB but significantly higher than in mPBMC from healthy adults. Lower expression of IL-1β, IL-3, and IL-6 and higher expression of IL-8 and IL-9 was observed from CB and mPBMCs from CP children rather than in healthy adults. Lower expression of IL-1β, and higher expression of IL-8 was observed from mPBMCs from CP children rather than in healthy adults.
Summary and Conclusions: The altered expression of neurotrophic factors and anti-inflammatory cytokines in mPBMC in CP children and CB from healthy children could provide a new potential source for cellular therapy for individuals with neurologic diseases.

Gene therapy, cellular immunotherapy and vaccination

PB1912

THE SECOND EPISODE OF ADOPTIVE IMMUNOTHERAPY WHEN REPEATED RELAPSE OF ACUTE MYELOID LEUKEMIA (AML) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (ALLO-BMT)

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Background: Chemotherapy followed by donor lymphocyte infusions (DLI) allow to achieve remission in the majority of patients with relapse AML after allo-BMT. Even so, repeated relapse of leukemia is the main cause of failure in adoptive immunotherapy.
Aims: The study the effectiveness of re-use adoptive immunotherapy in the second relapse after a first episode of adoptive immunotherapy. The T-cells for immunotherapy were obtained from a bone marrow donor.
Methods: 6 patients with AML aged 23-58 years (median 31), were diagnosed with a second relapse after allogeneic BMT from HLA-matched sibling donor. Median time after first successful treatment relapse following allo-BMT scheme chemotherapy + DLI (±IL-2) to the second relapse of AML was equal to 7 months (2-30 months). DLI the second relapse after chemotherapy was performed in the aplasia period (n=3) or in remission after chemotherapy (n=3). Total amount of the $CD3^+$cells varied from 1 to 41x10^7$CD3^+$cells/kg (median 28x10^7$CD3^+$cells/kg). After every DLI performed intravenous IL-2 in a dose of 6 MUE. Interval between transfusion was 1-4 weeks. Each patient performed 1-4 (median 2) DLI. Chimerism was monitored by PCR analysis (VTTR and STR) and by FISH – analysis for centromers of X and Y - chromosomes.
Results: In 5 patients achieved remission with full donor chimerism, which last from 4 to 127 months (median 6 months). In two cases, the duration of remission after a repeat episodes adoptive immunotherapy, including chemotherapy and DLI has exceeded a previous remission duration. 3 patients alive in remission with full donor chimerism.
Summary and Conclusions: DLI after chemotherapy for AML patients with repeated relapse after adoptive immunotherapy may be achieved long-term remission with restoration of full donor chimerism. In 83% of AML remission with recovery of 100% donor chimerism, which lasts from 4 to 127 months (median 6 months) after successful treatment with a second relapse after allo-BMT.

PB1913

EXPANSION OF NK CELLS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS USING GMP-COMPLIANT SUBSTANCES WITHOUT FEEDER CELLS FOR ADAPTIVE NK CELL THERAPY

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Background: NK cell receptor (NKR)-expressing cells have cytolytic activity against leukemic cells, and solid tumor cells escape from T cell recognition because of the low expression level of HLA class I molecules in both allogenic and autologous settings. This characteristic nature of NK cell recognition of target cells in contrast with T cells provides a strategy to overcome tolerance in the tumor-bearing host. Furthermore, donor alloreactive NK cell which was induced by killer cell immunoglobulin-like receptor (KIR) ligand incompatibility between donor and recipient HLA class I in graft-*versus*-host (GVH) direction can attack leukemic cells and host antigen-presenting cells (APC), resulting in the enhancement of graft-*versus*-leukemia (GVL) effect and the suppression of graft-*versus*-host disease (GVHD) at the same time. Therefore, adoptive transfer of NK cells is a potential immunotherapy to induce an anti-leukemia/tumor effect. However, expansion of NK cells from peripheral blood mononuclear cells (PBMC) without feeder cells is not easy for adaptive NK cell therapy.
Aims: In this study, we tried to expand NK cells from PBMC with antileukemic activity using good manufacturing practice (GMP)-compliant substances without feeder cells. We used tacrolimus to inhibit T cell proliferation and dalteparin to support NK cell proliferation during NK cell expansion using IL-2, IL-15 and OKT3 *in vitro*.
Methods: PBMC ($1x10^6$/mL) were cultured with IL-15 (10 ng/mL)(PeproTech Inc., Rocky Hill, NJ, USA), IL-2 (5 ng/mL) (R & D Systems, Minneapolis, MN, USA) and anti-CD3 mAb (OKT3, 10-1,000 ng/mL, Janssen Pharmaceutical Company, Tokyo, Japan) with tacrolimus (0.02-0.1 ng/mL, Fujisawa, Osaka, Japan) and dalteparin sodium (Fragmin, 5-10 U/ml, Pfizer Japan, Tokyo, Japan) in culture medium SCGM (CeeGenix, Freiburg, Germany) which was produced under GMP with 5% human AB serum without feeder cells. Cell cultures were split approximately one-second to one-fourth after 3-4 days of culture, and fresh medium, cytokines and reagents were added.

Results: After 3-weeks culture of PBMC (1x10^6/mL) with IL-15, IL-2, anti-CD3 mAb, dalteparin sodium and tacrolimus without feeder cells, CD56$^+$CD3$^-$ NK cells had increased by more than 3,000 fold with about 80% purity (before *vs* after culture, CD56$^+$CD3$^-$; 6.6±3.7 *vs* 83.2±7.6, CD56$^+$CD3$^+$; 0.9±1.0 *vs* 9.5±5.3, CD56$^-$CD3$^+$; 54.6±17.1 *vs* 3.3±2.0,%, n=7). Finally, we could obtain about 80x10^6 NK cells from 1x10^6 unmanipulated PBMC under GMP conditioned medium with 5% human AB serum without feeder cells (before *vs* after culture, CD56$^+$CD3$^-$ NK cells; 0.025±0.012 *vs* 80.6±25.7 x10^6, n=7). These expanded NK cells expressed stimulatory NK cell receptor NKG2D and intracellular cytotoxic molecule granzyme (before *vs* after culture, NKG2D$^+$CD56$^+$; 0.9±0.8 *vs* 76.1±9.0, CD56$^+$Granzyme$^+$; 1.3±2.3 *vs* 72.9±17.7,%, n=7). Also, the expanded CD16$^+$CD56$^+$ NK cells expressed inhibitory NK receptors, but significantly higher levels of stimulatory NK cell receptors including NKG2D and NKp44 than the levels of these receptors on CD16$^+$CD56$^+$ NK cells in resting PBMC before culture measured by mean fluorescence intensity (MFI) were noted. The cytolytic activities of expanded NK cells were tested against ^{51}Cr-labeled K562 human leukemic cell lines using standard 4-hour ^{51}Cr release assays. The expanded NK cells had a high level of cytolytic activity against the K562 leukemic cell line with specific lysis of more than 50% under the condition of an effector: target ratio (E:T ratio) of 10:1.
Summary and Conclusions: In this study, we could obtain more than 80 x10^6 NK cells from 1x10^6 unmanipulated PBMC using IL-2, IL-15 and OKT3 with tacrolimus and dalteparin sodium without feeder cells (more than 3,000 fold increase). Thus, autologous or alloreactive KIR-mismatched NK cells from PBMC might be able to expand for adoptive NK cell immunotherapy to induce an anti-leukemia/tumor effect for patients with malignant diseases.

PB1914

EXTRACORPOREAL PHOTOPHERESIS (ECP) AS SECOND LINE TREATMENT FOR REFRACTORY GRAFT VERSUS HOST DISEASE (GVHD): PRELIMINARY SINGLE CENTRE EXPERIENCE

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Background: Steroid-refractory GVHD is a common cause of morbidity and mortality and remains a challenging therapeutic problem after allogeneic hematopoietic stem cell transplantation (HSCT). There is currently no standard of care for this complication. ECP is an immunomodulatory therapy which has been used in the treatment of acute and chronic GVHD. We present our experience of ECP as second line treatment option for GVHD post-HSCT.
Aims: The aim of this analysis was to evaluate the clinical effect of ECP, its impact on intensity of immunosuppressive therapy, safety and tolerance of the procedure.
Methods: Since May 2012 we have performed 140 ECP procedures with the Therakos Cellex® device that is an integrated "on line" system. 8 patients are evaluable with median age 56y (47-65) and weight 52.5kg (38-71) presented with acute (2 patients) or chronic (6 patients) therapy-dependent/refractory GVHD. Allogeneic HSCT was myeloablative in 7 cases and reduced intensity conditioning in one case. Five patients were diagnosed of acute myeloid leukemia, two myelodisplastic syndromes and one acute lymphoblastic leukemia. The transplant was from an unrelated donor in 4 cases and 4 from a sibling donor, all of them HLA matched. All patients but one (with exclusive skin presentation) had multiorgan involvement, all suffered from skin changes in combination with liver, lung, oral or ocular disease. As first line treatment they all received esteroids plus cyclosporine or mycophenolate mofetil. Median ECP per patient was 15 (4-35). ECP procedures were performed for two consecutive days, in initial phase weekly (in those with aGVHD), or every two weeks (cGVHD) and then monthly according to clinical response. Median of ECP treatment duration was 4 months (1-26). Response was evaluated by clinical assessment and reduction in immunosuppression.
Results: In the cGVHD group response was complete in 3/6 (50%), partial in 3/6 (50%), ECP led to ≥ 50% improvement in symptoms and signs of cGVHD and significant reduction or even withdrawal of esteroids was observed. The first response was seen in skin and mucosal symptoms. Therapy of aGVHD led to complete remission in 1 patient, who finally died because of pneumonia, and no response was seen in other. The tolerance to the procedure was excellent and the unique complications seen were related with two central venous catheter infections.
Summary and Conclusions: In our preliminary experience, the ECP is associated with excellent tolerance, significant response rates and successful reduction of steroids in patients with cGVHD. More experience is required to assess response on aGVHD patients.

Red blood cells and iron - Biology

PB1915

HB BURGOS [ALPHA1 CD64(E13)(ASP→ASN)]: A NEW HEMOGLOBIN VARIANT

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Background: The HbA1c test by HPLC is a useful tool for the follow-up of diabetes mellitus patients. Some structural hemoglobin (Hb) variants caused by abnormalities in the genes encoding the globin chains are known to cause analytical interference in glycated Hb measurements.
Aims: In this study, it has been characterized a new Hb variant in four patients during their regular glicosylated Hb control.
Methods: Four patients with abnormal Hb elution peak during a routine check of glycated Hb were referred to our section. Glycated Hb analysis was performed using cation-exchange HPLC (Tosoh G8). HbA2 and F quantification and the separation of abnormal Hb were performed by ion-exchange HPLC (VARIANT™). The abnormal Hb also was separated by capillary electrophoresis (Sebia). The study of the globin chains was performed by reverse phase HPLC. Hematological data were obtained on a haematology analyzer (Coulter LH750). Genomic DNA from peripheral blood (Biorobot EZ1) was used for the molecular study. The most frequent mutations were ruled out by a-*globin StripAssay* (ViennaLab). The molecular characterization was performed by specific sequencing in an ABI PRISM 3100 Genetic Analyzer using the ABI PRISM BigDye Terminator V1.1 Cycle Reaction Kit (Applied BioSystems).
Results: A new structural variant of hemoglobin (Hb Burgos) clinically silent was detected by cation-exchange HPLC with a retention time of 1.02 (Figure 1) during the routine HbA1c test of four diabetes mellitus patients. An abnormal Hb was observed both by capillary electrophoresis (Hb A, Hb X and Hb A2) and by ion-exchange HPLC (Figure 1). A 17% of the Hb X was eluted by ion-exchange HPLC. The study of the globin chains by reversed-phase HPLC showed only two normal peaks (a and b) (Figure 1). Selective a1 gene sequencing showed a mutation GAC>AAC at codon 64. This produces a change of aspartic acid (Asp) by asparagine (Asn) identified as Hb Burgos [a1 64(E13)Asp>Asn]. This mutation was found in heterozygous state in 3 patients and in homozygous state in the last one.

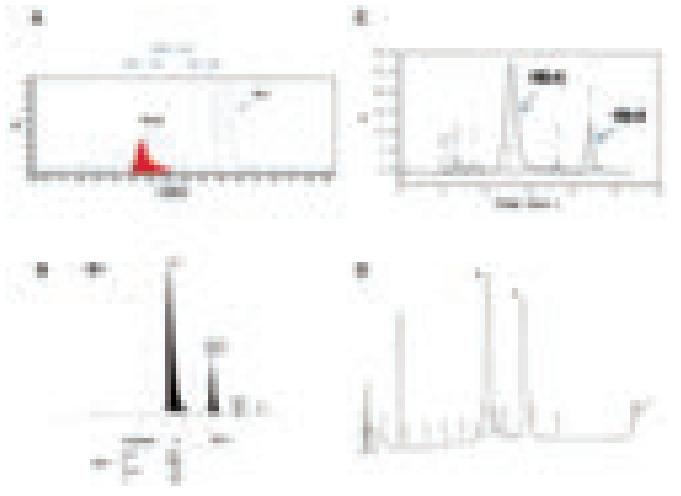

Figure 1.

Summary and Conclusions: The residue 64 is located on the outer surface of the molecule that is easily separated by capillary electrophoresis and HPLC. However, the change of Asp to Asn does not result in a functional change in the Hb that behaves as a silent hemoglobinopathy because it is precisely a change in the molecular surface. On the other hand, the percentage of Hb Burgos found by ion-exchange HPLC (13-17%) is somewhat lower than expected, which could be explained by the lower rate of a1 *versus* a2 gene expression. Besides, it has been found a homozygous case of Hb Burgos, where it has been found 34.3% of this variant by ion exchange HPLC. This finding corroborates the decreased expression of α1 *versus* α2 gene. This hemoglobinopathy and other structural Hb variants can be detected during the measurement of HbA1c and glycated Hb values may be altered. These cases, though rare, require examine the chromatograms thoroughly, for potential

interferences. Some pathological Hbs that interfere with the measurement of glycated Hb such as Hb G-Coushatta, Hb Queens and Hb Bologna-St.Orsola underestimate or overestimate the HbA1c levels which in some cases can be solved using a program with a longer elution time in the chromatography. However, since there are more than 1000 different Hb variants, many of which are rare, it is need to be aware when it comes to measuring HbA1c.

PB1916

VITAMIN B12 LEVELS IN SENIOR CITIZENS REMAIN STABLE AS THEY GET OLDER

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Background: The evolution of the vitamin B12 backup in senior citizens is ill defined but decrease in levels is anticipated from reduced haematinic provision and/or impaired intestinal uptake.
Aims: To recruit healthy senior citizens (722 f, 557 m) passing a severe selection process to exclude relevant comorbidities and to analyze in their fasting serum samples for Vitamin B12 and surrogate markers methylmalonic acid (MMA), holotranscobalamin (HoloTC) and homocysteine confirming/denying vitamin B12 deficiency.
Methods: Abbott Architect analyzer and SCIEX automatic platforms were used in our routine laboratory flow-charts. HoloTC was measured using an Axsym platform. The Mentzer index computes MCV related to RBC count.
Results: See Table 1.

Table 1.

	60-69 yrs	70-79 yrs	>80 yrs	P values*
MCV (fl)	89	90	91	<0.001
B12 (pmol/L)	239	230	237	0.09
HoloTc (pmol/L)	53	56	54	0.38
MMA nmol/L	196	215	234	<0.001
Glomerular FR (ml/min/1.73m2)	110	98	82	<0.001
Homocysteine (µmol/L)	12	14	16	P<001
Mentzer index	19	19	20	<0.001

Summary and Conclusions: Whereas the vitamin B12 levels remain steady over the age range, we see a significant increment of median MMA levels which go along with increments of homocysteine, a homologue of cysteine known to accumulate in vitamin B12 deficiency sometimes used as a biomarker to predict cardiovascular risk events. Whereas it is possible that the MMA levels rose because of GFR reduction or because of vitamin B12 deficiency remains to be determined.

PB1917

RED CELL INDICES FOR BETA THALASSEMIA TRAIT SCREENING

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Background: Isolated microcytosis and microcytic anemia due to β thalassemia trait are often wrongly diagnosed as iron deficiency anemia. Red cell indices such as red blood cells (RBC), Hemoglobin (Hb), middle cell volume (MCV), middle concentration of Hb (MCH) and Mentzer index (MI) may help for suspecting β thalassemia trait.
Aims: This study aimed to establish cut off values of these indices for the screening of β thalassemia according to Hb level.
Methods: From July 2001 to December 2012 the laboratory has received 2284 request for Hb exploration. Each patient beneficed of blood cell count, Hb electrophoresis at alkaline pH and dosage of HbA2 by ion exchange chromatography. The diagnosis of β thalassemia trait was based on a rate of Hb A2 ≥ 4% and the family study. Receiver operative characteristic curves were constructed to establish cut off values.
Results: A total of 835 subjects were considered carrying β thalassemia trait, among whose 697 (83.5%) have microcytic anemia. In patients with anemia (7< Hb<12 g/dl), RBC≥ 4.6 10^6/mm^3, MCV≤ 68 fl, MCH≤ 21 pg or MI≤ 14 allow to suspect β thalassemia trait with a sensitivity varying from 0.96 and 0.98. In patients without anemia (Hb≥ 12 g/dl), RBC≥ 5.2 10^6/mm^3, MCV≤ 71 fl, MCH≤ 23 pg or MI≤ 14 allow to suspect the β thalassemia trait with a sensitivity of 100%.
Summary and Conclusions: Red cell indices are very helpful for suspecting β thalassemia trait. The choice of optimal cut off values should take into account Hb concentration. Determination of HbA2 level remains necessary to confirm β thalassemia trait.

Red blood cells and iron - Clinical

PB1918

BONE MARROW IRON ASSESSMENT AND FAT QUANTIFICATION BY MRI-R2* IN PATIENTS WITH IRON OVERLOAD

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Background: Patients with iron overload suffer from different organ damage due to increased iron concentration. Liver failure, heart failure and loss of endocrine function are well known complications in iron overloaded patients. Iron overload in the bone marrow may play an additional role in these patients and is until now not well examined.
Aims: To perform MRI-R2* measurements in the vertebral bone marrow using water-fat chemical shift relaxometry for estimation of iron and fat content in comparison to hepatic and splenic iron concentrations in patients with red cell aplasia and hereditary hemochromatosis (HHC).
Methods: Patients with iron overload (n=112, mean age: 32 y, *e.g.* transfusion dependent thalassemia major (TM), Diamond-Blackfan anemia (DBA) and HHC) and 14 control subjects underwent MRI for determination of the transverse relaxation rate R2* assessed from ROI based signal intensities of one transversal slice (10mm) through the liver, spleen, and mid-vertebral bone marrow. Breathhold water-fat relaxometry (12 echo times, TE=1.3–26ms, FA=20°, bandwidth=1955Hz/px) was performed to determine apparent fat contents (aFC) and bone marrow R2*. Additionally, serum ferritin values and other hematological parameters were assessed.
Results: Relative to controls (n=14, R2* = $95s^{-1}$) and HHC (n=10, R2* = $95s^{-1}$), median bone marrow R2* rates were significantly increased in patients with TM (n=65, R2* = $398s^{-1}$, $p<10^{-4}$) or DBA (n=12, R2* = $252s^{-1}$, p=0.005). R2* of the bone marrow significantly correlated with serum ferritin (r_S=0.52, $p<10^{-4}$), splenic R2* (r_S=0.43, $p<10^{-4}$), cardiac R2* (r_S=0.43, $p<10^{-4}$), and hepatic R2* (r_S=0.37, $p<10^{-4}$). No significant correlation of aFC with marrow R2* could be obtained. However, these latter findings are currently spoiled by the ambiguity of fat or water dominance at 50%.
Summary and Conclusions: Determination of R2* rates for estimating iron overload can simultaneously be assessed in the liver, spleen and vertebral bone marrow. Water-fat chemical shift relaxometry allows precise determination of bone marrow R2* rates and estimation of the apparent fat content, which may add additional information to these patients. Patients with transfusion related iron overload have significant higher bone marrow iron content than patients with iron overloaded due to hereditary hemochromatosis.
Clinical Relevance: Water-fat chemical shift relaxometry has the potential for accurate determination of bone marrow R2* rates and allows monitoring of iron trafficking especially in patients with red cell aplasia under different transfusion and chelation treatment regimens

PB1919

TARTRATE-RESISTANT ACID PHOSPHATASE TYPE 5B AS A MARKER OF OSTEOCLAST ACTIVITY IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL DISEASE: RELATION TO BONE DISEASE

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Background: Bone involvement is a frequent cause of acute morbidity in sickle cell disease (SCD). It ranges from acute manifestations, such as painful vaso-occlusive crisis or osteomyelitis, to more chronic and debilitating complications, such as osteonecrosis, osteoporosis and osteopenia, impaired growth and chronic infections. Iron overload was reported to be associated with a lower bone mass index. However, current information is inconclusive for a possible correlation between bone mineral density (BMD) and iron stores. Tartrate-resistant acid phosphatase (TRACP) type 5 is an enzyme that is produced by bone-resorbing osteoclasts, inflammatory macrophages and dendritic cells. TRACP 5b is the bone resorption marker which is produced specifically by activated osteoclasts. Normal bone metabolism requires TRACP 5b expression. Adults with SCD have increased osteoclast activity and high TRACP 5b levels due to a potential role of inflammation rather than increased iron stores.
Aims: To assess BMD in young patients with SCD and asymptomatic sickle cell trait (SCT) siblings, determine levels of TRACP 5b and assess its possible relation with markers of hemolysis , iron overload and bone complications.
Methods: Thirty children and adolescents with SCD and 8 asymptomatic siblings with SCT were compared with 32 age- and sex-matched healthy controls. Patients were studied stressing on anthropometric measures, transfusion history, hydroxyurea therapy, hematological profile, serum ferritin, serum calcium and alkaline phosphatase (ALP). TRACP 5b was measured by

enzyme linked immunosorbent assay (ELISA). Assessment of BMD was performed for whole body and lumbar spine using dual energy X-ray absorptiometry (DEXA) technique.
Results: Among SCD patients, 66.7% received hydroxyurea and 60% were on chelation therapy. Bone fractures and avascular necrosis were found in 13.3% and 10% of SCD patients, respectively, and none in SCT .BMD was decreased in SCD patients as defined by Z score (mean -2.11±1.74) compared with SCT (mean- 0.79 ±1.1) and controls (mean -0.85±0.88); p=0.005, while no significant difference was found between the latter 2 groups. SCD patients had higher incidence of low bone density (40.7%) and osteoporosis (7.4%) than SCT group (12.5% and 0.0%, respectively) and controls (4.8% and and 0.0%, respectively); p=0.03. SCD patients with abnormal DEXA scan had higher age (13.30 years±2.82 *versus* 9.26±3.6 years), serum ferritin (3427.82±2401.6 *versus* 1738.79±2008.8 μg/L) and ALP (251.30±127.51 *versus* 149.16±58.2 IU/L) compared with SCD with normal BMD. Abnormal BMD was associated with higher incidence of splenectomy (36.4% *versus* 0.0%; p=0.005) and endocrine abnormalities (45.5% *versus* 5.3%; p=0.008). Serum TRACP 5b was significantly higher in SCD patients (4.10±2.72 U/L) than SCT (2.50±1.39 U/L) and controls (1.89±0.81 U/L) while levels were consistent between traits and controls. Hydroxyurea-treated patients as well as those on chelation therapy had lower TRACP 5b levels than untreated patients although levels did not reach a significant level. TRACP 5b was positively correlated to lactate dehydrogenase (LDH) (r=0.363, p=0.045) while no relation with other variables including serum ferritin, ALP,and BMD. BMD was positively correlated with body mass index (BMI) (r=0.624, p<0.001) while negatively correlated with platelet count (r=-0.686, p<0.001) and ALP (r=-0.402, p<0.001). There was positive correlation between whole body and lumbar spine BMD (r=0.919, p<0.001).
Summary and Conclusions: Ostopenia and osteoporosis frequently occur in SCD patients as reflected by decreased BMD and high ALP and TRACP 5b levels. The lack of correlation between abnormal DEXA scan and high TRACP 5b levels suggests that bone disease in SCD is multifactorial . Hemolysis and iron overload may be contributing factors in the occurrence of these complications .However longitudinal studies with large number of patients could identify the interaction between different factors contributing to decreased bone density in SCD.

PB1920

THE RED BLOOD CELLS' (WT-BETA+GAMMA)/ALPHA RNA RATIOS CORRELATES STRONGLY WITH THE BETA-THALASSEMIA MAJOR OR BETA-THALASSEMIA INTERMEDIA TYPES IN PATIENTS HOMOZYGOUS FOR THE IVS-II-1 MUTATION

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Background: Previously, we had studied the association of 5 genetic markers [beta-globin mutations (beta^{o},beta^{+},beta^{++}), co-inheritance of alpha-thalassemia (alpha-thal), and single nucleotide polymorphisms (SNPs) in the XmnI, BCL11A, and HBS1L-MYB loci] with the beta-thalassemia major (beta-TM) and intermedia (beta-TI) types in a cohort of 306 Iranian patients. Using multivariate regression analysis, a strong correlation was observed between beta^{++} mutations, the XmnI T allele, and the BCL11A rs4671393 (A) allele with beta-TI [Banan et al (2013) Hemoglobin 37(5), 413]. A scoring system based on odds ratios (OR) was used to facilitate the beta-thal type prediction (beta^{++} mutations=2 points, XmnI T allele=1 point, BCL11A rs4671393 (A) allele=1 point). Using this system, patients with a low score of (0, 1) were predominantly beta-TM whereas those having a high score (≥3) were beta-TI. Prediction, however, was especially difficult in the IVS-II-1 homozygous patients, largely composed of those having a score=2.
Aims: We hypothesized that variations in wildtype (Wt)-beta/alpha and gamma/alpha levels may account for differences in the disease severity of these patients. To gain insight, we determined the (Wt beta+gamma)/alpha RNA ratios in the RBCs of a subset of these patients (n=16 beta-TM, 15 beta-TI) as well as in healthy adults (n=4).
Methods: After acquiring informed consent, approximately 5 ml of fresh blood was obtained from patients in EDTA-containing tubes. RNA was extracted from red blood cells (RBC) as previously described [Kabanova et al (2009) Int J Med Sci 6(4), 156], and its quality assessed by gel electrophoresis. The RNA was DNase I treated and subjected to reverse transcription. For PCR of the full-length beta-globin cDNA, primers spanning exons 1 and 3 were used. Real-time PCR of the alpha-, beta-, and gamma-globin RNA were performed by using previously described primer sets (Smith *et al.* (2000) Blood 95 (3), 863.) and SYBR-green on an ABI 7500 system.
Results: PAGE and sequencing analyses of the full-length beta-globin cDNA suggested the presence of a number of alternatively spliced products in the IVS-II-1 homozygous patients. The predominant RNA products, however, consisted of an exon 1-3 RNA and the Wt exon 1-2-3 RNA. These results suggested that primers spanning exons 2 and 3 could be used to quantify the Wt beta-globin by qRT-PCR. Real-time PCR experiments showed variation in the beta/alpha and gamma/alpha RNA ratios among patients (from 0 up to7). Furthermore, in many cases the beta-TM patients had high (rather than low) levels of gamma/alpha transcript levels. Nevertheless, the (Wt beta+gamma)/alpha RNA ratios seemed to be a good predictor of the beta-TM/beta-TI type [control= 1.57±0.57; beta-TI= 2.12±0.67; beta-TM= 5.5±0.94.] Using a 2-tailed t-test to compare beta-TM and beta-TI, P <0.01.
Summary and Conclusions: The (Wt beta+gamma)/alpha RNA ratios seems to be a good predictor of the beta-TM/beta-TI types in the IVS-II-1 / IVS-II-1 patients. Furthermore, our results suggest that unidentified factors influencing beta-globin splicing (resulting in Wt beta-globin RNA production) and gamma-globin expression (other than the known HbF QTLs) may affect the beta-TM/beta-TI types in these patients.

PB1921

NON-TRANSFUSION DEPENDENT COMPOUND HETEROZYGOTES FOR THALASSAEMIC MUTATIONS. REPORT OVER A 15 YEAR- PERIOD IN NORTHERN GREECE

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Background: Greece is a Mediterranean country with a high frequency of haemoglobinopathies. The β-Thalassaemias constitute an heterogeneous group of disorders with a reduction of β-chain synthesis. The interaction of classic β-Thalassaemia mutations with mild β-globin gene mutations or several Haemoglobinopathies results in the clinical phenotype of Thalassaemia Intermediate. Since 1974 a nation wide program for Thalassaemia prevention has been implemented. Through population screening program that is performed, natives and immigrants are screened and counselled free of charge.
Aims: We report the non-transfusion dependent compound heterozygotes for thalassaemia detected over a 15 year period (1999-2013) in Northern Greece through the population screening program.
Methods: The carrier identification was carried out by a standard scheme, which included, CBC and red cell indices using the Coulter ONYX, a Cation Exchange HPLC variant system (Bio-Rad, Variant β-Thalassaemia Short Program), to determine HbA, HbA2 and HbF levels and the different abnormal structural haemoglobins, electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar, sickling test and tests for HbH inclusion bodies. Haemoglobin A2 was also quantified by column micro chromatography and serum ferritin levels were measured by micro Elisa technique. Biosynthesis of the α- and β- globin chains and DNA techniques are also performed on selected cases.
Results: From the total 35.797 subjects who underwent screening during this period we identified 34 cases of compound heterozygotes for thalassaemic mutations with mild phenotype, non-transfusion dependent. Nine cases of compound heterozygotes for severe β-thalassaemia mutations and the "silent" -101C-T mutation, 4 cases of common severe thalassaemia mutations and the +1480 C-G silent β-gene, 4 cases of beta thalassaemia and delta beta thalassaemia with mild phenotype, 11 cases of beta thalassaemia and triple α. They all had minimal bone changes and satisfactory growth.We also detected two cases of compound heterozygotes for beta thalassaemia/D-Punjab, 2 cases for β-thalassaemia/ O Arab, one case for α-thalassaemia/Hb E and one case of E-Saskatoon- β-thalassaemia. They all were non transfusion dependent except three cases of women than were transfused during their pregnancy. The two of them were compound heterozygotes of common severe thalassaemia mutations and the +1480 C-G silent β-gene and one carried the IVSI-1 and triple α. Pertaining to their ethnic background, they all were of Greek origin except one coming from Albania and one of Pomakic origin (β-thalassaemia/ O Arab).
Summary and Conclusions: The identification of such combinations is important in the genetic counseling of couples at risk, in a country such as ours where the high frequency of thalassaemia and haemoglobinopathies has a major impact on public health.

PB1922

PREGNANCY IN BETA-THALASSEMIA INTERMEDIA: A 20-YEAR EXPERIENCE OF A GREEK THALASSEMIA CENTER

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Background: Thalassemia is the most commonly inherited disease worldwide. Thalassemia intermedia (TI) represents a phenotype ranging from thalassamia major (TM) to thalassemia minor. Progress in the management of patients TI enabled increasing rates of pregnancies among TI women worldwide. Nevertheless, information regarding TI pregnancy management and outcome is quite limited in the literature.

Aims: The aim of this study was to report our experience regarding the maternal and fetal outcome of TI patients.

Methods: Approximately 250 patients with TM and 275 with TI are treated and followed up regularly in our center. We prospectively collected data for all pregnancies during the last 20 years. Therefore we analyzed our data recorded from 60 pregnancies in 34 women over this period of time.

Results: Thirty four patients achieved full-term pregnancies (mean maternal age±SD: 27.4±6.5 years) within 37±3 gestation weeks. Their mean hemoglobin value was 8.33±1.22 g/dl; 26.5% of patients were not transfused at all or they had been transfused only once during gestation. Caesarean section was performed in 29/44 (65.9%) of natural pregnancies and in 4/5 of IVF (80%) pregnancies. Six patients had more than two normal deliveries. There was one extra-uterine pregnancy while there were no stillborn births. There were eleven abortions (18.3%) including 6 spontaneous (10%) and 5 (8.3%) for medical reasons. The spontaneous abortions (5/11) were related to high HbF levels. Nineteen newborns (38.8%), which weighed 2-3 kg, required hospitalization to an intensive neonatal care unit for 1-3 weeks. Ten (29.4%) women required none or few transfusions (two units or less) during the first or the second pregnancy while they were transfused during the second or third pregnancy. Eighteen (52.9%) women were transfused up to 36 units during gestation. A 28 year old woman with beta/delta-beta thalassemia (FSC6/$\delta\beta^{Sic}$), no prior need for transfusions, normal spleen size and almost 100% HbF in Hb electrophoresis presented with decreasing Hb levels and platelet counts and progressive splenomegaly during the 6th month of her first pregnancy. The patient was regularly transfused until delivery because of hypersplenism and severe haemolytic anaemia. Due to worsening anemia and thrombocytopenia splenectomy was performed one month after delivery with full resolution of cytopenias. In another case, two consecutive pregnancies of a single patient were complicated by two episodes of spastic paraparesis due to compression of the spinal cord by extramedullary paravertebral masses. The woman developed asymmetrical weakness in her lower extremities, hypoesthesia, paresthesias, hyperreflexia, and bilateral Babinsky responses during the 6th month of pregnancy. A spinal MRI investigation revealed extensive bilateral paravertebral masses spanning from T2 to T9 together with corrosion of the vertebral bodies. The patient was successfully treated by hypertransfusion and local irradiation.

Summary and Conclusions: Although several complications can occur during a pregnancy in TI women, the careful and frequent monitoring by Special Units and multidisciplinary management by both haematologists and obstetricians can lead to successful deliveries.

PB1923

SAFETY AND EFFICACY OF COMBINATION OF DEFERIPRONE AND DEFERASIROX IN PATIENTS WITH THALASSEMIA MAJOR

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Background: Combination of two oral iron chelators, deferiprone (DFP) and deferasirox (DFX) looks promising as these target separate iron pools and do not require parenteral administration.

Aims: To study safety and efficacy of combination of deferiprone & deferasirox in thalassemia patients

Methods: Forty consenting patients (5-18 yrs) were randomized to 3 groups. Group 1 (n=10) and Group 2 (n=10) received DFP (75-100 mg/dl) and DFX (30-40 mg/kg/d) respectively. Group 3 (n=20) received both drugs administered sequentially every alternate week. Cardiac and liver MRI were done at beginning and end of the study. Serum ferritin was done every 3 monthly. CBC, liver enzymes and renal function tests were performed monthly.

Results: Mean cardiac MRI T2* at the beginning of the study were 28.67±4.56, 29.97± 4.01, 29.75±4.66 ms in group I, II, III respectively. Cardiac MRI T2* at the end of the study was slightly better in group I as compared to group III although was not statistical significant (p=0.07). Mean values for liver MRI T2* were 6.19±1.97ms, 5.89±0.70ms & 5.62±0.99 ms in group I, II and III respectively. Liver MRI T2* was not significantly different before and after the study. Serum ferritin reduced significantly in group I and II but not in group III. Group receiving combination therapy did not show any untoward side effects as compared to single drug regimen.

Summary and Conclusions: Combination therapy is safe but not more effective as either drug alone.

PB1924

COMPARATIVE ASSESSMENT OF LIVER IRON CONCENTRATION IN PATIENTS WITH THALASSEMIA BY MRI AND FERRISCAN

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Background: Iron overload is a significant problem in hemoglobinopathies. Iron accumulation affects mainly the endocrine glands, the liver and the heart leading to life-threatening complications. Therefore accurate monitoring of total body iron is clinically needed in thalassemia. Magnetic resonance imaging (MRI) gradient echo (T2*), the reciprocal of T2* (known as R2*) and spin echo (T2) techniques have been developed to quantify tissue iron in the liver and the heart. More recently St. Pierre *et al* (Blood 2005;105:855-61) described an accurate method of determining hepatic iron concentration (HIC) using R2 (where R2=1/T2) signal decay rates with a high correlation found between R2 values and biopsy results, commercially available as FerriScan.

Aims: The aim of this study is the comparative assessment of liver iron concentration in patients with thalassemia by MRI and by the more recent technique FerriScan.

Methods: We prospectively studied 15 patients (7M/8F, median age 45 years, range 40-61 years) with thalassemia major who are followed-up in our Center. All patients had MR examinations of the liver that were performed on a General Electric 1.5T Signa HDxt scanner (GE Healthcare, Milwaukee, USA). The pulse sequence used to estimate the T2* relaxation time of the examined tissues is a multi-echo fast gradient echo which has the ability to acquire 3 to 16 echoes in a breath hold time of 12 to 18 sec. The post processing of the data is performed with use of relevant software on a GE Advantage Windows 4.6 workstation according to the relevant literature. On the same day serum ferritin was also measured while hepatic iron concentration (HIC) was obtained using FerriScan. More specifically, images were transmitted to the FerriScan Analysis Centre in DICOM format according to the FerriScan protocol. Images were analyzed off-site, as previously described by St Pierre et al, and average HIC results were returned electronically to our center.

Results: Liver T2* and R2* values were (mean±SD) 7.82±6.38 mgr/gr and 303±257 Hz, respectively. HIC values as determined by FerriScan were 132±135 mmol/kg, while ferritin levels were 700±652 ng/ml. There was a strong correlation between R2* and FerriScan values (r=0.937, p<0.0001). Both R2* and FerriScan values strongly correlated with ferritin levels (r=0.709, p=0.004 and r=0.720, p=0.004 respectively).

Summary and Conclusions: We were able to demonstrate a very strong positive correlation between R2* values and FerriScan determined HIC. This could potentially lead to more timely patient results and cost savings.

PB1925

CORRELATION BETWEEN CLINICAL FEATURES AND HEMATOLOGICAL-COAGULATIVE PARAMETERS IN PEDIATRIC CARRIERS OF SICKLE CELL DISEASE

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Background: Sickle Cell Disease (SCD) is the most frequent structural hemoglobinopathy in the world.Sickle Cell Trait (HbSA) affects 300 million people worldwide and is considered as a benign state. However, several cases of heterozygous adult patients with the clinical manifestations of the SCD subjects were recently described, including: hematuria, renal papillary necrosis, ipostenuria, splenic infarcts, sudden death with rhabdomyolysis related to intense physical activity, acute chest syndrome (ACS) and painful vaso-occlusive crisis (VOC). In addition, an increased incidence of renal medullary carcinoma in HbSA patients has been reported.

Aims: 1) To describe a pediatric population with HbSA from a clinical, hematological and coagulative point of view. 2) To compare clinical and laboratory characteristics of HbSA children with those of SCD children (HbSS, HbSβ^0, HbSC) and healthy controls. 3) To possibly identify a subgroup of HbSA children who present clinical features of the affected SCD patients.

Methods: At our Center HbSA patients receive clinical evaluation and blood test once a year. Hematological and coagulation parameters were determined during the once a year blood test. Demographic, clinical and laboratory data of HbSA children were retrospectively collected from the Sickle Cell Database in use at our center and compared to those of the SCD children followed at our Center and of a group of healthy controls recruited at the coagulation Clinic.

Results: 41/88 consecutive HbSA children (mean age: 6,85; range: 1-15) were

enrolled from January 1st 2012 to July 31st 2013. 20 M and 21 F. 88% were Africans, 9,5% European. Hematological and coagulative parameters of SCD and HbSA patients are shown in Table 1 and Table 2 respectively. HbSA children had an hematological profile similar to the ones of HbSC patients (affected by a milder form of SCD), with mild anemia and similar levels of HbS, leucocytes and platelets (Tab 1). HbSA children had lower levels of endothelial activation and thrombin generation markers than patients affected by the most severe form of SCD (HbSS and HbSβ^0), but also completely different from those of the healthy controls. HbSA patients presented also similarities with HbSC patients from a coagulative point of view (Tab 2). 13/41 HbSA children presented manifestations characteristic of SCD: 3/41 ACS and 10/41 VOC; the events were managed at home (9/41) or by hospitalization (4/41). Children with history of ACS or VOC (Group 1), presented higher levels of Hemoglobin (12,47±0,81 gr/dl *vs* 11,41±1,05; p=0,01), MCV (79,28±4,13 fL *vs* 73,84±7,82; p=0,01) and P-selectin (37,24±12,30 ng/dl *vs* 23,39±10,92; p=0,04) than patients without these clinical events (Group 2).

Table 1.

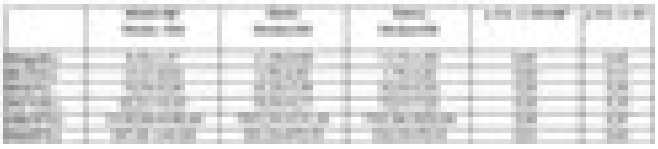
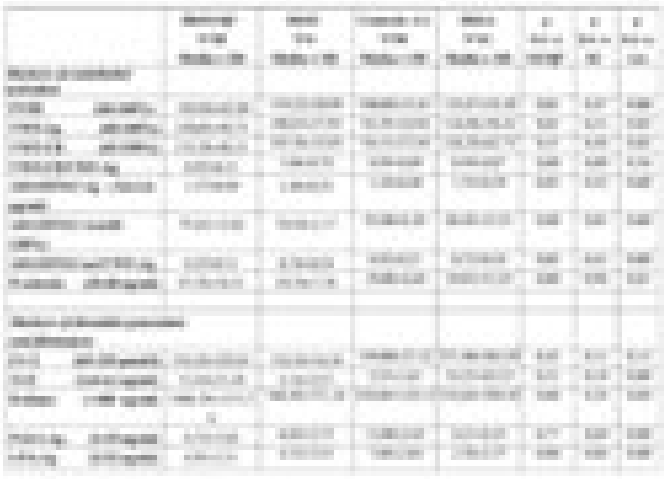


Table 2.



Summary and Conclusions: HbSA patients presented a typical hematological and coagulative profile, that is significantly different from healthy controls and similar to HbSC children, characterized by a mild activation of the coagulation cascade, but without severe anemia, leukocytosis and thrombocytosis typical of SCD. A subgroup of HbSA patients had also clinical manifestations characteristic of SCD at an early age. Those with clinical events presented higher levels of Hb, MCV and P-selectin, that could be explained by increased viscosity, hemolysis or endothelial activation, respectively. Therefore there is a subgroup of pediatric HbSA patients, that maybe needs a more intensive follow-up.

PB1926

STUDY OF THE EFFECTS OF DIFFERENT TYPES OF IRON CHELATION THERAPY ON N-TERMINAL PRO BRAIN NATRIURETIC HORMONE [NT-PRO-BNP] SERA LEVELS AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNORMALITIES IN PATIENTS WI

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Background: The prognostic predictive value of NT-pro BNP in patients with thalassemia major as a model disease with isolated diastolic dysfunction has been assessed where there were a direct relationship between the diastolic indices on echo Doppler and the serum level of NT-pro BNP.
Aims: This study was designed to study the effects of different types of chelation therapy on the LV systolic and diastolic functions using Tissue Doppler (TD) echocardiography and serum level of N-terminal pro brain natriuretic hormone (NT- pro BNP) in patients with β-TM.
Methods: The study was conducted on patients with β-TM (n = 80, age 15.7±8.9 years) and an age-matched controls (n =20, age 15.9±8.9 years). Patients with β-TM were classified into four groups according to regimen of iron chelation therapy. Group 1 was on desferoxamine, Group 2 on Deferiprone therapy, Group 3 on Deferasirox and Group 4 on combined regimen of desferoxamine and Deferiprone. Each group included 20 patients with β-TM. In all participants, TD echocardiography was performed and blood samples were withdrawn for measuring the serum level of NT-pro BNP, ferritin, and alanine transaminase.
Results: The mean serum NT pro-BNP in β-TM was significantly higher compared with controls (*P*< 0.05).There was significant decrease of the mean serum NT pro-BNP in combined iron chelation therapy than other groups of mono chelation therapy, while no significant difference among three groups of mono iron chelation therapy. The tissue doppler systolic wave (Sm) velocity and the early diastolic wave (Em) were significantly lower in β-TM group compared to controls. The tricuspid valve velocity was significantly higher in β-TM patients compared with controls .The tissue Doppler systolic wave (Sm) velocity and the early diastolic wave (Em) were significantly lower in three groups of mono chelation than combined iron chelation therapy.
Summary and Conclusions: We conclude that pro-BNP can be used as a sensitive cardiac biomarker in monitoring of cardiac dysfunction In B-TM, which is positively correlated with the early diastolic dysfunction (E/Em ratio). Group 4 had significant lower in pro-BNP and higher systolic wave (Sm) velocity and the early diastolic wave (Em) than those groups

PB1927

CLINICAL AND RADIOLOGICAL ASSESSMENT OF DEFERIPRONE RELATED KNEE ARTHROPATHY IN EGYPTIAN CHILDREN AND ADOLESCENTS WITH B-THALASSEMIA MAJOR

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Background: The most common clinical problem associated with Deferiprone therapy is arthropathy that mainly involves the knees. The cause of deferiprone-related arthropathy is not known. It has been hypothesized that the arthropathy is due to a toxic effect of deferiprone mediated by free radicals.
Aims: To assess knee arthropathy and its possible risk factors, both clinically and radiologically in patients with β- thalassemia major receiving Deferiprone monotherapy.
Methods: A longitudinal prospective study conducted on 80 patients with β-thalassemia major aged 8-18 years following up at in the Pediatric Hematology Unit, Ain Shams University Hospitals in the period from 2010 to 2013. It included 40 patients on deferiprone monotherapy and 40 patients on subcutaneous deferoxamine monotherapy .The chelation duration ranged from 1 to 8 years (mean 2.9±1.6 years).All patients were subjected to full history and examination mainly involving joint symptomatology and examination. Laboratory investigations included complete hemogram, serum ferritin, liver functions, viral hepatitis markers (B, C) and echocardiography. Knee joint assessment was done using modified hemophilia joint health score (JHS: total score of zero means normal and 40 means maximum affection), bilateral knee joints X-ray and ultrasound with particular comment on epiphyseal irregularities, synovial thickness and articular effusion. Knee MRI was done for patients in the deferiprone group with abnormal ultrasound findings.
Results: There was no significant difference between both groups as regards age, sex, transfusion index, , BMI, Tanner staging, hepatitis B and hepatitis C infection, pre transfusion hemoglobin, liver functions, serum ferritin and ejection fraction in ECHO .There was significant difference between both groups regarding *duration* of chelation therapy (p=0.000,longer duration for desferal), *compliance* (p=0.001,less compliance among desferal group) and *height* (p=0.002,desferal patients were shorter). Among the deferiprone group, 25 patients (62.5%) had knee symptoms compared to 17 (42.5%) symptomatic patients in deferoxamine group (p=0.309). Fourteen of the deferiprone asymptomatic patients (93.3%) had evidence of knee joint affection by JHS. Seven patients (17.5%) had symptoms of other joints' arthropathy. In deferiprone group ,knee symptoms were more prevalent with longer *duration of* therapy (p=0.049), *knee joint effusion in ultrasound* (p= 0.047) and *higher platelet* count (p=0.021), while in the deferoxamine group it was related to poor *compliance* (p= 0.009) and higher serum *ferritin* levels (p= 0.00). In the deferiprone group, the JHS ranged from 0-33 with 39 (97.5%) affected patients. JHS was positively correlated to *age*, *weight* ,*height , BMI*, *Tanner* staging and *platelet* count (p<0.01), and negatively correlated to

transfusion index (p=0.00) with no significant correlation to ultrasound or MRI findings. JHS was higher among *splenectomized* patients in both groups (p<0.01). There was no significant difference between both studied groups regarding JHS, premature epiphyseal fusion and ultrasound findings (p>0.05). US was 100% sensitive and 81.3% specific in detecting knee joint synovial thickening with 82.4% sensitivity and 100% specificity in detecting knee joint effusion.

Summary and Conclusions: Deferiprone induced knee arthropathy in patients with β thalassemia major could be asymptomatic. It increases with increased age, weight, height, BMI, platelet count, duration of treatment and lower transfusion index as well as among splenectomized patients. Ultrasound is good, sensitive, and specific for evaluation of knee arthropathy.

PB1928

ABSOLUTE RETICULOCYTES COUNT/IMMATURE RETICULOCYTE FRACTION RATIO, A NEW APPROACH IN THE SCREENING OF HEREDITARY SPHEROCYTOSIS

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Background: Hereditary spherocytosis (HS), the most common type of hemolytic anemia, is characterized by an increased fragility of the erythrocytes membrane due to the weakening of the vertical interactions connecting the cytoskeleton and the lipid bilayer. Main clinical signs are jaundice, enlarged spleen and anemia with reticulocytosis. With the last generation of full blood counters it is possibly to obtain the number of reticulocytes (RET#) and the immature reticulocytes fraction (IRF), and it has been observed that HS patients have increased RET# but not increased IRF. However, we have to be aware that IRF has different meanings in the different analyzers, and we need to have the reference value for each analyzer. Hereditary spherocytosis (HS), the most common type of hemolytic anemia, is characterized by an increased fragility of the erythrocytes membrane due to the weakening of the vertical interactions connecting the cytoskeleton and the lipid bilayer. Main clinical signs are jaundice, enlarged spleen and anemia with reticulocytosis. With the last generation of full blood counters it is possibly to obtain the number of reticulocytes (RET#) and the immature reticulocytes fraction (IRF), and it has been observed that HS patients have increased RET# but not increased IRF. However, we have to be aware that IRF has different meanings in the different analyzers, and we need to have the reference value for each analyzer.
Aims: With the objective of looking for HS screening tools based on hematological parameters, we analyzed the reticulocytes counts and the immature reticulocytes fraction available on our routine analyzer ABX HORIBA Pentra DX 120.
Methods: ABX HORIBA Pentra analysers employ Thiazole Orange technology to reticulocyte count (RET#) and via "RET channel" differentiate 4 sub-populations: RETL Low fluorescence, low RNA content; RETM Medium fluorescence, variable RNA content; RETH High fluorescence, high RNA content and IMM Immature reticulocytes/erythroblastic (NRBC) population (DNA). Immature Reticulocyte Fraction (IRF) = (RETH#+RETM#+IMM#)/RET#. Peripheral blood samples (n=150) processed on EDTAK3 in analyzer Pentra DX 120, levels of RET# and IRF determined in a cohort of 25 confirmed HS and in 125 samples divided in 5 groups: Autoimmune hemolytic anemia (AIHA) = 15; iron deficiency anemia (IDA) = 30; Intermedia Beta Thalassemia (IBThal) =8; Cord blood samples (CB) = 30; healthy subjects (control) =42 and then compared with the HS group. Statistical analysis of RET#, IRF, RETH#, RETM#, IMM#. ROC curve analysis for the sensitivity, specificity indices using GraphPad Prism 5.
Results: See Table 1.

Table 1.

Median x10^9/L	Control	AIHA	IDA	HS	IBthal	CB
RET#	48.5	220	50	299	215	187
RETH#	1.7	1.2	2.1	0.7	11.2	7.5
RETM#	7.5	10.1	8.3	5.8	22.6	20.4
IMM#	0.03	0.01	0.03	0.04	0.79	0.1

Summary and Conclusions: HS presented high RET# count (median=299) and IRF (median=12.8). RET#/IRF cut-off was >10 with excellent sensitivity (100%) and specificity (91%). The increased IMM in the IBThal group is due to high number NRBC. Despite increased IMM the RET# in the cord blood are unexpected near normal. This simple methodology can be used to screen HS using the HORIBA ABX PENTRA Hematological instruments. However, the diagnostic of HS still has to be confirmed by more specific and accurate tests such as, eosin-5'-maleimide (EMA) binding test and cryohemolysis.

PB1929

GROWTH HORMONE – INSULIN-LIKE GROWTH FACTOR-I AXIS AND BONE MINERAL DENSITY IN ADULTS WITH THALASSEMIA MAJOR

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Background: Bone disease and short stature are frequent clinical features of patients with beta-thalassaemia major. Dysfunction of the GH-IGF-1 axis has been described in many thalassemics children and adolescents with short stature and reduced growth velocity
Aims: to investigate GH secretion in adult thalassemic patients in relation to their bone mineral density (BMD) and serum ferritin concentrations
Methods: We performed clonidine stimulation test in 30 thalassemic patients (18 males, 12 females) with a mean age of 31.5± 7.2 years. The cut-off level for GH response was set at 7ug/l, according to the literature. Serum ferritin, IGF-I, liver enzymes, alkaline phosphatase (ALP) and type 1 Collagen Carboxy Telopeptide (CCT1) were also determined
Results: We diagnosed GH deficiency (GHD) in 12 patients (40%) and IGF-I defi ciency (IGF-I SDS <-2) was diagnosed in 20 patients (67%). Adult patients with TM had signifi cantly decreased IGF-I concentrations and bone mineral density (BMD) at the femur neck and lumbar spine compared to normal controls. Thalassemic patients with GHD and IGF-I deficiency had significantly lower BMD T score at the lumbar spine compared to patients with normal GH and IGF-I levels
Summary and Conclusions: GH status should be tested in adult thalassemic patients especially those with short stature and/or decreased BMD. Clonidine test appears to be effective and safe in adults with TM. If the diagnosis of adult GHD is established, GH treatment may be considered for possible improvement of bone mineral density and heart function in patients with TM.

PB1930

HYDROXYUREA IN THE MANAGEMENT OF PEDIATRIC B-THALASSEMIA INTERMEDIA: EIGHT YEARS' FOLLOW-UP IN EGYPT

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Background: One of the promising approaches for pharmacotherapy of β-thalassemia Intermedia (TI) is fetal hemoglobin (HbF) synthesis stimulating agents**.** Hydroxyurea (HU) has been shown to increase fetal hemoglobin (HbF) in TI patients. Its effects in increasing total hemoglobin (Hb) have been inconsistent. Studies of long term HU therapy in pediatric TI patiants are rather uncommon.
Aims: This study aimed to evaluate the overall response to HU therapy among Egyptian TI patients with variable clinical severity. The impact of genetic background and other disease variables on the likelihood of response were also studied.
Methods: An observational retrospective study, included 100 β-TI patients; 82 were children (mean age 9.9±4.1 years, range 2-18 years) and 18 adults (mean age 24.8±4.6 years). All patients were treated with hydroxyurea (mean dose 20.0±4.2 mg/kg/day, range 10-29 mg/kg/day) over a follow-up period of 4 to 96 months (mean 35.4±19.2 months). Minimum set of recorded clinical and laboratory data included baseline, at end of first year of therapy and/or last results at the time of the study. Clinical data included age, duration of disease, splenic size, frequency of transfusion, side effects and compliance to treatment. Laboratory studies included total hemoglobin, fetal hemoglobin percent by HPLC, serum ferritin levels by ELISA, liver and kidney functions. Molecular characterization was performed for 42 patients.
Assessment of Response: Major responders transfusion-independence and/or increase in their hemoglobin level by more than 2g/dl; minor responders: having a reduction in transfusion frequency by 50% or more and/or showing increase hemoglobin level by 1-2g/dl**.**
Results: Responders constituted 79/100 (79%); 64/82 (78%) children and 15/18 (83.3%) adult patients. Major and minor responders were 33% and 46% respectively. A significant decrease in transfusion frequency and increase in hemoglobin level. Response was evident in 25/79 (32%) of responders at 1-3 months with a peak reached at 12 months. Study of HbF and serum ferritin in 45 of responders revealed significant changes. *Transfusion dependency:* after one month of HU therapy, 11/25 (44%) transfusion dependent patients received no further blood, 12/25 (48%) needed less frequent transfusions. *Splenic Status:* Nineteen patients were splenectomized before starting HU**.** Decreased splenic size was found in 30/81 (37%) while increased in 6/81 (7.4%). *Molecular Characterization:* Molecular study of 42 patients showed that I*VS* 1.6 T>C was the most prevalent genotype among responders (n=28/36). *Pediatric vs. Adult response:* Both pediatric and adult groups were comparable regarding response

rate and parameters of response. *Predictors of Response:* Bivariate analysis carried out to identify predictors of response to HU in TI patients failed to show significant predictors. *Adverse effects:* Two patients developed single episode of neutropenia resolved upon temporary interruption of HU. One patient had increased liver enzymes improved spontaneously when decreasing HU. All pediatric patients showed good compliance to HU therapy (Table 1).

Table 1. Clinical and laboratory data of HU responders (n=79).

Summary and Conclusions: Hydroxyurea is a good therapeutic modality in management of pediatric TI patients as in adalts. It can minimize or stop transfusion needs with concomitant iron overload and blood-born viral transmission especially in developing countries like Egypt. Compliance to HU was good with minimal manageable side effects.

PB1931

EFFICACY OF DEFERASIROX IN CARDIAC IRON OVERLOAD OF B-THALASSEMIA MAJOR PATIENTS AFTER FIVE YEARS OF TREATMENT

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Background: B-thalassemia major patients suffer from iron overload due to regular red blood cell transfusions. The efficacy of Deferasirox (DFS), as a chelation agent, in reducing or preventing cardiac iron burden was retrospectively investigated in 26 patients with β-thalassemia major after five years of therapy.
Aims: Efficacy of Deferasirox in cardiac iron overload of β-thalassemia major patients.
Methods: All regularly transfused patients had MRI T2* evaluation of their cardiac iron load before starting Deferasirox and after a period of at least 5 years. Cardiac iron burden was categorized into 3 groups: A) increased load when T2*<8 ms, B) intermediate iron load when T2*=8-20ms, C) low /no iron load when T2*>20ms.Ferritin levels and left ventricular ejection fraction (LVEF) were also monitored.LVEF>60% was considered normal and was evaluated along with the MRI. Deferasirox was administered in starting dose of 30mgr/kgr/day and never increased more than 40mgr/kgr/day.
Results: Among 26 patients 17 were women (mean age 32, 8 years old) and 9 were men (mean age 33, 7 years old).Before starting deferasirox(DFS), 24 patients had MRI T2*cardiac iron>20msec, 2 had MRI T2*8-20ms and no one had MRI T2*<8ms.Mean serum ferritin levels were 1583, 73 ng/m L before DFS and 1668, 15 ng/ m L after 5 years of therapy. MRI T2* cardiac iron load before DFS was 32 ms and after was 37,5ms. LVEF was 66,2% before treatment with DFS and 64,8 after DFS therapy.Ferritin levels in patients with intermediate iron load was 3139,5 ng/m L before DFS and 1938 ng/m L after. MRI T2* in this group was 15,5ms before and 26,0ms after. No serious adverse events were seen.
Summary and Conclusions: Deferasirox is considered an effective chelating agent used as monotherapy even in patients with intermediate cardiac iron load.

PB1932

DEFERASIROX AND RENAL FUNCTION IN THALASSEMIC PATIENTS:A TEN YEAR EXPERIENCE OF A SINGLE CENTER

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Background: Data regarding renal function in thalassemic patients was limited until recently. Renal involvement is now beginning to be recognized in this patient group and is related to multiple factors, such as chronic hypoxia, iron overolad and / or chelation nephrotoxicity - especially in the context of new chelator use.
Aims: The aim of the present study was to retrospectively evaluate the presence of renal dysfunction in young, transfusion dependent thalassemic patients receiving chelation with deferasirox (Exjade®) during a ten year period.
Methods: The study included 47 patients receiving deferasirox during the 10 year period assessed. Deferasirox was given orally at a dose of 20 – 30 mg/kg/day. Information reported concerned sex, age at initiation of deferasirox, duration of deferasirox treatment, values of creatinine and estimated glomerular filtration rate (eGFR – Schwartz formula) at the time of chelation initiation and at the end of follow-up. In addition, the presence of hypercalciuria during the last year of deferasirox treatment was examined (defined as urine calcium of > 4mg/kg/day) and the urine calcium/creatinine rate during the same period was measured (normal values $U_{Ca}/U_{Cr} \leq 0.21$). Moreover, yearly ultrasound renal evaluations were assessed for the given 10 year period.
Results: Mean age at deferasirox initiation was 8.2±4.6 years (range 2 – 19.5 years) and mean duration of treatment 44.9±33.1 months (range 2.5 – 115 months). With regards to glomerular filtration paramenters, a statistically significant elevation of creatinine value was noted between start of drug and end of follow-up (0.52±0.11 και 0.65±0.16 mg/dl, respectively, $p<0.05$). In addition, mean eGFR presented a statistically significant decrease (136±20 ml/min/1.73m^2 and 125.9±21.9 ml/min/1.73m^2, respectively, $p=0.005$). Hypercalciuria was detected in 23/47 (48.9%) patients, of which 8/23 were put on hydrochlorothiazide treatment after conservative measures failed to improve urine calcium status. In 5/23 patients drug discontinuation we decided because of renal function deterioration, with subsequent laboratory parameter normalization. Kidney stone formation was detected on ultrasound evaluation of 2 patients while on deferasirox treatment, none presenting with parathyroid dysfunction.
Summary and Conclusions: This single center ten-year experience of deferasirox use shows that the drug can be safely administered to young thalassemic patients, given that close renal monitoring is performed. Relative renal abnormalities can be easily managed with conservative measures in the majority of patients, while discontinuation of the drug reverses abnormal laboratory parameters.

PB1933

DEFERASIROX AND OTOXICITY: A SINGLE CENTER 10 YEAR EXPERIENCE

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Background: The incidence of audiologic impairment in thalassemic patients has been well described and has mainly been associated to desferrioxamine chelation treatment. Although ototoxicity is a possible side effect of the newest chelator, deferasirox, literature has focused on other toxicities of the drug -*i.e.* nephrotoxicity.
Aims: To evaluate the effect of deferasirox on hearing function in young patients with transfusion dependent beta-thalassemia
Methods: Thalassemic patients receiving deferasirox chelation treatment during the period June 2003-June 2012 were retrospectively evaluated. Inclusion criteria were deferasirox treatment for at least 2 years and audiologic evaluation using pure tone audiometry prior to drug initiation and yearly during treatment. Data recorded consisted of sex, age at initiation, duration of treatment and audiometry results. In addition, mean hemoglobin and mean ferritin values were recorded, as well as deferrasirox dose at initiation, at time points of abnormal results and at the end of follow-up
Results: Out of 30 patients receiving deferasirox during the study period, 18 full-filled inclusion criteria. Mean age at initiation was 8.7 years (4.5-14years), mean treatment period 71.4 months (33-117 months), mean initial dose 21.44 mg/kg/day and mean initial ferritin 1417 ng/ml. One patient presented at the beginning of treatment with mild bilateral sensorineural hearing loss, but recovered 3 months later and remained impairment free until the end of follow up. Out of 18 patients 4 (22.2%) presented new findings related to sensorineural hearing loss during the study period. In 3 out of 4 cases intervention with dose reduction or drug discontinuation resulted in full recovery, while in one case improvement but not full recovery was noted after dosing intervention.
Summary and Conclusions: The study indicates the need for close audiologic monitoring in thalassemic patients receiving deferasirox so that early changes may be recognized and treatment may be judiciously adjusted in order to prevent or reverse hearing impairment

PB1934

SAFETY AND EFFICACY OF DEFERASIROX IN MANAGEMENT OF TRANSFUSION RELATED IRON OVERLOAD IN SICKLE CELL PATIENTS; SINGLE CENTER EXPERIENCE

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Background: Regular blood transfusion for patients with sickle cell disease (SCD) is a major supportive and preventive measure. Eleven percent of SCD patients will develop stroke by the age of 20 years. Chronic Regular blood transfusion therapy significantly reduces the risk of primary and secondary stroke in pediatric patients with SCD. However risk of iron overload is increased, which has been associated with morbidity and mortality in SCD patients. Iron chelation for SCD patients once liver iron concentration increases to 7 mg Fe/g dry weight, if serum ferritin more than1000 ng/l, or if patients have received cumulative transfusions of at least 20 top-up transfusions. Deferasirox is a once-daily, oral iron chelator that has shown effectiveness in reducing iron burden in SCD patients with acceptable safety profile.
Aims: Prospective single arm at a single center study, to assess Safety and efficacy of Deferasirox in management of transfusion related iron overload in Sickle cell patients. Report of One and two years results
Methods: SCD patients on regular blood transfusion, 2-15 years old and Serum ferritin levels >1000 ng/mL or received > 10 top up blood transfusion were included. Safety has monitored by Complete blood count, blood chemistry, liver enzymes, renal function parameters and urine examination for proteinurea was estimated at study entry and subsequently every 3 months. Efficacy has monitored through measurement of Serum ferritin level every 3 months. Hepatic and cardiac iron load was measured by magnetic resonance imaging (MRI) T2*, R2 and R2*.
Results: Twenty three SCD patients have enrolled in this study. Eighteen patients have continued study. Mean age was 9.8±5 years, 11 males and 7 females. Deferasirox dose range from 10 to 40 mg/kg/day (mean 25±6.5). Baseline mean serum ferritin level was 1451±594 and median was 1219 ng/mL (1006 -3950). At the end of the first year of Deferasirox treatment, serum ferritin levels had reduced in 14 patients and increased in 4 patients. Mean serum ferritin level was 1180±868 ng/mL, and median was 870 ng/mL (241 - 2978). Median serum ferritin reduction from baseline to the end of the first year was 707 ng/mL (78-1279), with a median reduction rate of 40%. Eight patients have completed the second year of Deferasirox, where serum ferritin levels had reduced in all 8 patients. By the end of the second year mean serum ferritin level was 660±670 ng/mL, and median was 313 ng/mL (145 - 2039). Median serum ferritin reduction from the end of the first year to the end of the second year was 615 ng/mL (96-2061), with a median reduction rate of 26%. While the median serum ferritin reduction from the baseline to the end of the second year was 1219 ng/mL (341-2160).with a median reduction rate of 93%. MRI T2*, R2 and R2* to assess liver and heart iron load has been planned to be done at baseline and every year after. The first 10 patients (56%) have shown normal results at baseline. Median cardiac MRI T2* was 35 ms (31-40) and median liver MRI R2* was 5 ms (4.5-8). This result lead panel to re-assess this tool benefit in the study where panel has decided to eliminate MRI tool from efficacy assessment. Normal MRI T2*, R2 and R2* results has been reported before. One patient had > 5 folds increase in liver enzymes, which has returned to normal after Deferasirox discontinuation. One patient had 43% increases in serum creatinine level at one occasion. Adverse reactions happened in 6 patients, vomiting in 2 patients, abdominal pain in 2 patients, and painful crisis in 2 patients. Tested trace elements have not been affected (Figure 1).

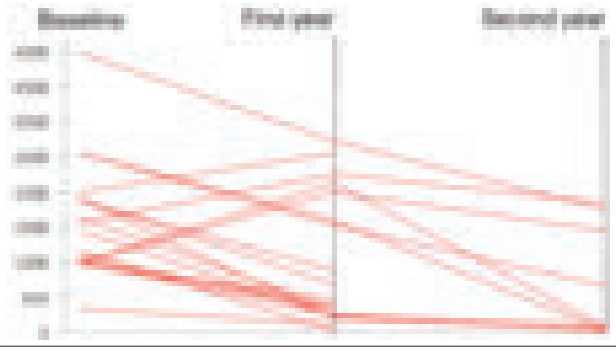

Figure 1.

Summary and Conclusions: Safety and efficacy of Deferasirox in SCD patients has investigated in 18 patients. Serum ferritin levels had reduced by 40% and 93% by end of first and second year respectively. No major adverse effects has reported. Deferasirox was safe and effective in management of transfusion related iron overload in sickle cell patients.

PB1935

ADULT-ONSET DIAMOND-BLACKFAN ANEMIA (DBA) WITH A *DE NOVO* NOVEL MUTATION IN THE EXON 5 OF RPL11

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Background: DBA is a rare disease characterized primarily by normochromic macrocytic anemia and reticulocytopenia. Classical DBA affects about7/1000000 live births and presents during the first year of life. Recent advances in identifying the genotype that underlies DBA have shown involvement of genes encoding both large and small ribosomal subunit proteins. However, as mutated genes have been discovered in DBA, non-classical cases with less distinct phenotypes are being described in adults as well as in children
Results: Case Report. 35-year-old male patient, with congenital hypoplasia of the thumb of the right hand and syndactyly between first and second fingers of the left hand, attended the emergency department complaining of progressive fatigue and headache for the past 15 days. A normocytic normochromic reticulocytopenic anaemia was disclosed (Hb7.2, MCV92.9, reticulocyte13x109/L).Iron, ferritin, transferrin saturation, B12vitamin and folic acid were normal. Was excluded thalassemia. Renal, liver function, direct and indirect antiglobulin tests, autoimmune studies, biochemical parameters of hemolysis, HIV, HBV, HCV, syphilis and EBV, B-19 Parvovirus, PNH clone, CT-body scan, ecocardiogram were normal or negative. Inmunophenotypic study of peripheral blood was negative for clonally B and T cells. BM aspiration showed moderate hypoplasia of the erythroid lineage without any signs of dysplasia. Perls' reaction in the BM smear was negative. Skeleton plain radiographs showed fusion of the phalanges of the finger with the thumb metacarpal in the left hand and absence of the first finger in the right hand. Fanconi Anaemia was excluded. A molecular study for DBA genes was performed and a heterozygous mutation C489_490delGCinsT in exon 5 of the RPL11 gene was detected. This mutation produces a variation in the composition of the last 16 a.a residues of the normal protein. This is previously NON-described mutation for DBA development. Molecular studies of the RPL11 gene were done in both parents and her sister and disclosed normal results. Our patient also exhibited other variations of the RPS19 (IVS4+14G>A) and RPS26 (5'UTR-22C>G) genes, that were considered as polymorphism and not pathological variations. According to the diagnostic criteria, our patient should be classified as sporadic non-classical form of DBA. The patient was initially treated with prednisone, folic acid and vitamin B12. He needed regular red-blood cells transfusions and iron chelation with deferasirox was started. We decided start cyclosporine to reduce the number of transfusions and reduce steroids effects. Also was added Danazol. With this combination, we observed a progressive erythroid response and transfusions were avoided. A Familiar HLA typing was performed and his only sister was found to be HLA genetically identical. Molecular studies of the RPL11 gene was performed on both parents and his only sister and was all normal. Until now the possibility of performing an allogeneic hematopoietic stem cell transplantion has been postponed.
Summary and Conclusions: We described a real clinical case of DBA in adulthood. Our case illustrates that late onset is not incompatible with the diagnosis of DBA. In the last 20 years, different mutations and deletions have been discovered and being associated with a development of DBA. Our patient has a previously undescribed mutation in exon 5 of RPL11. It was a de novo mutation. The RPL11 gene mutations have previously been associated with congenital bone malformations as those presented at the birth in our patient.

PB1936

GLUTATHIONE S-TRANSFERASE GENE POLYMORPHISMS (GSTM1, GSTT1 AND GSTP1) IN EGYPTIAN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) complications are associated with oxidative stress. Glutathione S-Transferases (GSTs) are a group of enzymes that protect against oxidative stress.
Aims: To evaluate the prevalence of GSTM1, GSTT1 and GSTP1 gene polymorphisms among homozygous sickle cell anemia (HbSS) patients and to investigate the possible association between the presence of those polymorphisms and SCD severity and complications.
Methods: This cross-sectional study included 50 Egyptian pediatric steady-state homozygous sickle cell patients (HbSS) aged 3-18 years. Fifty age and sex matched children free from hemoglobinopathies were included as a control group. All subjects were recruited only after informed consents were freely obtained from their guardians. Genotyping the polymorphisms in GSTT1 and GSTM1 genes was performed using multiplex PCR method. The GSTP1 ILe105Val polymorphism was determined using PCR-RFLP.
Results: No statistically significant difference was detected between patients and controls regarding prevalence of GSTM1, GSTT1 and GSTP1 genotypes

(p=0.153, 0.469 and 0.110 respectively). The GSTM1 null genotype was the most prevalent genotype among controls and sickle cell patients (68% and 52% respectively), followed by the mutant GSTP1 genotype (46% and 44% respectively) and the GSTT1 null genotype (18% and 26% respectively). GSTM1 null genotype was significantly associated with increased risk of severe VOC (Odds ratio=1.52, 95% Confidence Interval= 0.42-5.56, p=0.005). We found no significant association between GSTs genotypes and frequency of sickle cell-related pain, transfusion frequency, disease severity or hydroxyurea treatment.

Summary and Conclusions: GSTM1 gene polymorphism may be associated with risk of severe VOC among Egyptian SCD patients.

PB1937

IDENTIFICATION OF A NOVEL MUTATION IN THE B-GLOBIN GENE 3' UNTRANSLATED REGION [HBB: C.*+118 A>G] IN SPAIN

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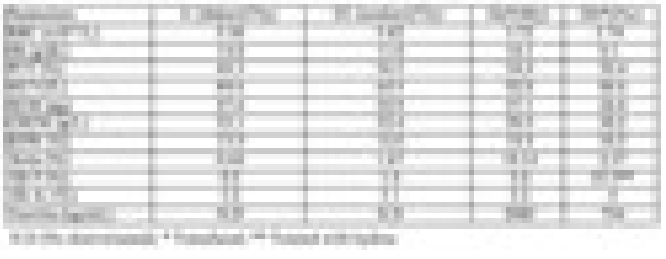
Background: The 3' UTR region is well known to be associated with mRNA stability because of its associations with 3' end processing, polyadenylation and mRNA capping. The 3' end processing of the pre β-mRNA involves some proteins bound to the 3' UTR, known as PTB, hnRNP, PAP and CstF. The αCP and nucleolin areas are also associated with the 3' UTR and involved in β-globin gene mRNA stability. Mutations located in this area cause a β-thalassemia phenotype compatible with β^+-thalassemia.

Aims: We describe one novel mutation located in the 3' UTR of the β globin gene and attempt to elucidate the resulting phenotype.

Methods: Two brothers, the first a 49-years-old male and the second a 41-years-old male. Both, were diagnosed with β-thalassemia intermedia at 2 years old. The oldest at the following year underwent a splenectomy. Both receive monthly erythrocyte transfusions. They are undergoing chelation therapy. Their parents have also been studied. The hematological data were obtained with an automated cell counter. The Hb A_2 and Hb F levels were measured by HPLC-VARIANT™. Hemoglobin was studied by capillary zone electrophoresis and cation exchange HPLC. The isolation of genomic DNA was made with an automatic method. Most frequent α globin mutations were screened with a commercial Alpha-Globin StripAssay kit. The β globin gene from promoter regions to the 3' UTR was automatic sequenced. All studies were made with the prior informed consent of the family.

Results: Both brothers were compound heterozygous for HBB:c.*+118 A>G and HBB:c.118C>T mutations according to DNA sequencing. Their mother was a carrier of HBB:c.118C>T mutation, the father was a carrier in the 3' UTR of the HBB:c.*+118 A>G mutation. The mother had mild anemia, clinically asymptomatic. Her hematological data correlated with the β-thalassemia trait. The father did not have hematological parameters associated with the β-thalassemia, and his blood smear revealed normocytosis and normochromia of the red blood cells (Table 1).

Table 1.



Summary and Conclusions: The adenine at position +1592 or +118 bases downstream of the termination codon is highly conserved among primates and placental mammals, as it is located between the poly(A) Signal (PAS) and the poly(A) Cleavage (PAC) site. Given its location, it is likely that this mutation would interfere with mRNA maturation; however, the clinical data of the heterozygous carrier show virtually no significant alterations in MCV or MHC, which were at the lower limit of normality. Therefore, we suggest that the impact on CstF recognition of the mRNA sequence would be minimal and not significantly alter polyadenylation. Therefore, the mRNA is likely stable enough that β-globin chain synthesis is not substantially affected. However, based on the observed β-thalassemia intermedia phenotypes, the accompanying mutation in compound heterozygous patients and the blood indices of the carriers, we believe that this novel β globin gene 3' UTR mutation is a silent mutation.

PB1938

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) LEVEL IN BETA THALASSEMIA PATIENTS

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Background: Oxidative status has been reported in beta-thalassemia patients due to iron overload as iron plays a critical role in the formation of Reactive Oxygen Species. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is known for its capacity to bind siderophores, transporting them inside the cells to activate cytoplasmic iron-dependent pathways, thus protecting the same cell from oxidative stress. It has also been found to have a role in kidney development and tubular regeneration after injury.

Aims: To evaluate serum levels of neutrophil gelatinase-associated lipocalin(NGAL) in beta-thalassemia patients in order to determine its possible relationships with iron status and iron chelation therapy and its value as indicators of renal function.

Methods: A prospective case control study was conducted on thirty thalassemic patients (14males and 16 females) aged 5-15 years during their regular follow-up visits and twenty age- and sex-matched healthy children as a control group at the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals - Sharkia, Egypt in the period from January 2012 to January 2013.
Thalassemic patients were randomized into three groups according to the type of iron chelation therapy .All patients and control were subjected to full medical history , thorough clinical examination and laboratory investigations in the form of complete blood picture, serum Iron and ferritin levels , renal functions and creatinin clearance and plasma concentrations of Neutrophil Gelatinase-Associated Lipocalin (NGAL) protein (ng/ml) by ELISA method.

Results: Our results showed that 63.3% of our patients were on Desferal, 26.7% on Kelfer and 10% on Exjade . The serum level of of NGAL was highly significant elevated among cases compared with controls. There were highly significant positive correlations between urea , creatinine , ferritin and duration of chelation and serum level of NGAL while there was non-significant relationships between types of iron chelators and serum NGAL level.

Summary and Conclusions:
the potential use of serum NGAL measurements among beta-thalassemic patients may be of great potential, but the findings made in the present study are only preliminary. Therefore, no clinical application can be contemplated without, for instance, specifically evaluating the effect of chronic inflammation on circulating NGAL levels, and making an effective cost-to-benefits analysis since rapid NGAL measurement incurs considerable economic costs.

PB1939

TIME TO LOOK AT COMBINATION CHELATION. EFFICACY AND SAFETY OF DIFFERENT IRON CHELATION COMBINATIONS IN TRANSFUSION DEPENDENT ANEMIAS

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Background: Routine MRI screening of hepatic and extrahepatic iron stores allows us to tailor the use of all three iron chelators according to specific organ loading. In most cases this is usually monotherapy with one of the three available medications, deferoxamine (DFO), deferiprone(DFP) or deferasirox (DFX). The decision on which chelator to offer is based on the severity and pattern of iron loading in the different organs, the amount of iron they are receiving through transfusions, adverse effects associated with the use of any of the compounds and patient preference. In cases where there was combined hepatic and extrahepatic loading, we have prescribed combination therapy with the oral agents DFP and DFX (N=14), DFP and parenteral DFO (N=2), as well as DFO and DFX (N=1).

Aims: The aim of this analysis was to determine the efficacy and safety of the chelation regimes used

Methods: We received IRB approval for retrospective review and anonymous publication. Data were acquired from 17 patients. Five with Sickle Cell Anaemia, 1 with pyruvate kinase deficiency , 1 with HbE thalassaemia and 10 with thalassaemia major. The age range was 9 to 36 years. Time of combined therapy ranged from 9 to 96 months. All patients were reviewed according to intention to treat. The numbers in each subgroup of combination of chelators were too small for statistical evaluation of each subset according to medications prescribed and compliance. We therefore report a single case, the outcomes in good compliers who had cardiac iron at the start of combination and simple

statistics on 7 patients who were good compliers on combination. Of those seven who did not have excess cardiac iron, the 5 were offered combined therapy because of excess hepatic and pancreatic iron

Results: One patient with SS was prescribed deferiprone and deferasirox with almost zero compliance and over a 20 month period his liver iron concentration(LIC) deteriorated from 29-34 mg/g dry weight (gdw), pancreatic iron from R2*90-450 herz and ferritin from 5050 to 18,500. Cardiac T2*changed from 31.6 to 20.5ms, indicating a downward trend. In two of the good compliers with combination DFO + DFP, cardiac T2* improved from 4.8ms to 5.4 in 12 months with left ventricular ejection fraction (LVEF) improving from 50.8 to 60.4% and 4.4 to 22.4 in 58 months with LVEF from 46.1% to 56.7% and one with DFX + DFP, T2* improved from 8.6 to 21.4 in 18 months with stable LVEF. In 6 compliers with excess hepatic and pancreatic iron the change in LIC per month (/m) was 0.6± 0.45 gdw (p=0.015), pancreatic R2* change/m was 24.0 ±18.6 hz(p=0.003) and ferritin reduced by 82.3±56.3 per month (p<0.001) It is clear that all three combinations are effective in reducing the iron content in areas of concern when patients adhere to the regimen. Every patient with better than 50% adherence significantly improved target organ iron burdens. Even in the patients with poor adherence (results not shown), there may be some cardiac protection as evidenced by the maintenance, and in some cases, improvement in LVEF. The adverse events associated with the combination therapy were no different from those with the use of each agent as monotherapy and there were no new previously unreported toxicities. The most common were gastric upset with both DFP and DFX and some very mild neutropenia in one patient on DFP. There were no elevations in creatinine or increased proteinuria in any patients on DFX.

Summary and Conclusions: The tailoring of mono and combination chelation therapies according to MRI assessment of hepatic and extrahepatic stores offers the ability to bring all patients to normal iron levels, prevent morbidities, and even potentially reverse existing functional deficits. Clearly, adherence remains the dominant challenge in effective chelation. It would be valuable for a registry to collect data on all patients on such combinations in order to confirm the efficacy and safety.

PB1940

EVALUATION OF CHILDREN WITH THALASSEMIA MAJOR WITH DEPRESSION SCALE AND SELF-CONCEPT TEST.

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Background: Beta Thalassemias are a group of inherited blood disorders caused by reduced or absent synthesis of the beta chains of hemoglobin, resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Individuals with beta thalassemia major usually present within the first two years of life with severe anemia, poor growth and skeletal abnormalities during infancy. Affected children will require regular lifelong blood transfusions. Thalassemic patients are vulnarable to emotional problems. Unfortunately, there are few psychological studies referred on the literature.

Aims: The study aimed to determine the prevalence of depression and low self concept in patients with thalassemia major and compare them with healty children.

Methods: The study was done with 40 voluntary children with thalassemia major having regular blood transfusions and iron chelation treatment and 20 healthy children between the ages of 9-16 years. Psychological data, including depressive disorder and low self concept were assessed by Child Depression Scale and Piers-Harris Self Concept Scale. Demographic data included age, sex, mothers and fathers educational levels, school achievement, serum ferritin levels,prevalence of transfusion, iron chelation treatment. Statistical analysis was performed by chi-square test, using the SPSS software.

Results: The prevalance of depressive disorder in thalassemic patients were more then control group: 27.5%, 10% respectively. However self concept scales in the study group were lower then the control group, but there was no statistically significant difference.

Summary and Conclusions: Beta thalassemia major, a chronic, genetically determined hematological disorder causes emotional and behavioral problems. This study indicated that the prevalance of depressive disorder and low self scales were more common in thalassemia major patients.

PB1941

VITAMIN E AND SELENIUM IN EGYPTIAN THALASSEMIA AND SICKLE CELL DISEASE PATIENTS: ARE THEY DEFICIENT?

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Background: Increased oxidative damage is well known in hemoglobinopathies due to increased resting oxygen consumption and circulating pro-oxidative free hemoglobin. It is important to know whether the level of antioxidants differ in beta thalassemia and sickle cell anemia or contribute to differences in the severity of oxidative damage in these two diseases.

Aims: To study the levels of vitamin E and selenium as antioxidants in transfusion dependant Egyptian beta thalassemia major (TM) and sickle cell disease (SCD) patients and whether these variables differ among these two diseases or correlate with iron overload status or transfusion requirements.

Methods: A case-control study conducted at the hematology clinic of the children hospital, Cairo University, Egypt. The study group consisted of 30 thalassemia patients (mean age 12.9±3.2), 30 SCD patient (mean age 11.8±2.9) and 30 healthy controls. After obtaining a written informed consent, blood samples were withdrawn for measuring vitamin E, selenium and Lipid profile (cholestrol, HDL, LDL, triglyceride (TG)) in addition to serum ferritin, AST, ALT, CRP, retix and LDH.

Results: β-Thalassemia group showed significantly higher mean transfusion frequency when compared to SCD group (p=0.005). LDH was nearly five times larger than normal values and C-reactive protein (CRP) was also almost 3-fold larger in SCD patients and SCD patients showed statistically significantly higher values of LDH and CRP when compared to TM group (p<0.05). Thirteen percent TM patients exhibited abnormal values of AST and ALT exceeding 2 folds *versus* none of SCD group. All TM and SCD cases had below normal Selenium level *versus* 11 (36.7%) of the control group, and mean Selenium level was comparable between Thalassemia and SCD groups (p>0.05) and both groups showed significantly lower levels when compared to the control group (p<0.05). Similarly, all TM and SCD cases had below normal vitamin E level *vs.* none of the control group, and mean vitamin E level was comparable between TM and SCD cases (p>0.05) and both groups had significantly lower levels when compared to the control group (p<0.05). Total cholesterol, LDL-cholesterol, as well as TG were comparable in patients with TM and SCD (p>0.05) and all were significantly lower than relevant controls (p<0.05). However, there was no significant differences between mean HDL- cholesterol levels in the three groups (p>0.05). Among TM group; serum ferritin and selenium levels didn't correlate with any of the tested variables including other antioxidants. Levels of vitamin E were proportionally correlated with ALT values *(r* = 0.4; p=0.049) and AST *(r* = 4; p=0.039), however, no other clear correlation was found between vitamin E and other variables. Transfusion rate correlated positively with CRP (r=0.341, p=0.065), AST correlated inversely with TG (r=-0.389, p=0.034) and Retics correlated negatively with Hb (r=-0368, p=0.046) and positively with ALT (r=0.424, p=0.020). Among SCD cases, serum ferritin, selenium and vitamin E levels didn't correlate with any of the tested variables.

Summary and Conclusions: These results demonstrate that Children with TM and SCD have increased oxidative stress and depleted antioxidants relative to healthy controls. However, levels of these antioxidants did not correlate with indices of iron overload, hemolysis, or inflammation in chronically transfused TM and SCD patients

PB1942

ASSOCIATION BETWEEN DUFFY ANTIGEN EXPRESSION AND DISEASE SEVERITY IN SICKLE CELL DISEASE PATIENTS

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Background: Sickle cell anemia (SCA) is associated with a pro-inflammatory state, characterized by an elevated baseline leukocyte count & inflammatory cytokines. White blood cell adheres to vascular endothelium with subsequent endothelial injury and repeated ischemia-reperfusion injury contributes to disease pathogenesis. Identification of genetic polymorphisms that may modulate disease severity in SCD has become an active area of research.
The Duffy blood group system, Fy*A and Fy*B, are codominant alleles, located on chromosome 1. Four phenotypes are present on the RBCs: Fy(a+b–), Fy(a+b+), Fy(a–b+), and Fy(a–b–). The positive Duffy blood group antigen thwarts the activation of white blood cells and hinders the dissemination of chemokines from blood into organs reducing the inflammatory process.

Aims: This paper investigates the effect of the RBCs Duffy antigen expression and its genetic polymorphisms on modulating Sickle cell disease severity as well as its complications.

Methods: Cross-sectional study included 100 patients with SCD. Patients were divided into complicated and non complicated SCD individuals. Complications were determined according to scoring system (0-5) based on the presence or absence of each of the following: 1) pulmonary dysfunction; 2) avascular necrosis of the hip or shoulder; 3) central nervous system (CNS) abnormality; 4) kidney dysfunction,; and 5) history of leg ulcers. For each item, one point was assigned if present. The expression of the Duffy phenotype was detected by indirect anti-globulin test using both: tube and gel methods. The Duffy genotype was determined with PCR-Restriction Fragment Length Polymorphism (RFLP) assay.

Results: In regards to Duffy genotype in SCD patients, the frequency of FY positive genotype was 56% and FY negative genotype was 44%.Total leucocytic

count was strongly associated with the Duffy genotype. Duffy-positive patients had significantly higher WBCs, than those detected in Duffy-negative patients (p=0.002).Comparison between each measure of disease severity (pulmonary dysfunction, avascular necrosis, CNS dysfunction, kidney dysfunction and leg ulcers) in Duffy positive and negative patients in our study revealed no statistical significant difference.
Summary and Conclusions: Our study suggests that RBC DARC expression increases the plasmatic levels of white blood cells in SCA patients and that Duffy genotype at the GATA site may not be a potential biomarker for early end-organ damage in SCD and no association was detected between RBC DARC quantitative phenotype and the occurrence of the studied SCA complications.

PB1943

RELATIONSHIP BETWEEN BCL11A SEQUENCE VARIATIONS AND RESPONSE TO HYDROXYUREA THERAPY IN IRANIAN BETA-THALASSEMIA INTERMEDIA PATIENTS

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Background: Increased HbF levels can ameliorate the disease severity in β-globin gene disorders such as β-thalassemia intermedia (β-TI).Hydroxyurea (HU) is a chemotherapeutic agent that have been known as a fetal hemoglobin (HbF) induction agent that can alleviate the symptoms in β-TI patients. The HbF level is influenced by many loci inside or outside the β-globin gene cluster. BCL11A gene on chromosome 2p16 is one of the three major loci that may play a role in the regulation of HbF levels.
Aims: The aim of this study was to assay the possible relationship of HU responder and BCL11A gene sequence variations in Iranian patients affected with β-TI.
Methods: In this cross sectional study 102 β-TI patients who were taking HU with a dose of 8□15 mg per kg body weight per day for a period of about 6 months to 13 years were randomly selected between February 2012 and October 2013 in southern Iran. Based on the need to blood transfusion and hemoglobin level, our patients were divided into two groups: good responder and poor responder. Response to HU treatment was defined based on decrease or cessation of the need to blood transfusion as well as evaluation of Hb level.
Results: We compared demographic and clinical variables between good and poor responders. There were no statistically significant association between hematological data and also age with response to HU (P>0.05).None of the evaluated SNPs were not significantly associated with response to HU(P>0.05).
Summary and Conclusions: In this study none of the evaluated SNPs showed significant association with response to HU, but further larger studies as well as evaluation of other genes are suggested.

PB1944

REPORT OF 15 YEARS OF GENETIC COUNSELLING FOR HAEMOGLOBINOPATHIES IN NORTHERN GREECE

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Background: Genetic counselling is defined as a communication process which deals with the human problems associated with the occurrence, or risk of occurrence, of a genetic disorder in a family. Greece is a country with an 8% carriers of thalassaemia and 1-1,5% carriers of Sickle cell trait. Since 1974 a population screening program is performed for Thalassaemia and Sickle cell disease prevention. Through this program, natives and immigrants are screened and counselled free of charge and the genetic counselling is mainly focused in the prevention of thalassaemia and sickle cell syndromes. We find the couples at risk for giving birth to a thalassaemic offspring and convey to the individuals information about risks, the consequences of the testing procedure and the potential choice of continuing or discontinuing the pregnancy. We also try to facilitate the decision making although the personal values and beliefs vary.
Aims: We report our experience of the genetic counselling of couples at risk for Haemoglobinopathies over a 15 year period (1999-2013).
Methods: The carrier identification is carried out by a standard scheme, which included, CBC and red cell indices using the Coulter ONYX, a Cation Exchange HPLC variant system (Bio-Rad, Variant β-Thalassaemia Short Program), to determine HbA, HbA2 and HbF levels and the different abnormal structural haemoglobins, electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar, sickling test and tests for HbH inclusion bodies. Haemoglobin A2 is also quantified by column micro chromatography and serum ferritin levels are measured by micro Elisa technique. Biosynthesis of the- α and β- globin chαins and DNA techniques are also performed on selected cases.
Results: From the total 35.797 subjects who underwent screening during this period we found 3883 couples that came for screening and counselling. The pregnancies at risk for the birth of a child suffering from thalassaemia or sickle cell disease, involved 315 couples, out of the 360 that both partners carried abnormal genes. Few had a positive family history, while the rest were identified through preconception and prenatal carrier screening at our Thalassaemia Prevention Unit. Prenatal diagnosis was mainly carried out by chorionic villous sampling (CVS) at 11-12 weeks of gestation, and in few cases by amniotic fluid sampling, collected at 16-18 weeks. Very few late comers were tested by foetal blood sampling at 20-24 weeks of gestation. During the same period, prenatal diagnosis was not carried out in 45 pregnancies, because it was not indicated as the combination phenotype is clinically very mild. Gene interactions in these cases were as follows:α thalassaemia with β-thalassaemia, α thalassaemia with δβ-thalassaemia, silent β-thalassaemia with β-thalassaemia, α thalassaemia with sickle cell trait, α thalassaemia with α thalassaemia, Hb Agrinio with β-thalassaemia, Hb E with Hb E, HbE-Saskatoon / HbS , HbE-Saskatoon/β-thal, HbO-Arab/HbO-Arab, HbD-Punjab/α-thal, HbD-Punjab/β-thalassaemia.
Summary and Conclusions: As is the case for all genetic diseases, counselling of parents with the potential danger of bearing a sick child requires extreme sensitivity, in relation to psychosocial and ethical issues.

PB1945

SAFETY AND EFFICACY OF INTERFERON AND RIBAVARIN THERAPY IN HCV POSITIVE THALASSEMIC PATIENTS

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Background: Liver disease in thalassemia is secondary to transfusional iron overload and viral hepatitis (hepatitis B and C). Universal immunization against HBV has markedly reduced the incidence of hepatitis B, hence hepatitis C remains the main risk factor for development of liver fibrosis and hepatocellular carcinoma. Treatment of chronic hepatitis C has been controversial due to increased transfusion requirement secondary to ribavirin induced hemolysis resulting in increased iron overload.
Aims: To determine safety and efficacy of interferon and ribavirin combination therapy in thalassemia patients with chronic Hepatitis C (genotype 3).
Methods: Fourteen thalassemia major patients with chronic hepatitis C (genotype 3) who gave informed consent for the treatment and were negative for HBsAg and HIV were included in the study. Patients were treated with Pegylated interferon α 2a and ribavirin for 24 weeks. Monitoring of treatment efficacy was based on HCV RNA measurements done at 4, 12, 24 and 48 weeks. CBC, liver and thyroid functions were assessed regularly during the treatment. Transient elastography was done at baseline and 1 year later to assess the liver fibrosis.
Results: The mean age of the patients was 15.14 (7-28) years. Ten (71.4%) were male and 4(28.6%) were female. Twelve (85%) patients achieved rapid virological response. All patients achieved complete early and end of treatment response. SVR was attained in 7/13 (53.8%) patients. Response rate in patients being treated for the first time was 60% (3/5) as compared to 50% (4/8) in non responders. Flu like symptoms is the most common adverse effect seen. Two patients required growth factor support for neutropenia. Eight (57%) out of 14 patients developed thrombocytopenia (platelet count<1, 00,000/mm3). Transfusion requirement increased by 35% during the treatment.
Summary and Conclusions: The efficacy of interferon and ribavirin combination in achieving SVR in thalassemia patients with chronic hepatitis C (genotype 3) is lower than that in general population.

PB1946

RED BLOOD CELLS ANTIOXIDANT SYSTEM COMPENSATES OXIDATIVE EFFECT OF HYPOXIA DURING CHRONIC INFLAMMATORY LUNG DISEASES

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Background: Red blood cells (RBCs) disorders (sickle-cell anemia, thalassemia) increase oxidative damage as result of release heme and iron from hemoglobin. Nevertheless, RBCs oxidative stress is not the main reason

of chronic hemolytic complaints at patients with these diseases because RBCs have tolerance of oxidative damage. This extensive antioxidant system prevents of formation of reactive oxygen species (ROS), scavenges ROS, and repairs of oxidized molecules. A partial defect in any of these processes can harm RBCs, but is without chronic hemolytic complaints too (van Zwieten R et al, 2013). Persons with hereditary RBCs disorders can get chronic inflammatory lung diseases (these pathologies have high morbidity). Chronic obstructive pulmonary disease (COPD) and bronchial asthma (BA) could be additional causes of peroxidation. For example, chronic inflammatory lung diseases lead to a hypoxia as a result of bronchial obstruction. Hypoxia is a condition to macrophage activation and ROS production. During exacerbations of chronic diseases inflammation is accompanied by growth amount of neutrophils and degranulation. Probably patients with RBCs pathologies need in more intensive medical care in the case of great role of RBCs in prevention of blood oxidant-antioxidant imbalance during COPD or BA .

Aims: to determine whether RBCs can compensate oxidative effect during chronic inflammatory lung diseases.

Methods: Informed agreement was received from all subjects. All subjects (mean age 45±15) were without RBCs disorders and with/without lung pathology. Patients received standard therapy in City clinical hospital No. 70 of Department of Healthcare of Moscow. There were 20 COPD,12 BA and 12 community-acquired pneumonia (CAP) patients on study. Control group include 10 health subjects. There were two times to blood assay – the 1 day (exacerbation) and 10 day at a hospital. Blood samples were collected in 4 ml tubes, 60 USP Units of Lithium Heparin. We study blood superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase activity in COPD and BA in comparison with CAP (acute inflammation) and Control. Blood processing (cells washing with isotonic saline solution) means that main source of antioxidant enzymes is RBCs. SOD activity was measured with using RANSOD kit, Randox. GPX activity was measured with using Paglia DE and Valentine WN method, 1967 (RANSEL kit, Randox) . Catalase activity was measured with spectrophotometric detection of the breakdown of hydrogen peroxide (Beers J, Sizer IW, 1952). We determined thiobarbituric acid reactive substances (TBARS) level in plasma to estimate oxidative stress by thiobarbituric acid test (Uchiyama M, Mihara M, 1978). Statistical analysis was applied using Statistica 6.0 and GraphPad Prism5. Data are expressed as the mean +/− standard deviation (SD). * - P value <0.05 was accepted as significant in comparison with Control.

Results: According our data in patients with lung diseases antioxidant enzyme activity changes similarly (results in Figure 1). Despite low activity of GPX (1 day Kruskal-Wallis test p=0.017) we can't say about oxidative damage in all groups. High plasma TBARS concentration shows enhanced oxidative stress during acute lung inflammation (1 day CAP group in comparison with Control, Mann-Whitney's criterion p=0.015). However there was no increasing of this parameter during exacerbation of COPD or BA. PlasmaTBARS concentrations in COPD (1 day p=0.51) and BA (1 day p=0.78) didn't differ from normal values. Therefore blood antioxidant system can prevent of oxidant-antioxidant imbalance in the case of chronic inflammation.

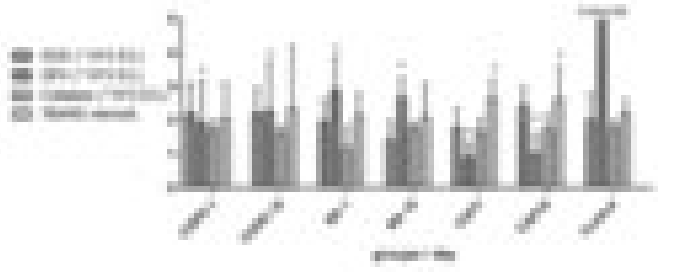

Figure 1. Antioxidant enzymes activity and TBARS concentration.

Summary and Conclusions: The results testify to blood antioxidant system compensates oxidative damage during COPD and BA. Therefore hereditary RBCs disorders can influence an oxidant-antioxidant balance and outcome of chronic inflammation. So the further proteomics investigations in RBCs antioxidant defense can help to improve quality of life.

PB1947

IRON DEFICIENCY IS NOT ASSOCIATED WITH INCREASED BLOOD CADMIUM IN INFANTS

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Background: Because iron is absorbed via mechanisms similar to those of other divalent metal ions, including cadmium, manganese, and lead, a dietary deficiency in iron can lead to excess absorption of cadmium, a widespread toxicant with detrimental effects on health. Indeed, iron deficiency has been found to predispose animals to cadmium toxicity by increasing gastrointestinal cadmium absorption. Several studies have suggested an association between iron status and blood cadmium concentration in adults, especially pre-menopausal women, whereas few studies have been conducted in children.

Aims: We assessed whether blood cadmium concentrations are higher in iron-deficient than in control infants, and whether treatment of the former with iron supplements affects cadmium concentrations.

Methods: Thirty one infants with iron deficiency, ranging in age from 6 months to 2 years, were selected from infants being treated at an ambulatory pediatric hematology clinic at Ulsan University Hospital, Ulsan, South Korea. Thirty-six healthy, age- and sex-matched control subjects, with serum ferritin concentrations higher than 15 µg/L, were selected from among the infants visiting a general pediatric clinic in the same hospital. Subjects were excluded if they were delivered preterm or at low birth weight; had a history of any disease, or had concurrent acute or chronic infection or inflammation; or if their parents had a history of occupational exposure to cadmium. All 31 iron-deficient infants were treated with ferric hydroxide-polymaltose complex (6 mg/kg Fe^{3+}/day) for 1-6 months. Blood cadmium concentrations were determined in all control subjects and in all iron-deficient subjects prior to iron supplementation. Blood cadmium concentrations were assessed again in 19 iron-deficient infants after their ferritin concentrations returned to the normal range; the other 12 iron-deficient infants were lost to follow up.

Results: Age and gender distribution were similar in the iron-deficient and control groups, as were their GM blood cadmium concentration. However, hemoglobin concentrations, hematocrit levels, and serum ferritin levels differed significantly in the two groups. Mean duration of breast feeding was longer in iron-deficient than in control infants. All the iron-deficient infants were treated with an iron supplement, and 19 were tested again for blood cadmium concentrations after their ferritin concentrations reached the normal range. The GM blood cadmium concentration in these 19 infants was not significantly altered by ferric hydroxide treatment, while their hemoglobin, ferritin, and Fe/TIBC (%) levels were significantly higher after than before treatment (Table 1).

Table 1. Laboratory features of iron-deficient infants before and after iron therapy.

Group characteristics	Before iron therapy (n=19)	After iron therapy (n=19)	*P* value
Cadmium (µg/L)	0.58 (0.06-1.40)	0.47 (0.06-1.38)	0.529
Hemoglobin (g/dL)	10.49±1.75	12.38±0.85	0.001
Hematocrit (%)	32.77±3.80	36.87±2.24	0.002
Ferritin (µg/L)	7.03±3.16	20.83±7.0,4	<0.001

Summary and Conclusions: These findings indicate that iron deficiency does not increase blood cadmium concentrations in infants, in contrast with the effects of iron deficiency on manganese and lead concentrations.

PB1948

CLINICAL AND BIOCHEMICAL ASPECTS OF ANEMIA IN CANCER PATIENTS

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Background: Although anemia further weakens the cancer patients and is also associated with poorer outcome, it can be easily ignored in practice. Actually, multifactorial pathogenesis is involved in anemia of cancer patients and defining the causes of anemia is not always simple.

Aims: In this study, we looked at cancer anemia from two perspectives including clinical and biochemical aspects.

Methods: There are two separate data sets for the analysis. First data for analyzing clinical aspects of anemia is collected from the 345 patients who were treated in Samsung Medical Center for the 4 major cancers in Korea (gastric, colorectal, lung cancer and hepatocellular carcinoma) between Jan 2012 and Apr 2012. Second data for biochemical aspects of anemia is from the patients having storage serum samples. Twenty-nine stored frozen serum samples from 4 types of cancer (gastric cancer, biliary tract cancer, lung cancer, and lymphoma) were used to analyze ferritin, soluble transferring receptor (sTfR), CRP, and hepcidin. Anemia at diagnosis was defined by hemoglobin (Hb) of 11g/dL or below. During treatment, Hb ≤11g/dL and a drop of Hb 2g/dL or more is also defined as anemia for patients whose baseline Hb level was over 11g/dL.

Results: Of the total 345 patients, there were 152 lung cancer (44.1%), 101 gastric cancer (29.3%), 69 colorectal cancer (20.0%), and 23 hepatocellular carcinoma (6.7%) patients. Forty nine patients (14.2%) had anemia at their initial diagnosis of cancer, and 129 patients (37.4%) experienced anemia during 1st line anti-cancer treatment. Among these 129 patients, 34 patients (26.4%) were

treated for their anemia, almost by RBC transfusion. For analyzing biochemical aspects of cancer anemia, we included patients with serum samples, which included 11 gastric cancer (37.9%), 6 biliary tract cancer (20.7%), 8 lung cancer (27.6%) and 4 lymphoma patients (13.8%). When comparing to the reference value from general population, cancer patients tended to show higher ferritin, sTfR and CRP level and lower hepcidin level. Among the cancer patients, anemic patients showed significantly higher level of sTfR compared to non-anemic patients (p=0.011). Although not statistically significant, the level of ferritin, CRP, and hepcidin was more elevated in anemic patients.

Summary and Conclusions: This study shows that cancer patients are being insufficiently paid attention for their anemia. Consistent increase of three inflammatory markers (ferritin, hepcidin, and CRP) in anemic patients with cancer implies that cancer anemia seems to be closely related to inflammatory process (ACD). Of note, however, we can also identify that iron deficiency and/or iron restricted erythropoiesis also contributes considerably to pathogenesis of anemia in cancer patients.

PB1949

CLINICAL SIGNIFICANCE OF THE NEW BECKMAN-COULTER PARAMETERS IN THE DIFFERENTIAL DIAGNOSIS OF ANEMIA

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Background: The differential diagnosis of anemia is based some biochemical indicators of iron metabolism. These are parameters of erythrocytes, ferritin, transferin saturation, transferin, serum iron and total iron binding capacity. These parameters are useful parameters to determine iron status, but they may not provide conclusive criteria for determining iron deficiency because of their high variability and low sensitivity.

Aims: In recent years, some studies reported that these convantional parameters is weak or relatively, so the researchers recommended that more sensitive tests (RSf, LHD, MAF, Transferrin/ Log Ferritin) are used in the differential diagnosis of anemia. In our study, we aimed to evaluate the these parameteres in the differential diagnosis of anemia.

Methods: The study was conducted at Eskişehir Osmangazi University, Faculty of Medicine, Department of Hematology. After obtaining the approval of the Ethics Committee and informed consent, 136 patients with iron deficiency anemia(IDA) (serum hemoglobin <12 gr/dl, TSAT <20% and serum ferritin <20 ng/ml), 50 patients with anemia of chronic disease (ACD) (serum hemoglobin <12gr/dl, TSAT >20% and serum ferritin >50ng/ml), 34 patients with beta thalassemia , 56 patients with anemia of chronic kidney disease (CKD) and 166 patients with healthy controls were included the study. These parameters are calculated with the following formulation, RSf= √(MRV X MCV), MAF={(Hgb X MCV)/100}, LHD= 100 X $\sqrt{1-\{1/(1 + e^{1.8\ (30-MCHC)})\}}$, Transferrin/Log Ferritin.

Results: Present measurements showed that RSf and MAF levels were lower in patient with iron deficiency anemia and beta thalassemia compared to patients with anemia of chronic disease and anemia of CKD. LHD and Transferrin / Logaritmic ferritin were markedly higher in patients with IDA when compared to patients with ACD and CKD. A statistically significant difference was seen in each group with RSf, MAF, LHD and T/LF. RSf and MAF showed a significant positive correlation with hematocryte, MCV, MCH, MCHC, serum ıron, transferrin saturation and serum ferritin level. But these parameters showed negative correlation with transferrin and total iron binding capacity. As well as, LHD and T/LF showed a significant negative correlation with hematocryte, MCV, MCH, MCHC, serum ıron, transferrin saturation and serum ferritin level. But these parameters showed positive correlation with transferrin and total iron binding capacity.

Summary and Conclusions: In recent years, some studies reported that these convantional parameters is weak or relatively. For example,Serum ferritin is acute phase reactant and it levels are increased in acute/chronic inflammation. Serum iron level is decreased in infection, inflammation and malignancy but it increased for used iron supplement, ineffective erythrophoesis and liver diseases. Transferrin saturation fluctuates due to the diurnal variation . Parameters used in routine differential diagnosis of anemia , as well as the current need for more sensitive and robust indicators available. In our study, we showed that these parameter that can be confidentially used in the differential diagnosis of anemia.

PB1950

DECREASED LIPID PROFILE IN PATIENTS WITH SICKLE CELL ANEMIA IS NOT A GOOD PROGNOSTIC MARKER

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Background: Children with sickle cell disease (SCD), even in steady-state, have differences in several biomarkers as compared to healthy age-matched children. Those differences are related to numerous mechanisms associated with infection, inflammation and vaso-occlusion in the disease. Concerning Lipids, it was observed that changes in lipid profile in SCD patients were associated with hemolysis, inflammation and renal metabolism.

Aims: to evaluate the lipid profile in SCD and sickle thalassemia patients in correlation to various complications encountered in those patients.

Methods: A case- control study was performed in the Pediatric Hematology Clinic, Ain Shams University hospital in the period from October 2011 to June 2012.It included 25 patients with SCD and sickle thalassemia in steady state, and 25 age and sex- matched healthy controls.Patients were subjected to medical history taking and revision of hospital records for history of blood transfusion, number of hospital admissions and their causes, transfusion index, number of vaso-occlusive episodes, number of strokes details of hydroxyurea theapy, packed RBCs transfusion and chelation therapy. Full clinical examination was done and laboratory investigations including CBC, lipid profile and serum ferritin.

Results: We found a significant lower median HDL 35.6 (29.25-43.25) mg/dl (P= 0.015), median LDL 70 (54.25-81.25)mg/dl (P=0.037), mean cholesterol levels 127.96±32.45 mg/dl (P= 0.038) in sickle cell patients compared to control group where median HDL 44 (39.5-51) mg/dl, median LDL 81 (66.75-97.25)mg/dl, mean cholesterol levels 146.64±29.5 mg/dl, with no significant difference concerning triglycerides. Triglycerides levels were significantly lower in patient with sickle cell disease 112 (80.5- 126.5)mg/dl compared to patients with sickle thalassemia disease 69 (52-94) mg/dl (P=0.020).In patients group, a positive correlation was observed between triglycerides with HbF (r= 0.550, P= 0.005), ALT (r= 0.467, P= 0.019) and LDH (R= 0.410, P=0.047), while a negative correlation with pre-transfusion hemoglobin (r= -0.527, P= 0.025) was found. A negative correlation was found in patient with sickle cell disease between total cholesterol and Hb S level (r= -0.829, P= 0.042) and a positive one between total cholesterol and mean ferritin level (r= 0.431,P=0.032). A significant positive correlation was found between LDL and WBCs (r= 0.492, P=0.012) and direct bilirubin level (r= 0.494,P= 0.012). A positive correlation was noticed between HDL with total number of hospital admission due to vaso-occlusive crises and infections (r= 0.520,P=0.011) and dose of hydroxyurea (r= 0.845,P= 0.034).By classifying the patient group according to HDL level (>,<40 mg/dl) we found significant increase in hospital admission with increasing HDL level 6.5 (3.5-18.75) (P= 0.037).

Summary and Conclusions: Significant decrease in cholesterol, HDL and LDL levels was found in patients with sickle cell disease and sickle thalassemia, HDL level was associated with increasing hospital admissions, and triglycerides level was associated with increased hemolytic markers. Increasing HDL level is not a good prognostic factor in patients with SCD as in normal population.

PB1951

HFE MUTATIONS C282Y AND H63D IN IRANIAN POPULATION WITH TYPE 2 DIABETES

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Background: HFE gene, which is also responsible for Hereditary Hemochromatosis (HH), is involved in iron metabolism. It has revealed that there is a link between type 2 diabetes (T2D) and HH so that 20 to 50% of patients with HH also develop T2D.

Aims: Regarding the relationship between type 2 diabetes and hereditary chromatists, we conducted a genetic analysis on two previously reported mutations C282Y and H63D related to the *HFE* gene in our population.

Methods: Altogether, 145 patients with type 2 diabetes and 145 healthy controls were examined. A Genotyping assay performed using electrophoresis of the DNA digestion products from MboI and RsaI for H63D and C282Y, respectively.

Results: Results showed a significant difference between case and controls regarding C282Y (P value=0) and H63D genotypes (P value=0.013). We also found a relationship between both mutations and nephropathy. Moreover, the difference between C282Y genotypes of patients with retinopathy and healthy controls were statistically significant (P value=0.020) while there was no association between H63D and retinopathy. In addition, observed differences of both mutations were significant when nephropathic patients compared to the controls. No relationship detected between blood parameters and typed mutations.

Summary and Conclusions: Our study showed a significant association between H63D and C282Y mutations and the risk of type 2 diabetes in Iranian population

PB1952

IMPACT OF GENOTYPE ON ENDOCRINAL COMPLICATIONS IN BETA THALASSEMIA MAJOR PATIENTS

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Background: β-thalassaemias are a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chains. Some mutations cause a complete absence of β-globin chain synthesis termed β0 thalassemia, while others may allow some β-globin production and are termed β+ or β++thalassemia. The homozygous state results in severe anemia, which needs regular blood transfusion. The combination of transfusion and chelation therapy has dramatically extended the life expectancy of thalassemic patients who can now survive into their fourth and fifth decades of life .On the other hand, frequent blood transfusion in turn can lead to iron overload which may result in several endocrinal complications.
Aims: We aimed to investigate the impact of genotype on the development of endocrinal complications in β thalassemia major patients.
Methods: A cross sectional study was conducted on 100 thalassemia major patients (54 males, 46 females) aged over 10 years, who were registered in and followed up at pediatric hematology unit of Zagazig university hospital. Data abstraction form was designed to capture the appropriate information from the individual medical records including full clinical, laboratory, transfusion and chelation data. Genotype and hematologic phenotype of patients were also identified.
Results: The mean age of our patients was 14.2±1.37 years. Patients with β0β0 hematologic phenotype showed earlier age of start transfusion and chelation as well as more frequent transfusions compared to those with β0β+ and β+β+ hematologic phenotypes. Also, Patients with β0β0 hematologic phenotype had significantly higher prevalence of growth retardations, hypogonadism, hypothyroidism and hypoparathyrodism compared to those with β0β+ and β+β+ hematologic phenotypes ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.037$ respectively). Patients with homozygous IVS-1 mutation had significantly higher prevalence of growth retardations, hypogonadism, hypothyroidism and hypoparathyrodism, while patients with homozygous IVS-1-110 mutation and homozygous IVS-1-6 mutation had significantly lower prevalence of growth retardations, hypogonadism ,hypothyroidism and hypoparathyrodism ($p=0,039$, $p=0.013$, $p=0.006$, $p=0.008$ respectively). Patients with homozygous IVS-11-745/VS-11-745 mutation had significantly higher prevalence of diabetes ($p=0.001$).
Summary and Conclusions: Endocrinal complications were common in β thalaasemia major patients with a clear association between hematological phenotype, genotype and clinical disease severity.A significant relationship was also observed between serum ferritin levels and the presence of endocrine complications, emphasizing the important role of iron overload in the development of these complications.

PB1953

IRON OVERLOAD IN ACUTE LEUKEMIA LONG SURVIVORS

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Background: Iron overload, primarily related to RBC transfusions, is a relatively common complication in patients receiving induction/consolidation chemotherapy for acute leukemia.
Aims: The rate and amount of tissue iron accumulation are the two most important factors that determine the risk of iron-related organ toxicity. Estimation of liver iron concentration (LIC) by liver biopsy or imaging is recommended after regular transfusion to determine the need for iron-chelation therapy. Although serum ferritin and transferrin saturation is not as reliable as LIC for estimating body iron stores, the risk of cardiac disease and early death is increased in patients with serum ferritin of more of 2500 ng/ml while clinically significant iron overload is uncommon in patients with serum ferritin level less than 1000 ng/dl.
Methods: In our institution we studied, approximately 2 years after the last transfusion, the iron status of 32 evaluable adult acute myeloid and lymphoid leukemia (AL) survivors who were treated between 2005 and 2009 with intensive chemotherapy and in 3 cases with first remission allogeneic bone marrow transplantation. 26 patients showed high ferritin levels.
Results: Their mean (+/-SD) age was 47 +/- 16 years (range, 35 - 67 years). The ratio men to woman was 1.45 (16 man and 11 woman).They received an average of 28 RBC units each (range, 18 - 45), the median serum ferritin level for all patients was 1870 ng/ml (normal range 30- 365 ng/ml) and transferrin saturation was 47% (normal range 15% - 45%).
Summary and Conclusions: We suggest that iron build-up is present also some years after chemiotherapy and transfusional treatment. Transferrin saturation values confirm the suspicion of iron overload, as for patients with hemochromatosis. During a median 33 months (range between 13 and 47 months) of follow-up no patients expressed cardiac diseases (documented with ECG), transaminases elevation or increased rate of infections. During this period of observation we are enable to suggest for patients an iron-depleting therapy with phlebotomy or iron chelators because remain a number of unanswered questions about the short and the long term fibrosis impact of iron overload in adult leukemia survivors. Furthermore is not clear if performing this kind of therapy during or after transfusion treatment. Our observation could be compared with collaborative multi-institutional long term studies to better understanding the contributions of iron overload on late effects of leukemia treatments.

Infectious diseases, supportive care

PB1954

EMPIRICAL ANTIBIOTIC THERAPY IN FEBRILE NEUTROPENIA OF ACUTE LEUKEMIA: EPIDEMIOLOGIC ORIENTED-STRATEGY IN A SINGLE DIVISION IN THE ERA OF MULTIDRUG RESISTANCE BACTERIA

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Background: In acute leukemia and high risk myelodisplastic syndrome treated with chemotherapy febrile neutropenia needs prompt treatment with antibiotic therapy. A delay in starting optimal empirical therapy can lead to septic shock and death in a few hours. An anti-pseudomonal beta-lactam combined with an aminoglycoside can be an adequate therapy. Recently a Cochrane review concerning a beta lactam-aminoglycoside combination *versus* beta lactam monotherapy in sepsis has been published. All-cause mortality rate was similar but risk of nephrotoxicity was more frequent in combination therapy. Guidelines for febrile neutropenia in high risk patients recommend monotherapy with anti-pseudomonal cephalosporins, piperacillin/tazobactam or carbapenem (ECIL 2011).
Aims: Aim of this study was to verify the applicability of published recommendations in our Division in presence of resistant bacteria.
Methods: Since June 2013 we have conducted an overseeing of carbapenemase-producing Klebsiella Pneumoniae species because of their emergence in our Hospital. All inpatients had a rectal swab searching for carbapenemase-producing Enterobacteriacee when they were admitted to the ward. Our empirical first-line therapy in febrile neutropenia was ceftazidime, in association with amikacin in presence of hemodynamic instability. We reviewed all febrile neutropenia episodes (absolute neutrophil count<500 x 10 9/L or expected to decreased<500 x 10 9/L during the next 48 hours) in 44 patients affected by acute leukemia or high risk myelodisplastic syndrome treated with aggressive chemotherapy in our Division from 1.1.2013 to 31.1.2014. All patients had prophylaxis with levofloxacin.
Results: There were 78 episodes of febrile neutropenia. Blood culture results were positive in 35 (45%) episodes. In 19 (54%) episodes sepsis was due to Gram-positive bacteria. In 16 (46%) episodes sepsis was due to Gram-negative bacteria. In 44% (7/16) of Gram-negative episodes, the isolated bacteria (4 Escherichia Coli, 3 Enterobacter Cloacae, 1 Klebsiella Pneumoniae) showed resistance to ceftazidime but sensitivity to amikacin.. All the isolated Gram-negative bacteria resistant to ceftazidime and sensitive to amikacin were sensitive to carbapenem. Only in 3/7 patients surveillance swab cultures confirmed the same bacterium as blood cultures (Escherichia Coli). One patient in monotherapy had a septic shock and died. In the surveillance of carbapenemase-producing Enterobacteriacee conducted in the Division we found 7 patients colonized by carbapenemasi-producing Klebsiella Pneumoniae but no bacteremia.
Summary and Conclusions: The increasing development of carbapenemase-producing Enterobacteriacee in our Hospital discourages the use of carbapenem as empirical first line therapy except in patients with surveillance cultures positive for ESBL. Choise of empirical antibiotic therapy for febrile neutropenia of leukemic patients should consider not only the epidemiologic data of the Division but also the epidemiologic data of the Hospital. In our epidemiological contest antibiotic monotherapy could be inadequate. Risk of nephrotoxicity due to the combination of a beta lactam with an aminoglycoside could be reduced by rapid de-escalation of aminoglycoside if possible.

PB1955

EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF BACTEREMIA IN ACUTE MYELOID LEUKEMIA (AML): EXPERIENCE IN A TERTIARY REFERRAL CENTRE IN PORTUGAL

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Background: Antibiotic (atb) resistance has become a major public health concern. Atb efficacy is vital for the management of AML patients (pts) because they are at high risk for infection. Local surveillance of atb resistance, atb consumption, and patient outcomes is essential for defining the best empirical approach for infection treatment and also to minimize the selection of resistant pathogens. Bloodstream cultures are a very useful model for surveillance of atb resistance and its clinical impact.
Aims: The incidence and pathogenesis of bacteremia episodes (BE) in AML pts were analyzed, as well as the atb resistance profile (ARP) of the pathogens. We also analyzed pts' characteristics, clinical outcomes and the end-point of BE.
Methods: We conducted a retrospective analysis of all BE in AML pts treated in our Institution between Jan 2011 and Dec 2013. Our pts are usually treated in an open medical ward (2-4 pts each), with filtered air.
Results: A total of 107 episodes of bacteremia occurred in 65 patients. Seventy one percent (n=76) of the pathogens responsible for BE in our institution were gram negative (GN), 26% (n=28) gram positive (GP) and only 3% (n=3) were fungal. We observed a high fluoroquinolone (FQ) resistance among our isolates (52%), even in pts without FQ prophylaxis, which was done in 20% of our pts. Additionally, an important 3rd generation cephalosporins resistance among GN (27%) was noted, as well as the prevalence of ESBL producers (21%). Piperacillin-tazobactam (Pip-taz) resistance was 24% (n=18) among the GN, but there were much lower rates of Amikacin resistance: 8% (n=6). Concerning GP, 32% (n=11) were *Enterococcus* species, with the rest being mostly *non-aureus Staphylococcus.* We documented only 2 BE associated with methicillin-sensitive *S. aureus*. Four out of eleven of the *Enterococcus* species were VRE. The frequency of multidrug resistant organisms (MDRO) was 29% (n=32), but in 66% of the cases this occurred in the setting of prior very recent broad-spectrum atb, with only 5 of this 32 isolates not having prior atb. Surprisingly, the majority of BE (48.6%) were recorded after post-remission therapy, 25.2% in the context of relapsed/refractory (R/R) hematologic disease and only 15.8% after induction therapy, indicating that, for this type of infection in AML pts, an important contributor factor might be a cumulative risk associated with the typical nosocomial infections. Sixty percent of BE were associated with indwelling venous devices, and 81.3% have occurred in the context of severe neutropenia. Almost 35% progressed to sepsis or septic shock. The overall mortality rate was 15,3%, with 8 out of 9 pts dying in the setting of R/R disease. There was a cumulative incidence of 13 BE per thousand days of hospitalization, with 15% of admissions for AML registering BE. The average length of stay (ALS) in AML pts with BE was 25,29 days, significantly higher than the overall ALS in AML pts - 14,01 days ($p<0.001$). It is also interesting to note a remarkable decrease in the ALS in AML pts with BE between 2011 and 2013 (32,46 days in 2011, 24,24 in 2012 and 20,67 in 2013), which may reflect an improvement in our care.
Summary and Conclusions: BE due to GN organisms are the dominant type of bacteremia in our AML pts, with an increasing epidemiological burden of severe infections by MDRO. It is crucial for the management of such infections, not only the choice of prophylaxis – whereas our resistance pattern to FQ makes us question its value – but also the initial atb therapy. Among our ARP, Pip-taz resistance is of major importance since this is our first line atb for neutropenic fever. Despite this, the low resistance to amikacin is reassuring because we use it in association with Pip-taz when there are signs of clinical severity.

PB1956

EPIDEMIOLOGY AND ANALYSIS OF ANTIFUNGAL PRESCRIPTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A SINGLECENTER REAL LIFE EXPERIENCE

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Background: Invasive fungal infection (IFI) causes morbidity and mortality among patients with hematological malignancies who receive cytotoxic chemotherapy or hematopoietic stem cell transplantation (HSCT).
Aims: Invasive fungal infections generally constitute a major clinical and therapeutic challenge associated with high morbidity and mortality rates in the course of cytotoxic treatment of hematological malignancies. In this retrospective study we aimed to check out our IFI rates and antifungal treatment modalities.
Methods: We retrospectively evaluated the diagnoses, antifungal prescriptions, and incidence and treatment outcomes of proven andprobable IFI in our center between October 2012 and December 2013 following the recent European Organization for Research andTreatment of Cancer/Mycosis Study Group (EORTC/MSG) consensus criteria.
Results: We evaluated 511 patients with hematological malignancies. Systemic antifungal agents were used 126 (24.6%) of 511 patients. Seventy five (59.5%) of the 126 patients received prophylaxis. IFIs were diagnosed in 37 (29.3%) patients (9 proven, 28 probable) of all, including 9 patients who underwent HSCT. Thirty nine patients had received mold-active prophylaxis, consisting in 35 with posaconazole and 4 with voriconazole. Thirty six patients received no mold-active prophylaxis with fluconazole. Liposomal amphotericin B (n=69) was the most common single-agent therapies. In 56 (44.4%) of all patients, first antifungal agent was changed another (Table 1). Seven (18.9%) of 37 patients with proven (3 patients) and probable (4 patients) IFI were died in one month. 28 patients of all were diagnosed with probable pulmonary IFI as determined by computed tomography scan and positive galactomannan assay. Serum galactomannan antigen was detected in 40 (31.7%) patients of all; 9 (7.1%) had positive cultures (5 Aspergillus sp, 2 Candida sp and 2 Mucor sp), and 55 (43.6%), abnormal computed tomography scans. One patient who received posaconazole prophylaxis was diagnosed proven IFI and 7 patients were diagnosed probable IFI. Also 2 patients received fluconazole prophylaxis were diagnosed proven IFI and 7 patients were diagnosed probable IFI. Four

patients who received voriconazole prophylaxis were diagnosed probable IFI (2) and possible IFI (2).

Table 1. First and second line antifungal agents and reasons for change.

Summary and Conclusions: First of all, results indicated that our probable and proven IFI incidences are significantly higher than literature (29.3% *vs* 2-8%) although one- month mortality still remains similar (18.9% *vs* 13%). This crucial inference prompted us to determine the underlying potentials of actual engrossing consequence. Mainly it may be related with diagnostic explication differences and local clinical conditions. Retrospectively we noticed that High-Resolution Computed Tomography (HRCT) findings were usually non-specific however prepossession of radiologist might lead to interpret the diagnosis on the side of IFI. Furthermore with a slight probability, our galactomannan assays occasionally might be conflicting due to technical problems or patient-related conditions like wide-spread use of penicillin-based antibiotherapy. High incidence of proven IFI may be related to managing diagnostic invasive procedures properly and successful mycological culture practice. Environmental and host-related factors may also be effective. Clinical awareness, competent assessment of risk factors, appropriate prophylactic implementation, determining right antifungal choice, time of treatment initiation and length of therapy are the critical steps in the administration of this clinical process. Improved strategies are required to reduce the frequency of IFI and to increase the proven IFI.

PB1957

LOW RELIABILITY OF GALACTOMANNAN ANTIGEN ASSAYS IN ROUTINE CLINICAL CARE – CLINICAL TRIAL RESULTS ARE NOT EASILY REPRODUCED

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Background: The diagnosis of invasive fungal infections (IFI) in neutropenic and non-neutropenic hematologic patients remains a challenge, with many patients treated empirically within the context of suspected, probable or presumptive diagnoses. For IFI by Aspergillus spp., the serum galactomannan antigen assay (GalAg) – detecting a cell wall molecule - has been proposed as a biomarker to orient preemptive antifungal therapy, with favorable results in clinical trials, more so when used in conjunction with other diagnostic tests. Nevertheless, its efficacy in everyday clinical practice differs markedly across centers and its routine use is debated, with starchy foods and Penicillum-derived antibiotics inducing false-positive results.
Aims: We aim to evaluate the results of GalAg testing outside clinical trials, in routine clinical practice, to clarify its utility and reliability, and thus its ability to orient antifungal use.
Methods: We reviewed all GalAg detections performed on hematology patients in our center between 1-1-2012 (when the test was started in our Lab) and 31-12-2013. According to our local practice, culture was considered the gold standard for the diagnosis of IFI – cultures were considered relevant for the interpretation of GalAg results if performed up to four days before or after GalAg testing; GalAg results without a relevant culture within the time-frame thus defined were excluded. According to the manufacturer's specifications, positive tests were subjected to confirmatory testing.
Results: We obtained 953 GalAg determinations with relevant cultures over the two-year study period; in 4.8% of these cases, a positive culture for fungus was observed. Considering Negative GalAg results, cultures were positive for fungi in 3.6% of cases. Considering Positive GalAg results, 56.2% of cultures were negative, 22.9% were negative for fungi but positive for bacteria, and 22.9% were positive for fungi, although all were positive for Candida spp. and none for Aspergillus spp. Although three cases observed during the time-frame were positive for Aspergillus spp. (niger and flavus), all three were negative for GalAg; two (A. flavus) were being treated with antifungals, while the third (A. niger) was not; no other cause for false-negativity could be identified in the latter. Considering bacteria-positive GalAg-positive results, only 22% of patients were being treated with antibiotics known to originate false-positive results in GalAg determinations; in the remaining 78% of patients, no known causes for false-positivity were identified (although the patients' diet could not be thoroughly reviewed).
Summary and Conclusions: In our series of non-selected, retrospective patients treated outside clinical trials, results obtained with GalAg assays were markedly inferior to the published results that led to its approval. We found that GalAg was unable to detect any of our confirmed Aspergillus infections. While in 2/3 cases, a source of false-negative results was suspected (treatment with antifungals), in 1/3 cases no cause was identified. Over half of GalAg-positive cases had negative cultures; on the other hand, while almost a quarter of GalAg positive-cases tested positive for fungi, all corresponded to Candida spp., which should not cross-react with the GalAg assay. A quarter of cases were positive for bacteria, but a cause for false-positivity could only be identified in a quarter of those. We suggest that GalAg testing remains an unreliable tool, with low reproducibility, associating with very disparate results across centers. Local dietary traditions (in particular the use of starchy foods) could account for the wide variation in false-positive rates and, therefore, the decision to use GalAg testing to orient empirical-vs-preemptive antifungal treatment should be established locally. It remains an ancillary test, unable to replace established diagnostic tests.

PB1958

OUTPATIENT EXPERIENCE WITH BIOSIMILAR FILGRASTIM IN PATIENTS WITH LYMPHOID NEOPLASMS: LESSONS FROM ROUTINARY USE

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Background: Filgrastim derived biosimilars are now extensively used in routine clinical practice and together with their originators they are recommended for the prophylaxis of febrile neutropenia (FN) in cancer patients undergoing chemotherapy.
Aims: A monocentric observational study to investigate the efficacy and safety of biosimilar filgrastim in patients with lymphoid neoplasms at various stages of their disease treated with different non-myeloablative chemotherapy regimens on an outpatient basis.
Methods: Data from 141 patients affected by chronic lymphoproliferative disorders and treated with a non-myeloablative chemotherapy regimen from January 2012 to December 2013 were obtained from our institutional database. Median age was 56 (range: 15-86) years. Diffuse large B-cell lymphoma (47 patients), Hodgkin's disease (29 patients) and follicular lymphoma (16 patients) were the prevalent subtypes; among the remaining, there were 12 indolent non-follicular lymphomas, 11 mantle-cell lymphomas, 7 T-cell lymphomas and 2 Burkitt's lymphomas; 9 patients had hairy cell leukemia, 7 chronic lymphocytic leukemia, and 1 had Langerhans' cell histiocytosis. A total of 148 chemotherapy lines were administered (7 patients received two lines): 123 were delivered first-line. The most represented chemotherapies were: CHOP/COMP (38 cases), ABVD (25), fludarabine-containing regimens (22), third-generation CHOP-like regimens (MACOP-B/VNCOP-B, 20), cladribine (8) and ifosfamide-containing regimens (IEV, 14). Other regimens were: bendamustine, ibritumomab, lenalidomide, high-dose cyclophosphamide or cytarabine. Filgrastim biosimilar was applied to prevent or treat FN in accordance with the European Society of Medical Oncology guidelines.
Results: Overall, 1,806 vials were used, at an average of 12.9 vials per patient. The number of vials/patient for each regimen is detailed in the Table 1. Biosimilar filgrastim was used for the prophylaxis of FN in 143 cases; in 5 cases (3.4%) it was used for the therapy of FN. All chemotherapy-induced cytopenias were managed on an outpatient basis; hospitalization rate was 5.4%, due to intensive treatment for FN. No treatment was interrupted because of persistent neutropenia or unrecovered myelotoxicity. Neutropenia was always transient and FN easily manageable with biosimilar filgrastim and intravenous antibiotics. No septic shock nor infection-related deaths were documented. Adverse events were infrequent and mainly consisting of bone pain (4%), fever (1.3%) and tachycardia (0.7%). Drug administration was interrupted in only 1 case due to an adverse event.

Table 1.

Regimen	Patients treated	Vials used	Vials/patient (range)
CHOP/COMP	38	436	11.5(3-18)
ABVD	25	442	17.7 (3-40)
Fludarabine-Mitoxantrone	15	184	12.3 (3-18)
VNCOP-B	14	160	11.4 (2-21)
IEV	14	83	5.9 (4-10)
Cladribine	8	150	18.8 (8-42)
Fludarabine-Cyclophosphamide	7	52	7.4 (3-13)
MACOP-B	6	54	9.0 (2-20)

Summary and Conclusions: Biosimilar filgrastim is easy and safe to use on an outpatient basis, with no clinically relevant adverse events reported and without any significant difference from its originator. It shows efficacy in both the prevention of FN among several chemotherapy regimens and its treatment.

PB1959

FIRST EXPERIENCE WITH THE THERAKOSTM CELLEX® PHOTOPHERESIS SYSTEM

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Background: At our department, the TherakosTM Cellex® photopheresis system (CLX) has partially replaced the Therakos Uvar XTS (XTS) system for extracorporeal photochemotherapy (ECP) since 25 September, 2013. The Cellex technology incorporates several innovative developments, providing higher level of safety and patient-focused care for the patients (pts).
Aims: We present our first 4 months experience with TherakosTM Cellex® photopheresis system and the opinion of our pts.
Methods: 103 ECP treatments in 25 pts were performed by CLX during the first 4 months period. Data of these CLX treatments were compared with data of 150 XTS procedures performed in the same 25 pts.Patient's characteristics: there were 18 males and 7 females. The median age was 41 years (range:20-67) and the median weight was 66 kg (range:36-100). Indication of ECP was graft *versus* host disease:18 pts, cutan T cell lymphoma:4 pts and graft rejection after solid organ transplantation:3 pts (lung:2, heart:1). Procedures characteristics: selection of technique for pts was random, however, there was a slight tendency to use CLX for pts being in poorer condition and having worse venous access. CLX group: the apheresis volume was fixed at 1500 ml as determined by the system. One arm procedure was used in 26% of cases. Peripheral veins were used in 94% of procedures. XTS group: XT125 bowl was used for all cases. Six treatment cycles were performed for pts with body weight > 50 kg and 5 treatment cycles for patients with body weight<50 kg. Peripheral veins were used as venous access in 95% of procedures. One of our nurses conducted a telephone survey of pts. Two questions were asked: 1: Did they experience any difference between CLX and XTS? 2: Which machine would be preferable for them?
Results: Set up and priming are much faster in CLX than XTS giving more time to nurses to care the patient. Buffy coat: volume was significantly less in CLX than XTS (median 163 ml *vs.* 263 ml, p=1,17E-72) needing significantly less Uvadex (median 2,8 ml *vs.* 4,5 ml, p=2,4E-74) for CLX treatments. Photoactivation time: was significantly shorter in CLX than XTS (median 21 min, *vs.* 27 min, p=2E-07), which provides sparing of UVA lamp hours. Procedure time: was significantly less in CLX than XTS (median 95 min, *vs.* 166 min, p=8E-29). This was also shorter in one arm procedures (median:151 min *vs.* 166 min, p=0,2), however this was not statistically different. Platelet loss: post-apheresis decrease of platelet count was significantly less in CLX than XTS (median:-12%, *vs.*-20%, p=2,5E-07). Success of treatments: All CLX treatments were finished successfuly. There were 2 early treatment abortions due to venous access problem in XTS group. Side effects: The venous access issue was the most frequent problem. CLX was less sensitive for this. One female pt had mild vasovagal reactions in 2 CLXs and 1 XTS treatments. There were no other clinically relevant side effects in either group. Patients' survey: 21/25 pts were interwieved successfuly. 18/21 pts market CLX to be preferable, all of them indicated the higher speed to be the major advantage. 2/21 pts responded that there is no difference and there was only 1 pt who liked more the old XTS. 4/25 pts did not participate in the survey 2 of them due to death.
Summary and Conclusions: Our data prove that the innovation of TherakosTM Cellex® system was very successful providing faster and safer machine for ECP. Our opinion well coincided with the pts' very positive attitude.

PB1960

EFECTIVENESS OF ANTIFUNGAL PROPHYLAXIS WITH NEW AGENTS COMBINED WITH A DIAGNOSTIC-DRIVEN ANTIFUNGAL THERAPY STRATEGY IN HIGH-RISK NEUTROPENIC PATIENTS. A SINGLE-CENTER PROSPECTIVE STUDY

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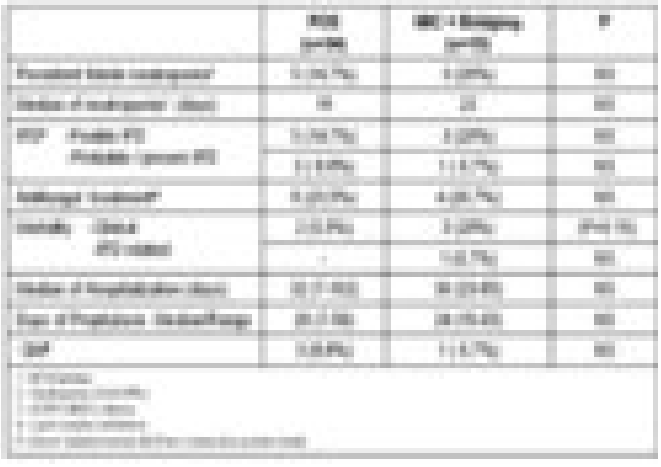
Background: Invasive fungal diseases (IFDs) are a leading cause of morbi-mortality in haematological patients undergoing intensive chemotherapy. Previous studies have shown that primary antifungal prophylaxis (PAP) with new agents reduces the incidence of proven/probable IFD to less than 3%. Despite this efficacy, 30-60% of these patients receive a subsequent empirical antifungal therapy (EAT), a strategy associated with unnecessary costs.
Aims: To compare the efficacy of PAP with posaconazole (POS) and micafungin (MIC) in a real-life setting of high-risk neutropenic patients.
Methods: Between January 2012-January 2014, all patients receiving intensive chemotherapy and a mold-active PAP for at least 7days were included in the study patients were hospitalized in rooms with HEPA filtration and positive pressure. Our PAP policy was as follows: oral POS (200mg/tid) was our standard PAP, but it was replaced by iv MIC (50mg/day) in patients with ALL receiving vincristine and MIC was also used as bridging in patients with severe mucositis and/or significantly elevated liver function tests (POS-MIC). A surveillance-driven strategy was applied (galactomannan twice-weekly all the period of neutropenia). High-resolution CT scans were performed when an IFD was clinically suspected. Antifungal therapy (liposomal amphotericin B) was administered only when a diagnosis of IFD was made according to 2008 EORTC/MSG definitions. EAT was reserved to patients with persisting febrile neutropenia, a negative intensive diagnostic work-up and worsening clinical condition. Endpoints were incidence of IFD, EAT, persistent febrile neutropenia, as well as IFD-free and overall survival.
Results: 49 episodes were included; median age of 54 years (range 32-83); disease distribution was as follows: 30 AML induction, 12 AML consolidation/Intensification, 4 ALL induction and 2 Burkitt's L. and 1 AA; 34 of 49 (69%) received POS for a median time of 26 days and 15 of 49 (31%) received MIC (9 MIC and 6 POS-MIC for a median time of 24 days). The main Patient's clinical characteristics and outcomes are summarized in Table 1. No significant differences were observed in the incidence of proven/probable IFD (POS 8.8% *vs* MIC 6.7%), possible IFD (POS 14.7% *vs* MIC 20%), antifungal therapy (POS 23.5% *vs* MIC 26.7%) and IFD-associated mortality. No patient received EAT as a result of our diagnostic-driven antifungal therapy strategy. There was no grade III/IV CTCAE toxicity related to PAP.

Table 1. Treatment success by type of prophylaxis.



Summary and Conclusions: Both prophylactic regimens are feasible, safe and effective. The use of an overall strategy combining both an intensive diagnostic approach (to reduce the underdiagnosis of IFD) and a diagnostic-driven antifungal therapy may reduce the requirement for EAT, and saving additional costs.

PB1961

DETECTION OF FUNGAL DNA IN LYSIS-CENTRIFUGATION BLOOD CULTURE FOR THE PREVISION OF RESPONSE TO ANTIFUNGAL IN AML PATIENTS WITH PERSISTENT NEUTROPENIC FEVER: A PILOT SUBSET

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Background: Several studies have demonstrated that a prompt addition of a systemic antifungal therapy in AML (Acute Myeloid Leukemia) patients suffering Invasive Fungal Disease (IFD) improves clinical outcome, anyway there are not clear tests able to distinguish patients that will benefit an empiric antifungal treatment and patients that will not.
Aims: We investigated the possible diagnostic role of pan-fungal DNA PCR for the early detection of IFD in patients receiving antifungal prophylaxis.
Methods: 28 patients with AML have been analyzed: 15 patients underwent a prophylaxis with posaconazole 200 mg tid per os and 13 patients a prophylaxis with amphotericin-B 10 mg bid per aerosols and Nystatin 600,000 IU orally tid.

The serological monitoring was performed twice a week through the beta-D-glucan (BG) (Fungitell) and the antigen galactomannan (GM) (Platelia ™ Aspergillus EIA) detection. Chest CT was performed in presence of fever lasting more than 72 hours. Invasive Fungal Disease (IFD) was diagnosed according to the EORTC criteria. The empirical antibiotic and antifungal therapy were administered according to ISDA guidelines. The fungal DNA was evaluated on whole blood after lysis of the white blood cells according to[1] two times a week and was interpreted as positive if revealed in two consecutive detections. The qualitative data were analyzed using the chi-square test.

Results: Eight patients (32% of the total) had fever unresponsive to antibiotic therapy, 4 of which had positive fungal DNA. 5 patients received antifungal prophylaxis with posaconazole, 1 of these responded to antifungal treatment (voriconazole) and had a positive fungal DNA. 3 patients received prophylaxis with amphotericin-B per aerosols and Nystatin, two of which were sensitive to antifungal treatment (1 treated with voriconazole and caspofungin in combination and the other with liposomal amphotericin-B), both with positive fungal DNA , 1 patient with high-risk AML resistant to induction therapy with positive BG and fungal DNA did not respond to the combination of caspofungin and liposomal amphotericin –B. Except for the latter patient, no other patients refractory to antifungal therapy had positive fungal DNA. No differences emerged with regard to the rate of fever resistant to antibiotics between the 2 groups (p=0.68). Overall, fungal DNA positivity conferred a greater likelihood of responding to the antifungal therapy compared to negative (p=0.03) (Table 1).

Table.1

Data analyzed	Response to antifungal	No response to antifungal	Total
DNA pos	3	1	4
DNA neg	0	4	4
Total	3	5	8

Chi-square test: p=0,03

Summary and Conclusions: The performance of DNA-PCR assay will be of great help in the early detection of patients with antibiotic resistant fever with a great probability to respond to antifungal empiric therapy.

Reference

1. Trovato L, Betta P, Romeo MG, Oliveri S. Detection of fungal DNA in lysis-centrifugation blood culture for the diagnosis of invasive candidiasis in neonatal patients. Clin Microbiol Infect. 2012 Mar;18(3):E63-5. doi: 10.1111/j.1469-0691.2011.03731.x. Epub 2011 Dec 22. PubMed PMID: 22192484.

PB1962

MONITORING THE INTRODUCTION OF MICAFUNGIN IN THE DEPARTMENT OF HAEMATO-ONCOLOGY AT BARTS HEALTH NHS TRUST

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Background: In acute lymphoblastic leukaemia (ALL), azoles are contraindicated for prophylaxis of invasive fungal disease (IFD) because of the vinca-alkaloids in ALL regimens. We changed our guidelines to include Micafungin as a prophylactic alternative to AmBisome in first induction therapy of ALL.

Aims: This audit aims at monitoring the introduction of Micafungin, including adherence to the guidelines and its use for other indications.

Methods: Data was collected from Jan to Dec 2013. The selection criteria included patients with ALL and patients receiving Micafungin in the Haemato-Oncology Unit. 40 patients were identified; some with multiple admissions (*i.e.* separate episodes of IFD risk, such as cyclical intensive chemotherapy). We evaluated prophylactic drug use and, for each episode of treatment of suspected IFD, chest CT or Galactomannan (GM) results.

Results: 48 episodes from 40 patients were included. Mean age was 45 yrs, range: 21-67; 18 were female and 22 were males. The primary diagnosis was ALL (n=24, 60%), AML (7, 18%) and other (9, 22%: 2 HD, 3 lymphoma, 1 CLL, 1 CML, 1 MDS and 1 myeloma). In 10 episodes of ALL induction therapy, the antifungal prophylaxis was: Micafungin (n=1) AmBisome (2), Fluconazole (6) and no prophylaxis (1). No patient had their prophylaxis changed. In the other 38 episodes, prophylaxis was: Fluconazole (23) Micafungin (3), AmBisome (2), Posaconazole (2) and no prophylaxis (8). Suspected IFD was treated in 19 episodes: Micafungin (n=8), Ambisome (10) and Caspofungin (1). In 3 cases, AmBisome was changed to Micafungin (1 - due to liver abnormaities, 2 – not stated); and 1 from Caspofungin to Micafungin, as per guidelines. Fungal diagnostics: in 4/19 (21%) episodes of treatment, no CT/GM testing was performed. In the remaining 15, 4 patients started treatment prior to CT/GM testing. Overall, in 15/19 cases, the clinical suspicion of IFD was not supported by CT, GM or cultures. In 22/48 (46%) episodes, no antifungal treatment was given, of these, 11 cases a CT chest/GM test were performed with no evidence of IFD. 8 patients (20%) died, 5 in ICU. 6/8 had CT chest/GM testing - with only one positive CT scan for IFD. Despite the lack of evidence, 7/8 patients were treated for suspected IFD: Micafungin (n=6), AmBisome (1). There was missing/incomplete drug information in 7 episodes of the total 48 (15%).

Summary and Conclusions: There was a lack of adherence to the guidelines for ALL IFD prophylaxis in 1st induction therapy. 90% of Micafungin use was outside of the guideline indication, which can be explained by removal of Caspofungin from the formulary to avoid having two echinocandins in our Unit. Fungal investigations were largely performed appropriately, with only 4 antifungal treatments starting before CT/GM testing. However, in 15/19 episodes of treatment, there was no CT/GM evidence of an IFD. Following re-training of the clinical teams on the antifungal guidelines, better adherence for ALL prophylaxis is expected and this is being re-audited. Nevertheless, the data shows that the clinical decision to treat for suspected IFD often occurs with negative CT/GM results. Perhaps a way forward to implement a diagnostic-driven approach is to have a small, dedicated team responsible for all fungal management, *i.e.*, the concept of antifungal stewardship.

PB1963

NATIONAL SYSTEMATIC APPROACH TO THE MANAGEMENT OF ASPLENIA: THE SET UP OF THE ITALIAN NETWORK ON ASPLENIA.

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Background: Asplenia is a condition due to spleen absence or dysfunction, which leads to high risk of infections and thrombotic events with significant chance of mortality and morbidity. A number of national and international recommended interventions in asplenic or hyposplenic patients has been published but adherence to recommendations was shown to be poor in several studies. The cost to the health system of asplenia- related complications can be significant, and systematic approaches to prevention were demonstrated to be cost-effective. In Europe very little experience exists in comprehensive national program for management of asplenia. Particularly, in Italy no common policy of patient care has yet been developed, and management of asplenia is mainly case or locally directed.

Aims: Our aim was to investigate the feasibility of a systematic national integrated program for the management of asplenia in Italy and to create a national working group focused on asplenic patients.

Methods: Centers of the Italian Association of Pediatric Hematology Oncology (AIEOP) and the Italian Society of Thalassemia and Hemoglobinopathies (SITE) were invited to participate in the Italian Network on Asplenia. The coordinating centre sent a registration form to all doctors who formally agreed to the proposal.

Results: Twenty-seven pediatric care centers and two adult care centers formally agreed to the project during a recruitment period of six months. During the following four-month period, twelve centers sent back the completed forms containing data about reason and duration of asplenia, type of surgery and post-surgery complications for splenectomized patients, long term infectious and thrombotic complications, antibiotic and vaccine prophylaxis and causes of death. At the last update, the database included data from 318 patients. Meetings of involved parties were held to discuss key points in the management of asplenic patients and to develop a consensus on recommendations for patient care.

Summary and Conclusions: This is the first systematic approach to the management of asplenia in Italy and the most recent study in Europe, as previous reports are long-standing. This preliminary analysis shows that a comprehensive national project is feasible in Italy. Recruiting patients through representatives of national scientific societies is an effective way to involve the main national centers where the majority of asplenic patients are followed up. A call for participation in the Italian Network on Asplenia will be addressed to other national scientific societies involved in the asplenic patient care, such as the Italian Society of Hematology, Oncology, Surgery, Cardiology, Pediatric Cardiology, ect. The disparity of participation between pediatric and adult care centers suggests a deeper awareness of risks due to asplenia among

pediatricians and the need for more active involvement of doctors treating asplenic adults. Till now the main concern for asplenic patients has been the high risk of overwhelming infections, but there is a growing body of knowledge on thrombotic risk in asplenic patients. The novelty of this project consists in collecting data regarding this issue, which has been neglected so far. Finally, this project has the potential for implementing research and public health purposes in this target population at a national level.

PB1964

BACTEREMIA AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION – RESULTS OF A 13 YEARS SINGLE CENTRE STUDY

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Background: Successful management of bacterial infections after hematopoietic stem cell transplantation (HSCT) is mandatory for improving outcome of transplant recipients. One of the most frequent bacterial pathogens' isolation site in neutropenic HSCT patients is bloodstream, therefore the spectrum of microbial strains isolated, as well as their antibiotic susceptibility has major implications on both prophylactic and empirical antibiotherapy choices.
Aims: To evaluate the incidence, outcome and pattern of bacteremias after HSCT with emphasis on the identified pathogens and their *in vitro* susceptibility to antibiotic agents.
Methods: In a 13 years retrospective study we have included 287 consecutive patients, both children (n=83) and adults (n=204) admitted between 2001-2013 for HSCT (autologous-232, allogeneic-55). Anti-infective prophylactic measures included: nursing in laminar air-flow or HEPA-filtered sterile rooms, sterile diet, gut decontamination with quinolones and weekly immunoglobulin substitution. In addition, all the patients received a short course of amoxicillin/clavulanic acid and metronidazole. Bloodstream cultures were drawn from a central venous line in all patients with febrile neutropenia before initiation of empirical antibiotic therapy. Susceptibility and resistance of isolated microbial strains were evaluated using either disk-diffusion method or an automated blood culture system.
Results: Cumulative incidence of bacteremia was 14% (auto-13%, allo-20%), overall being registered 55 episodes of bacteremia (auto-38, allo-17) in 41 patients (auto-30, allo-11). The median time from the day of stem cell graft infusion to the day of bacteremia was 126 days (1-2610 days), with more than 90% of episodes occurring in the first 100 days after HSCT. In both autologous and allogeneic transplant setting, gram-negative pathogens were more frequently isolated than gram-positive ones (73% *vs.* 27%). Gram-negative strains included: *Escherichia coli* (auto-39%, allo-47%), *Pseudomonas aeruginosa* (auto-11%, allo-12%), *Klebsiella pneumoniae* (auto-16%, allo-0%, p=0,04) and *Enterobacter spp*. (auto-11%, allo-6%). Coagulase-negative staphylococci represented 73% from the gram-positive bacteria, followed by *Staphylococcus aureus* (13%). Over 80% of identified gram-negative strains were resistant to quinolones, while 33% of *Klebsiella pneumoniae* and 17% of *Escherichia coli* produced extended-spectrum beta-lactamases. Methicillin-resistance was found in 70% of *Staphylococcus spp* isolates. Multi-drug resistant gram-negative strains represented 30% of the gram-negative isolated rods; all the gram-negative pathogens retained their susceptibility to colistin. Crude mortality in patients with bacteremia after HSCT was 51% (auto-40%, allo-82%, p<0,01) with a mortality attributable to bacteremia of 22% (auto-23%, allo-18%), related only to gram-negative pathogens: *Pseudomonas aeruginosa*-44%, *Escherichia coli*-33%, *Klebsiella pneumoniae*-11%, *Enterobacter spp*.-11%.
Summary and Conclusions: Although, recent published studies describe a shift to predominance of gram-positive strains responsible for severe infections after HSCT, in our study, both the incidence and direct mortality of pathogens isolated in blood cultures of HSCT recipients were dominated by gram-negative life-threatening multi-drug resistant gram-negative rods.

PB1965

DEVELOPMENT OF EARLY INFECTIONS DURING AZACITIDINE THERAPY FOR HIGH-RISK MYELODYSPLASTIC SYNDROMES.

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Background: The incidence and risk factors for infection during azacitidine treatment are only partially known. Specifically, risk factors for infection during the first cycles of treatment, when haematological improvement has not been achieved yet, and the impact of comorbidities have not been addressed.
Aims: To analyse factors associated to early infection in a cohort of myelodysplastic syndromes (MDS) patients treated with azacitidine as front-line treatment.
Methods: Patients with a diagnosis of high-risk MDS and those with an intermediate-low risk IPSS and absolute platelet count (plt) <30 x10^9/L or absolute neutrophil count (ANC)<0.5 x10^9/L receiving azacitidine as front-line treatment were studied. Only the first 4 courses of azacitidine treatment were analysed for the purpose of the study. Univariate comparisons between cycles with and without infection were done using a Chi-square or a T-test as appropriate. A propensity score to match patients according to their probability of receiving prophylaxis was calculated. Variables with a significant difference were included in a logistic regression model, and odds ratios (OR) with their 95% confidence interval were computed.
Results: From 15th September 2007 to 1th December 2013, fifty patients received azacitidine and 188 episodes of treatment were recorded. Median age at the beginning of treatment was 69 years (range 25-88). According to IPSS score, 75% of patients were classified as high risk and 25% as intermediate risk. Sorror comorbidity score was low in 36%, intermediate in 26% and high in 38% of the patients. Azacitidine was administered during 7 consecutive days in 78% of the patients, 18% received a 5-0-2 scheme and 4% received 5 days of treatment for each cycle. Regarding prophylaxis, no antimicrobial agent was administered in 55% of the cycles; a single antimicrobial agent (either antibacterial or antifungal) was administered in 14% and a combined antibacterial plus antimicrobial regimen in 31% of the cycles. There were significant differences in age, blast number and ANC among patients receiving prophylaxis or not. ANC, platelets and haemoglobin (hb) at the beginning of each cycle were 0.8 x10^9/L (range 0.05-11.14), 95 x10^9/L (range 11-885) and 94 g/L (range 55-142). During the first 4 cycles, 30 infectious episodes occurred and 8% of the patients died as a result of infection. Seventy percent of the infectious episodes occurred in the first 2 cycles of azacitidine. In univariate analysis, a high comorbidity index, alternative schemes of administration of azacitidine other than 7 consecutive days and the number of packed red blood cells and platelets concentrates transfused were associated with infection. A multivariate analysis was done including those variables with significant differences in the univariate analysis and the computed propensity score. Predicitive factors for infection in this model were: a high comorbidity score {p=0.008, OR 2.41 (CI 1.28-4.84)} and ANC below 0.5 x10^9/L {p= 0.056, OR 5.13, (CI 0.91-27.89)}. Combined antifungal and antibacterial prophylaxis showed a trend towards a decreased risk of infection {p= 0.06, OR 0.07 (0.003-1.24)}.
Summary and Conclusions: Comorbidity index is the most important factor in predicting infection during azacitidine treatment for high-risk MDS. The impact of neutropenia and antimicrobial prophylaxis must be studied in larger population.

PB1966

COMPREHENSIVE PULMONARY REHABILITATION IN PATIENTS WITH PULMONARY CHRONIC GRAFT VERSUS HOST DISEASE

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Background: Pulmonary chronic graft *versus* host disease (cGVHD) is a serious late complication of allogeneic hematopoietic stem cell transplantation (HSCT), which hampers quality of life and increases mortality of survivors. Medical treatments have limited efficacy at improving pulmonary function.
Aims: We investigated the therapeutic effects of comprehensive pulmonary rehabilitation (CPR) in patients with deteriorated lung function due to pulmonary cGVHD .
Methods: We retrospectively analyzed the medical records of patients who had pulmonary cGVHD and participated in the CPR program, which consisted of aerobic exercise, inspiratory muscle strengthening, accessory respiratory muscle strengthening and mechanically assisted coughing. The subjects were planned to undergo maximal cardiopulmonary exercise test and a body composition test with bioelectrical impedance analysis at baseline and 3 months. Exercise testing parameters, skeletal muscle mass of lower extremities and body mass index (BMI) were analyzed.
Results: Nine patients were assessed (two males, seven females; median age 40 (range 24–64) years). Donor lymphocyte infusion was performed in two patients. All patients had skin, mucosal and visceral manifestations, in addition to pulmonary cGVHD. When starting CPR, the median FEV1 was 25% (range 18% – 51%). Eight patients received concomitant corticosteroid and three received imatinib. At a median follow-up of 11 (range 2–16) months, the patients had participated in the CPR program for a median of 40.5 (range 10–148) days. Eight patients (88.9%) had completed more than 20 sessions of the CPR program. The rehabilitation programs were readily tolerable and had no adverse effects. The median change in the maximum oxygen consumption (VO_{2max}) was 28.5% (range –31.8% to 60.1%) and the muscle mass of the lower extremities changed by 3.0% (range –0.2% to 24.1%). All four patients who had an increased muscle mass in the lower extremities had an increase in VO_{2max}. There were no marked changes in BMI.

Summary and Conclusions: Our comprehensive pulmonary rehabilitation program for patients with pulmonary cGVHD with limited lung function was feasible and improved the exercise capacity in a population of patients.

PB1967

GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR AND GRANULOCYTE COLONY STIMULATING FACTOR AUTO-ANTIBODIES IN CHRONIC HEPATITIS -C VIRUS INFECTED PATIENTS WITH NEUTROPENIA

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Background: .HCV infection is frequently associated with hematological disorders such as anemia, leucopenia and thrombocytopenia. Neutropenia is common during combination antiviral therapy for chronic hepatitis C. Haematopoietic growth factors are generally well tolerated and they may be useful for managing hematological side effects of anti-HCV therapy.G-CSF and GM-CSF are mainly responsible for the proliferation of granulocytes and macrophages.There is increasing evidence that spontaneous anticytokine autoantibodies are associated with chronic infections and autoimmune diseases.
Aims: Assessment of auto-antibodies against Granulocyte Macrophage Colony Stimulating factor (GM-CSF) and Granulocyte Colony Stimulating factor (G-CSF) in chronic HCV infected neutropenic patients.
Methods: Patients: (87) chronic HCV infected patients were included, the study group included (50) neutropenic (ANC<2.5 cmm) chronic HCV infected patients, while the control group included(37) chronic hepatitis C virus inrected patients without neutropenia. The following investigations were carried out: Complete blood count (CBC), biochemical assessment: Liver function tests, 3)Serological assessment: -Serum HCV antibody using anti HCV enzyme immunoassay 4-Detection of HCV –RNA by RT-PCR 5-Detreminaiton of serum (G-CSF) and (GM-CSF) level by using ELISA Kit 6-Assesment of anti (G-CSF) &(GM-CSF) by using ELISA technique.
Results: The mean value of serum (G-CSF) In neutropenic group (90.23±62.06 µg/mL) was significantly higher than the same for non neutropenic group (5.49±4.90 µg/mL) P value<0.001. The mean value of serum (GM-CSF) In neutropenic group(77.29±71.40µg/mL) was significantly higher than the same for non neutropenic group (0.80±0.65µg/mL) P value<0.001. Anti-G-CSF antibody was positive only in 5 patients (10%)of neutropenic group and negative (not detected) in non neutropenic group. Anti-GM-CSF antibody was positive only in 3 patients (8.3%)of neutropenic group; niether of both autoantibodies was detected in the in non neutropenic group.
Summary and Conclusions: · The higher level of serum G-CSF and GM-CSF in neutropenic group suggest intactness of G-CSF and GM-CSF physiologic feedback mechanism; in conjunction with the low level of anti-G-CSF and anti-GM-CSF suggest that the etiological factors for neutropenia in HCV infected patients could be confined to the committed hematopoietic stem cells and the early myloid proginators which should be targeted in future studie.

PB1968

THE EPIDEMIOLOGY AND PROGNOSTIC FACTORS OF FUNGEMIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Despite the growing antifungal armamentarium, fungemia remains an extremely poor prognosis in hematological patients and clinicians still face many challenges in diagnosing and treating fungal septicemia.
Aims: The objective of the current retrospective study was to investigate the epidemiology of fungemia and its prognostic factors in patients with hematologic malignancies.
Methods: We retrospectively analyzed patients with hematologic malignancy and positive cultures for fungi at the Nanfang Hospital in China (2003-2012). The medical and electronic records of patients were reviewed for demographic data and clinical information, including clinical presentations, the infecting fungi species, treatment and outcome.
Results: Forty-seven patients were identified based on blood cultures in our study, and other causes of death except fungemia-related cause were excluded. Acute myeloid leukaemia was the most common underlying disease in hematological patients with fungemia (61.7%). The majority of patients (91.5%) received chemotherapy and 32(68.1%) patients belonged to nosocomial infections. 42(89.4%) patients existed severely agranulocytosis, and the median time of agranulocytosis duration was 9 days (range, 4-40 days). 43(91.5%) patients were complicated by infections of other sites, and respiratory infections most frequently presented in 33/43 patients (78.6%). Non-albicans Candida strains (83%) was the predominant species found in our survey, and Candida tropicalis (57.1%) was confirmed the importance of this species as cause of fungemia. Outcomes were divided into success and death and the mortality rates was 25.5% (n=12). By multivariate logistic regression analysis, the duration of agranulocytosis, presence of shock, and the initiation time of antifungal therapy were the independent risk factors for death.
Summary and Conclusions: In order to prevent the prevalence of fungemia in hematological patients, a prophylactic strategy against fungemia in high risk patients should be considered. In those patients who had developed fungemia, providing timely and appropriate antifungal therapy, promoting recovery of agranulocytosis and preventing the shock will improve the prognosis of the patients.

PB1969

INCIDENCE AND OUTCOME OF SEPTIC SHOCK IN PEDIATRIC CANCER PATIENTS (CCHE EXPERIENCE)

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Background: Sepsis is the leading cause of death in cancer patients and may lead to Systemic Inflammatory Response Syndrome (SIRS). Severe sepsis, left unmanaged, may lead to the inevitable septic shock. Septic shock is severe sepsis with persistent hypotension despite adequate fluid resuscitation in the presence of a known infection.
Aims: To Identify incidence and cause of septic shock among pediatric cancer patients receiving chemotherapy and to survey the outcome of septic shock among pediatric oncology intensive care unit (PICU).
Methods: A study done on 92 patients below age of 18 years having cancer, receiving chemotherapy and admitted to CCHE PICU by septic shock and started inotropes in the period from October 2010 till March 2012.
Results: Total number of patients are 92.Their age ranged from 9 months to 18 years with a mean of 7.3±4.8 years. The included patients were 41 males (44.6%) and 51 females (55.4%). Blood cultures and sensitivity were performed during the infectious episodes:31/92 cultures (33.7%) showed Gram +ve organisms, 52/ 92 cultures (56.5%) showed Gram –ve organisms , 2 cultures showed Candida and another 2 showed mixed gram positive and Candida ,while 5/92 cultures (5.4%) showed no growth. Outcome of the studied patients was recovery of 54/92 (58.7%) &and death of 38/92(41.3%). Heart failure is the most common complication (27.2%) followed by renal failure (14.1%), typhilities(6.5%),and pancreatitis(5.4%).
Summary and Conclusions: Gram negative organisms are the most common cause of septic shock in pediatric cancer patients with the highest mortality rate. Heart failure was the most common complication associated with septic shock in pediatric cancer patients.

PB1970

VANCOMYCIN RESISTANT ENTEROCOCCI COLONIZATION AND INFECTION IN PEDIATRIC PATIENTS WITH CANCER

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Background: Vancomycin-resistant enterococci (VRE) may cause a serious hospital-acquired infection.
Aims: The study aimed to assess the VRE intestinal colonization status in children with cancer, the associated risk factors and resistance profile.
Methods: A cross-sectional study was performed in Ain Shams University, Pediatric Hospital, Hematology-Oncology Section. It included 50 children with cancer divided into two groups. Outpatient group including 25 patients with no symptoms and signs suggestive of infection and with neutrophil count more than 500/uL and 25 inpatients admitted for febrile neutropenia compared to 25 healthy age and sex matched control group. Data collection for the type of cancer, febrile episode during the previous 6 months, antimicrobial use, and bacteriologic studies . Colonization and infection studies were performed. For inpatient group stool culture , rectal swab and blood culture were done on admission, after 72 hours and one week post-admission and the concomitant neutrophil count. Outpatient group and control group were studied for rectal swab and stool culture. The analysis of samples included culture, isolation, identification and detection of susceptibility of isolates to antibiotics.
Results: No age or sex difference was found between studied groups. 38 patients had hematopoietic malignancies and 12 had solid tumors. Eleven inpatients(44%) had associated GIT symptoms and thirteen (52%) had respiratory symptoms. One week post admission only 3 (12%) were still had GIT symptoms and 5(20%) were still having respiratory symptoms. The median duration of admission was 15 days (9-60). 20% of blood culture showed VRE isolates. VRE were detected in one inpatient on admission, compared to 5 patients after one week (p=0.01). There was no significant difference in age ,sex, duration of neutropenia and duration of admission between infected inpatient and non infected inpatients (p>0.05), infected inpatients had significant

lower neutrophil count compared to non infected ones (p<0.001). They had significant higher usage of vancomycin ,flagyl and imipinem compared to non-infected ones (p<0.05). Stool culture were negative in 72% of inpatients after one week compared to 100% on admission, with detected enterococci in 4% 72 hours post admission, compared to 28% one week post admission . 18(72%) patients had negative rectal swab one week post admission, compared to 21(84%) on admission results. Stool culture showed in one (4%) inpatient 72 hours post admission, compared to 7(28%) one week post admission. 8(32%) inpatients had enterococcal colonization ,3(37.5%) of them were resistant to vancomycin. In control group, enterococci were detected in 2(8.0) and none of them were resistant to vancomycin. There was no significant difference in sensitivity pattern of stool culture and rectal swab to vancomycin between outpatients and control group.(p>0.05) .The frequency of sensitivity of detected enterococci to different antimicrobial in inpatient group was as follow; ciprofloxacin (28.5%), gentamycin (85.7%), azithromycin (28.5%), pipercillin/tazobactam (14.3%), ampicillin(42.8%), levofloxacin (28.5%), erythromycin (14.3%) . While sensitivity pattern in outpatient group was gentamycin (66%), azithromycin (33.3%), pipercillin/tazobactam (33.3%), levofloxacin (33.3%), erythromycin (0%).

Summary and Conclusions: children with Cancer are at high risk for VRE colonization and infections. Neutropenia, excess use of vancomycin long hospital stay are important risk factor; Newer less toxic antimicrobials and with broader spectrum could be an alternative to vancomycin

PB1971

CMV REACTIVATION IN MULTIPLE MYELOMA TREATED WITH BORTEZOMIB-BASED REGIMENS

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Background: Bortezomib therapy in multiple myeloma increases the risk of reactivation with herpes simplex and herpes zoster viruses and acyclovir prophylaxis is recommended to prevent reactivation. Recent data from the Rome Transplant Network suggest that multiple myeloma patients treated with bortezomib-based regimens are at higher risk of developing a symptomatic CMV reactivation after autologous stem cell transplantation. There is little or no data regarding CMV reactivation after bortezomib therapy in the initial treatment of myeloma.

Aims: To describe two patients with multiple myeloma who developed CMV reactivation after bortezomib therapy prior to autologous stem cell transplantation.

Methods: Patient 1: A 59 year old gentleman presented with loss of weight, cough and lump on the right side of the chest wall for 2 weeks. Laboratory evaluation revealed renal failure, hypercalcemia, anemia, lytic bone lesions, IgA lambda paraproteinemia, plasmacytoma and malignant pleural effusion. Bone marrow examination confirmed the diagnosis of multiple myeloma. He was treated with VCD (Bortezomib, Cyclophosphamide and Dexamethasone). During the second cycle of treatment he developed melena. Endoscopy showed an oesophageal ulcer, prepyloric erosions and duodenal ulcer. Biopsy was not performed because of concern that it may worsen bleeding. In view of oesophageal ulcers, blood was tested for CMV PCR which was positive at 480 copies/ml and peaked at 2700 copies/ml. He was treated with valganciclovir following which CMV PCR became negative. Acyclovir prophylaxis was reinstituted and there was no further reactivation. Paraprotein has disappeared after five cycles of VCD and autologous stem cell transplant is being planned. Patient 2: A 62 year old lady presented with chest and right shoulder pain for 2 weeks. Evaluation revealed osteolytic lesions and multiple rib fractures. Further evaluation revealed anemia, hypercalcemia and destructive vertebral lesions. MRI of the spine revealed cord compression.Bone marrow examination confirmed the diagnosis of multiple myeloma.She was treated with urgent radiotherapy to the spine and VCD. During the second cycle she developed odynophagia which was treated with empirical fluconazole. Upper GI endoscopy showed acute oesophagitis with ulceration and both biopsy and the blood was positive for CMV PCR. She was treated with Valganciclovir and there was complete resolution of the ulcers and CMV PCR became negative. Acyclovir prophylaxis was reinstituted and there was no further reactivation.She achieved stringent CR after five cycles of VCD, had an autograft and remains in stringent CR with no further CMV reactivation.

Results: We have described two cases of CMV reactivation with bortezomib treatment for multiple myeloma. Low level CMV reactivation may be common after Bortezomib but there are no data on its incidence. Patient 1 had low level CMV reactivation and treatment was given because of the presence of oesophageal ulcer although there was no proof the ulcer was due to CMV.Patient 2 had definite evidence of CMV oesophagitis.The reasons for no recurrence of CMV reactivation are not clear but may be due to recovery of immune function as there was excellent response to treatment in both patients.

Summary and Conclusions: CMV reactivation can occur with bortezomib therapy prior to autograft. Most of the reactivations are likely to be low-level without any clinical significance. However, if patients have clinical features suggestive of CMV disease(like oesophagitis, hepatitis or retinitis), reactivation has to strongly considered. Patients with multiple myeloma treated with bortezomib should be prospectively monitored for CMV reactivation in future studies. This will help us determine the incidence of CMV reactivation and CMV disease in this patient population.It will also help us determine whether CMV monitoring and preemptive treatment is needed in patients receiving bortezomib based regimens.

PB1972

VISCERAL LEISHMANIASIS MIMICKING LYMPHOPROLIFERATIVE DISORDERS

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Background: Visceral leishmaniasis (kala azar) usually occurs with fever, splenomegaly and blood exams abnormalities, such as cytopenias and hypergammaglobulinemia. Early infection typically presents with skin lesions, such as nodules or plaques.

Aims: To investigate Leishmania spp infection in patients observed with symptoms suggesting either new onset or relapsed aggressive lymphoproliferative disorders.

Methods: We report on 6 either immunocompetent or immunocompromised patients, admitted between September 2012 and January 2014, because of either cytopenias or fever or splenomegaly with or without adenopathies. All of them underwent bone marrow (BM) aspirate and/or trephine biopsy for morphologic and flow cytometry examinations, together with molecular analyses for either lymphocyte clonality or Leishmania spp detection by nested polymerase chain reaction (nPCR).

Results: All 6 patients (1 female and 5 males, aged 45 to 84 years) were living in the neighborhood of Modena and Bologna (Italy). One of them had a previous diagnosis of B-chronic lymphocytic leukemia (CLL), while the woman was HIV-seropositive. At least one cytopenia, usually leukopenia with mild to severe neutropenia and thrombocytopenia, was documented in all patients, while fever occurred in 5 cases. Three of them showed splenomegaly and adenopathies. One case presented with acute liver failure. Only the patient in follow-up for CLL had severe polyclonal hypergammaglobulinemia. BM smear cytological evaluation on May-Grünwald-Giemsa staining documented the presence of Leishmania amastigotes, either into macrophage cytoplasms or dispersed, in 3 out of 6 cases. In the remaining samples, in which parasites were not microscopically visible, features suggesting increased inflammatory response, such as macrophages activation and plasmocytosis, were described. Moreover, in one case a definite hemophagocytic syndrome was demonstrated. nPCR for Leishmania spp, carried out on BM samples, was positive in all cases, allowing a diagnosis of visceral leishmaniasis also in 3 patients in which Leishmania amastigotes were not found by microscopic observation. Targeted therapy with liposomal B amphotericine (3 mg/Kg/daily for 5 days) was started, immediately after diagnosis, namely the same day of BM aspirate when Leishmania amastigotes were microscopically observed, or within one week when parasite molecular detection was needed. Hematologic recovery and clinical improvement were seen in all patients by one month (Figure 1).

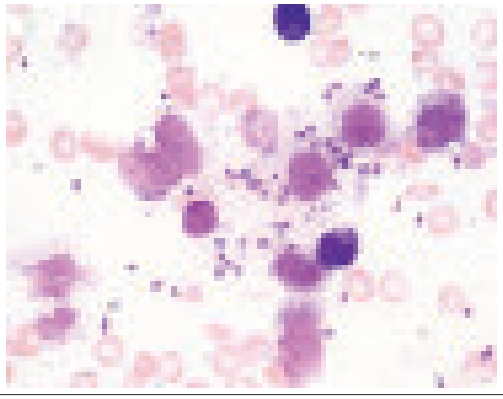

Figure 1.

Summary and Conclusions: Our small series suggests the importance to consider Leishmania spp protozoal infection in the differential diagnosis in patients presenting with at least one of the following symptoms: fever, one or more cytopenias, non homogeneous splenomegaly and/or lymph node enlargement. Specific features of our cases, diagnosed with visceral leishmaniasis, clinically mimicking aggressive lymphoproliferative disorders, in a non-endemic area, relies on the heterogeneous clinical manifestations, with disseminated infection in the absence of skin involvement and usually without hypergammaglobulinemia. Besides thorough clinical history, physical examination and imaging, diagnostic workup should also include BM examination in these cases. Of note, we would like to remark the relevance of collecting BM aspirate samples not only for morphologic examination, but also for molecular analysis, which is currently the highest sensitive diagnostic method for leishmaniasis. Prompt start of specific therapy against Leishmania is essential to obtain resolution of symptoms and parasitic eradication.

PB1973

PRE-TREATMENT BLOOD INFLAMMATORY MARKERS WITHIN NORMAL RANGE: AN INDICATOR OF LOWER PROBABILITY OF SYSTEMIC INFECTION DURING INDUCTION CHEMOTHERPAY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA?

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Background: For patients with acute myeloid leukemia (AML), systemic infection is a frequent and serious problem during dose-intensive chemotherapy. Blood inflammatory markers, such as C-reactive protein (CRP) and ferritin, are known to have a predictive value for the incidence of systemic infection in patients who underwent hematopoietic cell transplantation (Hong et al, Biol Blood Marrow Transplant 2013;19:994).
Aims: This study aimed at evaluating the role of CRP and ferritin in predicting the incidence of systemic infection and infectious treatment-related mortality (TRM) among adult patients with newly diagnosed AML who were treated with current standard induction chemotherapy.
Methods: Patients were included if they were: 1) age > 20 years, 2) diagnosed as AML according to 2008 WHO criteria, and 3) treated with induction chemotherapy within 5 days after diagnosis. Patients with APL or with a HIV infection, were excluded. Induction chemotherapy consists of continuous infusion of cytarabine 100 mg/m^2 /d for D1~7 with idarubicin 12 mg/m^2/d (or daunorubicin 90 mg/m^2/d) i.v. for D1~3. Identical prophylactic measures including prophylactic antibiotics/antifungals, use of G-CSF, use of isolation room with a low bacterial diet, were provided. Systemic infection was defined as an infection in which the pathogen is distributed throughout the human body rather than in localized areas, with systemic inflammatory response syndrome. Informed consent was waived by the institutional review board, considering no actual intervention for the patients.
Results: A total of 110 patients (median age 54.5 years, range 20-80) were included. Pre-treatment CRP was measured in all patients and baseline serum ferritin was acquired from 79/110 patients (71.8%). Thirty nine events of systemic infection were reported in 38 patients (38.5%). Twenty two patients experienced infectious TRM (21/110 = 19.1%). Elevation of serum CRP (p=0.032) and ferritin (p=0.002) were related to the incidence of systemic infection. Elevation of CRP was also related to infectious TRM (p=0.029), whereas elevation of ferritin was not (p=0.119). Elevation of CRP and ferritin were not related to age<or ≥ 60 years (p=0.259 for CRP and p=0.696 for ferritin, respectively). However They showed a borderline relationship with a performance status<or ≥ 2 (p=0.087 and p=0.065, respectively). In multivariate analysis, serum ferritin was an independent risk factor affecting the incidence of systemic infection (p=0.026), although CRP failed to reach statistical significance (p=0.161) despite a trend with the odds ratio of 3.31 (Table 1). Both pre-treatment CRP and ferritin level showed correlation with WBC count. However, elevation of WBC itself was not related to systemic infection or infectious TRM. More frequent incidence of systemic infection was observed in patients with elevated levels of the inflammatory markers, whether they had higher (≥10x10^3/μL) or lower (<10x10^3/μL) WBC counts. These finding implies that, although elevation of CRP or ferritin above their conventionally appointed normal range is predictive of systemic infection, higher blood level of inflammatory markers does not indicate a greater probability of systemic infection and it is possibly affected by the expansion of leukemic myeloblasts.
Summary and Conclusions: Pre-treatment CRP and ferritin within normal range showed an association with decreased probability of systemic infection in patients with AML who underwent induction chemotherapy. These cheap and easily testable blood markers could be useful in selection of a subgroup with a lower probability of systemic infection in those patients.

Table 1.

PB1974

CMV PROPHYLAXIS WITH GANCICLOVIR, FOSCARNET AND VALGANCICLOVIR IS HIGHLY EFFECTIVE IN PREVENTING CMV INFECTIONS AFTER IN VITRO CD3-DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Haploidentical hematopoietic stem cell transplantation (HHCT) has become a possible alternative treatment for patients with malignant or non-malignant diseases who lack an HLA-matched related or unrelated donor. Compared to transplantation from other sources, HHCT is limited by delayed immune recovery and subsequent infections. Cytomegalovirus (CMV) is the most prevalent infectious agent and one of the major causes of death after transplantation.
Aims: We compared the incidence of CMV reactivation and disease between before and after CMV prophylaxis to evaluate the efficacy of the prophylaxis in HHCT.
Methods: Between July 2008 and January 2013, 28 children and adolescents underwent a total of 35 HHCTs using *in vitro* CD3-depleted peripheral blood stem cells at AMCCH. In July 2011, we initiated CMV prophylaxis with ganciclovir (5mg/kg twice per day) prior to HHCT, and forcarnet (60mg/kg/day) from the time of infusion and up until engraftment. After engraftment, valganciclovir (25mg/kg/day) was administered until 100 days post-transplantation and until the recovery of CD4$^+$ cell numbers >100/μL. Therefore, among 28 patients, 15 received CMV prophylaxis per protocol, but 13 did not.
Results: CMV reactivation occurred in 15 of the 28 patients at a median of 47 days after their first HHCT, leading to a cumulative incidence (CI) of 53.6%. Four patients developed CMV diseases (retinitis in two, pneumonia in one, and encephalitis in one), and two patients died of CMV disease (pneumonia and encephalitis in one each). After July 2011, at which point CMV prophylaxis was initiated, only 5 out of the 15 patients experienced transient reactivation and none developed CMV disease. The CI of CMV reactivation was lower in patients who received prophylaxis than that of those who did not (33.3% *vs* 76.9%, *P*=0.014). In addition, the CI of CMV disease was significantly lower in prophylaxis group (0.0% *vs* 30.8%, *P*=0.033).
Summary and Conclusions: The CMV prophylaxis with ganciclovir, foscarnet and valganciclovir is highly effective in decreasing the incidence of CMV disease as well as its reactivation in HHCT. Given the delayed immune reconstitution in certain type of transplantation, CMV prophylaxis with anti-viral drugs is recommended in transplantation using T-cell depleted graft or umbilical cord blood.

PB1975

IMAGING OF PULMONARY INFECTIOUS COMPLICATIONS IN HEMATOLOGICAL MALIGNANCIES

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Background: Pulmonary complications frequently occurred in immunocompromised hosts, and specifically in patients treated for hematologic malignancies or undergoing hematopoietic cell transplantation. Infections are the most common (75%) and are associated with high morbidity and mortality. These infections occur despite routine prophylaxis for common pathogenic organisms and empiric therapy of febrile episodes during the early neutropenic period. High-Resolution Computed Tomography (HRCT) scans have high

degree of sensitivity (>85%), high negative predictive value (>85%) in detecting pneumonia with gain of 5 days compared with chest radiography.
Aims: The aims of this study were to examine characteristics and frequencies of high-resolution computed tomography (HRCT) patterns found in case of infectious pulmonary complications in patients with hematological malignancies and to examine the correlation between the etiological agent and a specific HRCT pattern.
Methods: 133 patients with hematological malignancies, **t**hat had pulmonary abnormalities on HRCT of the lungs images after treatment with chemotherapy or hematopoietic cell transplantation, were enrolled and retrospectively evalueted. Chi-square test was performed for the statistical analysis.
Results: Seventy-seven (58%) of the 133 patients, that showed parenchymal abnormalities on HRCT, had single patterns and 56 (42%) had mixed patterns. Fifty patients (38%) of 133 were positive at microbiological analysis. There were 27 cases (54%) of bacterial pneumonia, 7 (14%) of fungal pneumonia, 2 (4%) of viral pneumonia and 14 (28%) of mixed pneumonia. The statistical analysis showed a statistically significant association between the following HRCT patterns and the etiology: centrilobular micronodules and fungal pneumonias (p <0.01); mixed pattern solid nodules, consolidations and air-crescent sign and fungal pneumonias (p <0.05); nodules with halo and mixed pneumonia (p <0.05).
Summary and Conclusions: Centrilobular nodules (with or without tree-in-bud) and mixed pattern solid nodules, consolidations and air-crescent sign are suggestive of fungal pneumonias; halo sign pattern is suggestive of mixed pneumonia. Other high-resolution CT patterns are not helpful in distinguishing among the various types of infection seen in neutropenic – immunocompromised patients.

PB1976

FEBUXOSTAT FOR MANAGEMENT OF INTERMEDIATE RISK OF TUMOR LYSIS SYNDROME WITH HEMATOLOGICAL MALIGNANCIES.
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Background: Tumor lysis syndrome (TLS) is a rare but life-threatening oncological emergency, and controlling the serum uric acid level (S-UA) is crucial. In the guidelines, a xanthine oxidase inhibitor, allopurinol, is employed in the management of an intermediate risk of TLS. However, patients at risk of TLS are occasionally complicated with renal failure, which may increase the risk of adverse reactions to allopurinol. Febuxostat (FEB), a new non-purine xanthine oxidase inhibitor is a more selective and potent inhibitor of xanthine oxidase than allopurinol and applicalble to patients with renal insufficiency. Because the use of a potent UA-lowering agent rasburicaseis limited due to its immunogenicity and cost, FEB should be the choice for TLS.
Aims: In this single institute, short-term, and pilot prospective study, the efficacy of FEB in patients with an intermediate risk of TLS was evaluated.
Methods: A total of 10 patients who presented to our insutitute between April and December in 2012 were enrolled. All patients were evaluated with an intermediate risk of TLS using the 2008 guidelines of Coiffier, *et al.* FEB was administrated orally for 7 days at 60 mg for patients with eGFR ≥ 90 ml/min/1.73 m^2 and 40 mg for patients with eGFR between 30 and 90 ml/min/1.73 m^2. Induction chemotherapy was started within 48 h after the first administration of FEB. The primary endpoint was the reduction of S-UA to ≤ 7.5 mg/dl by day 5. As the secondary endpoints, the renal function, pharmacokinetics of FEB, and resultant increase in the metabolites xanthine and hypoxanthine by FEB were measured.
Results: The median age was 67 (range: 52 - 79), with 4 males and 6 females. They were all diagnosed with hematological malignancies including leukemia and lymphoma. Three patients received 60 mg of febuxostat, while 7 patients received 40 mg. The median S-UA at the baseline was 8.0 mg/dl (range: 3.2 – 10.6), and the median S-UA on the 5th day was 3.3 mg/dl (range: 1.1 – 5.8) (P<0.0001). All patients achieved S-UA levels≤7.5 mg/dl. Overall, S-UA levels differed among the patients but decreased over time despite chemotherapy-induced tumor lysis, thereby supporting the efficacy of febuxostat. The median S-Cr levels decreased from 0.86 mg/dl (range: 0.7 – 1.38) to 0.72 mg/dl (range: 0.57 – 1.35) (P<0.0003). The median eGFR value increased from 56.4 ml/min (range: 37.8 – 111.9) to 66.7 ml/min (range: 49.2 – 126.5) (p=0.038). Febuxostat and allopurinol are both xanthine oxidase inhibitors that inhibit the conversion of hypoxanthine to xanthine and inhibit the conversion of xanthine to UA. While S-UA decreased over time, serum hypoxanthine and xanthine concentrations increased on day 1 (5.6, 3.1 µg/ml, respectively) and persisted at these levels through day 4. Urinary UA concentration decreased over time, a trend which might correspond with changes in S-UA. The mean urinary hypoxanthine and xanthine concentrations increased on day 1 (94.3 µg/ml and 191.8 µg/ml, respectively) and decreased on day 7.Serum FEB concentrations at 2 h after administration were 767.9 ng/ml for the 40-mg dose and 770.6 ng/ml for the 60-mg dose (no significant difference). The trough levels achieved steady state at day 4, with values of 30.4 ng/ml (mean) for the 40 mg dose of febuxostat and 27.1 ng/ml (mean) for the 60 mg dose of febuxostat. Day 7 values were 42.5 ng/ml (mean) (p=0.20) for the 40 mg dose of febuxostat and 28 ng/ml (mean) (p=0.90) for the 60 mg of febuxostat, suggesting no drug accumulation during the treatment period. No patients exhibited TLS progression. No grade 3, 4 adverse reactions related to FEB were reported.
Summary and Conclusions: FEB successfully controlled S-UA without major adverse reactions together with improvement of renal function during chemotherapy for intermediate-risk TLS. The determination of oxypurines suggested the clinical efficacy of FEB to inhibit xanthine oxidase in the recruited patients. The present results strongly suggest that FEB is an excellent alternative to allopurinol for the management of TLS.

PB1977

APPLYING THE CONCEPT OF HEALTHCARE ASSOCIATED INFECTIONS IN HEMATOLOGY AND ONCOLOGY PEDIATRIC UNIT OF CASABLANCA
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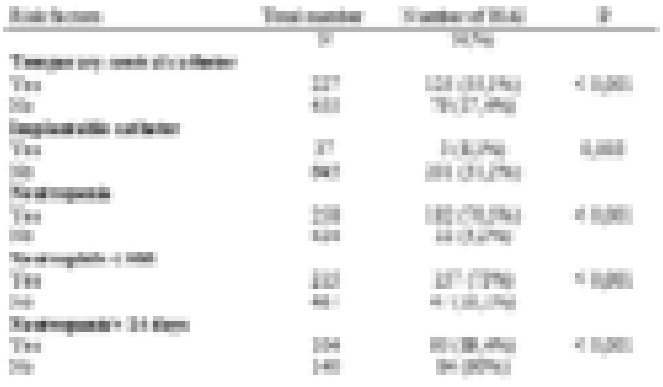
Background: Infections in neutropenic patients are a cause of morbidity and mortality. They represent in onco-hematology (9-12%) compared to not cancerous patients (6-7%). The incidence of nosocomial infections (NI) is about 20%. Unlike NI, data of healthcare associated infections (HAI), are less available. The rigging of a register for HAI monitoring in the Pediatric Hematology and Oncology Unit of Casablanca allow a better knowledge of care practices and develop a prevention strategyermettra une meilleure connaissance des pratiques de soins et l'élaboration d'une stratégie de lutte conte les IAS.
Aims: Objectives of this study were to determine the incidence rate of HAI, and define risk factors, and ecological and bacterial profile, and evolution of these patients.
Methods: Prospectively, study was conducted over a period of eleven months, between February and December 2012. Were included all patients older than 18 years, admitted to hospital in the adult and transplant sectors. An operating sheet was developed according to WHO criteria. Data analysis was performed by Epi Info software. HAI rate was calculated per 1000 patients days.
Results: 682 admissions were recorded in 338 patients, median age was at 38 years old, and sex ration M/F=1.5. Incidence rate of HAI was 38.4% against 30% for NI. Incidence density of HAI per 1000 patients days was 31.6% against 24.6 for NI. HAI were microbiologically documented in 41% with bacteremia in 25% of all HAI cases. Pneumonia was noted in 32.8% and Infection was of unknown origin in 22.5%. Gram negative bacteria were isolated in 35% and Gram positive bacteria in 50%, and fungal in 15%. Median duration of hospitalization in infected patients was at 22 days [2- 70] (p<0.001), and death by infection was at 6% (Table 1).

Table 1. Risk factors in the group of infected patients.



Summary and Conclusions: Results of systematic monitoring of HAI in onco-hematology constituted a fundamental step to release the constraints of implementation of the register. They also helped to identify risk factors and ecological profile for development of a prevention strategy.

PB1978

PEGFILGRASTIM VERSUS FILGRASTIM IN THE MANAGEMENT OF POST-CHEMOTHERAPY NEUTROPENIA IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA
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Background: Patients receiving cancer chemotherapy are at increased risk of neutropenia, leading to increased risk of infections and delay in subsequent chemotherapy treatments. Recombinant granulocyte colony stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils in patients receiving chemotherapy. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than nonpegylated G-CSF. Multiple Myeloma (MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment (*e.g.* Anthracyclines, Alkylating agents, etc), but myelotoxicity is the main expected side effect. In this context, G-CSFs are often necessary to counteract the risks of febrile neutropenia: their use is bound to frequent evaluation of neutrophil counts that may not be easy for patients in home-care. Avoiding severe neutropenia by prophylactic pegfilgrastim seems particularly useful in these cases.
Aims: The objective of this study was to compare the efficacy and safety of pegfilgrastim in patients affected by Multiple Myeloma in advanced phase of disease. In order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospedalization duration.
Methods: We have enrolled in our study 29 patients (16 male and 13 female) with a median age of 64.7 years (range 39-82) affected by multiple myeloma, all relapsed and refractory to a median of 6.3 lines of therapy (range 4-8), all previously exposed to Bortezomib, Lenalidomide, Melphalan and all relapsed after auBMT, treated with different chemotherapy regimens combining Bortezomib, Lenalidomide, Bendamustine, Melphalan, Doxorubicine.
Results: Since first course, received in our out-patient department, patients performed blood counts twice weekly and received, from day +8 to day +19 (considering "day + 1" the day in which the chemotherapy protocol starts), prophylactic oral chinolonic antibiotics and anti-fungal drugs. During neutropenia after first cycle of chemotherapy, Filgrastim (5 µgr/kg/day for 3 days) was given if neutrophils count was <1500 x 10^9 cells/L. Median number of filgrastim administrations was 4.7 (r. 3-6); nadir neutropenia was registered after a median of 11.3 days (r. 8-14); median of nadir neutrophil count was 1.16 x 10^9 cells/L (range 0.4 – 1.8 x 10^9 cells/L), with maximum duration of 13 days. From the second course of chemotherapy, all patients switched to prophylactic therapy with pegfilgrastim (6 mg), injected subcutaneously with a single administration on day +3 independently from the neutrophil count at that time. Primary endpoint of this study was the duration of neutropenia (neutrophil count<1.5 x 10^9 cells/L), comparing pegfilgrastim and filgrastim. During pegfilgrastim, neutropenia was never longer than 8 days, with a consequent reduction of neutropenia-related infections. Median nadir neutrophil count, evaluated for every patients for at least three courses of therapy (r. 3-6) registered at day +11, was 1.628 (range 0.93-2.25 x 10^9 cells/L); only four patients (13.7%) needed, one week after pegfilgrastim administration, a supplement of 3 administrations of filgrastim. During pegfilgrastim prophylaxis, neutropenia was shorter than during Filgrastim treatment. Besides the mono-administration, pegfilgrastim was well tolerated in all patients: main side effects in our patients were mild fever and bone pain, (5/29 patients,17%)
Summary and Conclusions: In conclusions, in patients affected by MM exposed to myelosuppressive agents in advanced phases of myeloma disease, pegfilgrastim seems to reduce the incidence of neutropenia and may increase the possibility to maintain the scheduled time of treatment.

PB1979

RELEVANCE OF PARALLEL USE OF GALACTOMANANN AND BETAGLUCAN ASSAY IN THE DIAGNOSIS AND FOLLOW UP OF FUNGAL INFECTION

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Background: Simultaneous detection of D-beta glucan, galactomannan and TAC are rarely performed jointly. We explore preventive strategy of fungal infection using these indirect markers of fungal infection in haematological patients
Aims: Retrospective analysis of positive determinations of galactomanann or betaglucan assays in patients with haematological diseases. We also compared with TAC images and calculate specificity of galactomann and betagulcan assays. Comparison between both test and TAC, survival after treatment, change of treatment after positivity of beta-D-betaglucan or galactomanan were analyzed.
Methods: Design and surveillance of patients at risk: All patients receiving AML treatment, ALL induction treatment, Bone marrow transplantation patients and patients with inmunosupressive drugs, such cyclosporine, anti-lymphocyte serum, or allentuzumab were monitoring in a preventive strategy every Monday and Thursday until neutropenia recovery or immunosuppressive therapy period. Patients: 48 patients admitted in Haematology from March 2010 to March 2013 were positive to beta-D-glucan or galactomanan assays. The median age was 65,7 year (16-91,69). The diagnosis of the patients were acute myeloid/lymphoid leukaemia: 21 (43%); multiple myeloma:9(18%), non and Hodgkin's lymphoma:10(20%), CLL/T-prolymphocytic leukaemia 5(10%); aplastic anaemia 2(4%) and haemolytic uremic syndrome 1(2%). The type of treatment was: Inmunosupression: 8 (16%), palliative: 4(8%) and chemotherapy 37(75%). The infection site reported were: Adenitis 1(2%), nail 1(2%), lung: 46 (94%), and lung+brain: 1(2%). Previous prophylaxis was performed in 29 patients(fluconazole 13(26%), itraconazole 14(28%) and posaconazole 2(4%)). Thirtynine (79%) of the patients were in respiratory insufficiency; median baseline oxygen saturation was 88(40-100). The median title of galactomanan was 0.8 ng/ml(0,5-7,6), and the median title of beta-D-glucan 291 pg/ml(7-781). The number of patients who were positive to galactoman: 43(89%) and beta-glucan positive in 43(89%). The number of positive determinations of galactomanan and betaglucan after treatment were 2,5 and 3 respectively. TAC images were: pneumonia 14(36%), cavity pneumonia: 9(23%), pleural effusion: 8(21%), nodular infiltrates: 6(15%), brain abscess+ lung cavity: 1(2,6), pulmonary embolism: 1(2,6%) and no images: 2 (5). Halo sign was detected in 12 (30%).
Results: Results: The first antifungal treatment was: Voriconazole in 35 (70%), Caspofungin in 10(20%), Fluconazole 2(4), Posaconazoles 1(2%), Ambisome 1(2%). Seven patients change of antifungal agents, 6 to Voriconazole and 1 to Ambisome; 11 patients were treated with combination therapy (7 with caspofungine+ voriconazole, 4 with ambisome+Voriconazole). Only 27 patients (62%) recover neutrophils>0.5x10^9/L. The results of treatment were death in 19 patients and curation of the lesions in 28 (58%). The median survival of the patients was 12,6 months (1,4 –non reached). The median survival time of the patients who recovered neutrophils was 22,2 months *vs* 4,4 months who did not (p<0,0001).Following EORTC criteria of invasive fungal infection: Proven 5 (6%), Probable: 40(83%) and false positive: 3 (6%). Specificity of galactomanan probe is 98% and betaglucan 96.5%. In 8 patients results of betaglucan and galactomanan were discrepant. T. ROC area unde the curve: 0.81. In one case, brain abscess and lung cavity, was detected after positivity to galactomanan .No fever, respiratory or neurological signs were detected before and was a surprise in TAC images.
Summary and Conclusions: A strategy of preventive treatment with antifungals (voriconazole) could be worthy using galactomanan and beta-D-glucan assays.

PB1980

EPIDEMIOLOGY OF VISCERAL MYCOSIS IN AUTOPSY CASES OF LEUKEMIA AND MDS IN JAPAN

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Background: There are many reports described an increase of invasive fungal infections. Moreover, patients are higher risk in case with leukemia and MDS. Although the risk should be considered in the present clinical settings, there are no detailed reports on visceral mycoses in autopsy cases in the survey at the national level.
Aims: It is important for us to clarify the recent trends concerning the frequency of mycoses in autopsy cases. We epidemiologically analyzed the data reported in the "Annual of pathological autopsy cases in japan".
Methods: The data on visceral mycoses reported in the "Annual of pathological autopsy cases in japan" were analyzed epidemiologically at intervals of four years in 2011 after 1989 and in 2011.
Results: The frequency rates of visceral mycoses decreased rapidly 1989(4.5%) and 1994(3.2%), but increased again (4.0-5.0%). The predominant causative agents were *Candida* and *Aspergillus.* Although the rate of candidosis showed a gradual decrease, the rate of aspergillosis exceeded that of candidosis in 1994. The rates of them still showed an increase. In addition, *Mucorales* increased gradually in recent years. In cases of leukemia and MDS, the rate of mycoses were higher (15-30%) in comparison with all of cases (4-5%). in each year. The rate of causative agents were *Aspergillus*(42%), *Candida*(14%), *Mucorales*(10%), and complicated infection cases(12%) in 2011. Interestingly, the rate of complicated infection cases increased gradually in recent years. The combination of causative agents in cases of complicated infection were *Asperugillus* and *Mucorales*(5.10%), and *Asperugillus* and *Candida*(4.08%) in 2011.
Summary and Conclusions: We epidemiologically analyzed the data reported in the "Annual of pathological autopsy cases in japan". We showed the data of visceral mycoses in Autopsy cases of leukemia and MDS in japan. The results of our study suggest that clinicians should increasingly focus on visceral mycoses.

PB1981

RATE OF FUNGAL INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA DURING INDUCTION TREATMENT PERFORMING TWO PROPHYLACTIC STRATEGIES: PRELIMINARY RESULTS

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Background: The prophylactic approach, consisting in a prophylactic treatment of at risk patients, has been gaining more and more diffusion. Recently the SEIFEM group has demonstrated that posaconazole-based prophylaxis can significantly reduce the incidence of fungal infection *to court*. Indeed this kind of prophylaxis has gained an "A" level of evidence, especially in naive patients and patients undergoing to allogeneic bone marrow transplantation. However, no studies have compared the effectiveness of Posaconazole *versus* topical therapy.
Aims: The aim of this study was to determine the differences in Invasive Fungal Disease (IFD) incidence and outcome between two prophylactic regimens, *i.e.* Posaconazole *vs* Aerosolized amphotericin plus nistatin.
Methods: We analyzed 28 patients with AML undergoing induction treatment: 15 received prophylaxis with posaconazole 200 mg tid per os. and 13 prophylaxis with amphotericin-B 10 mg bid per aerosols; Nystatin 600,000 IU orally tid. The serological monitoring was conducted twice a week through the demonstration of beta-D-glucan (BG) (Fungitell) and the antigen galactomannan (GM) (Platelia ™ Aspergillus EIA). The Chest CT was performed in presence of fever lasting more than 72 hours. Invasive Fungal Disease (IFD) was diagnosed according to the EORTC criteria. The empirical antibiotic and antifungal therapy were administered according to ISDA guidelines. The qualitative data were analyzed using the chi-square test and chi -square for trends tests.
Results: No differences in terms of age, sex, Charlson comorbidity index according to subtype of AML, risk category, refractory to induction therapy and promyelocytic morphology were recorded between the two groups. BG resulted positive in 17.5% of patients (5/28), all of them underwent prophylaxis with amphotericin-B per aerosols and oral Nystatin (p=0.013 , relative risk 2.87 , 95% CI 1.64 to 5.03). Of these, only two patients out of 13 (15%) have reached, due to the presence of infiltrates on chest CT fitting the EORTC criteria, the clinical evidence of an IFD. No cases of IFD emerged in the group who received posaconazole prophylaxis. Stratifying patients according to Maertens *et al.* 2012 (1), it was evident that patients who received posaconazole prophylaxis were more likely to remain in the group "A" and "B", while patients who received amphotericin-B per aerosols and oral Nystatin were more likely to be within the groups "C" and "D" (p=0.01) (Table 1).

Table 1.

	Aerosolized amphotericin plus nistatin	Posaconazole
A	4	9
B	1	4
CI	1	0
CII	3	2
CIII	2	0
CIV	0	0
D	2	0

Chi Square Test for Trend: p=0,01

Summary and Conclusions: Considering the new criteria for patient stratification, prophylaxis with posaconazole determines a lower probability to develop an IFD in respect to amphotericin-B per aerosols and oral Nystatin.

Transfusion medicine

PB1982

UP-REGULATION OF NKG2A IS INVOLVED IN NK CELL CYTOTOXICITY IMPAIRMENT IN B-THALASSEMIA MAJOR PATIENTS WITH LONG TERM BLOOD TRANSFUSION

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Background: Allogeneic blood transfusion can induce immunosuppression in recipients and natural killer (NK) cells play important role in this effect. Killer activated receptors (KARs) and killer inhibitory receptors (KIRs) are expressed on NK cells .Whether NK cells exert function or keep silence depends on which receptors(KARs or KIRs) and their following pathway take advantage.
Aims: The aim of this work is to explore possible mechanisms involved in NK cell cytotoxicity impairment in β-thalassemia major patients with long term blood transfusion.
Methods: 20 β-thalassemia major patients, 20 β-thalassemia minor patients and 20 healthy volunteers were included in this study and median ages were respectively 9,10 and 9 years. The cytotoxicity (CD107a expression), KARs□ NKP30,NKP46, NKG2D□and KIRs (NKG2A,CD158a) on peripheral NK cells were detected by multicolor flow cytometry. IFN-γ production by NK cells was detected by intracellular cytokine staining. Statistical analysis was performed by T test. P<0.05 is considered significantly different.
Results: Compared with β-thalassemia minor patients or healthy volunteers, CD107a were lower in β-thalassemia major patients(P< 0.05). Expression of NKG2A in β-thalassemia major was remarkably up-regulated(P< 0.05), but there was no significant difference in expression of NKP30, NKP46, NKG2D, CD158a and IFN-γ(P>0.05).
Summary and Conclusions: Up-regulation of NKG2A may related to NK cell cytotoxicity impairment in β-thalassemia major patients with long term blood transfusion .

PB1983

SEARCH FOR EXTENDED PHENOTYPE-MATCHED BLOOD UNITS TO TRANSFUSE ALLOIMMUNIZED PATIENTS – IMPLICATIONS FOR THE BLOOD BANK

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Background: Red cell antigen alloimmunized patients, especially sickle cell or thalassemia intermedia patients, need to be transfused with respect to their red blood cell (RBC) extended RBC phenotype, including mainly the Rhesus (DCE), Kell, Duffy, MNSs and Kidd systems.
Aims: The aim of our study was to register the reagents consumed and the labor needed to satisfy the need for transfusion of alloimmunized patients with respect to their extended phenotype and subsequently estimate the cost and identify possible alleviating strategies.
Methods: During a five month period (January through May 2013) our center was asked to supply 45 patients with extended phenotype RBC compatible units. We used the gel test technique (BIORAD's monoclonal antisera gel cards, or neutral gel cards with the addition of specific antisera), to screen blood units of the same ABO, Rhesus, and Kell phenotype for antigens of the Duffy, MNSs and Kidd systems, as well as Lewis, P, Lutheran, if needed. The RBC phenotype of each unit screened, was registered in the donor's card who was then added to our electronic registry. In all cases where the combination of antigens in the patient produced a rare phenotype, instead of screening random blood units, we used our rare donor registry, to call blood donors with the phenotype needed.
Results: A total of 1086 RBC units were screened for 1-6 red cell antigens each. 2090 red cell antigen phenotyping tests were performed in total, with a cost of 2.6-3.5 euros per test (without counting the cost of the labor man-hours). A frustrating fact is that not all patients are transfused with the units found proper for them, because the clinicians postpone the transfusion or their condition gets ameliorated by other means. The most illustrative example was a patient with acute myeloid leukemia who received 28 out of 165 RBC units found proper (of the phenotype needed). For each of the other 44 patients the number of transfused extended phenotype compatible units was 0-3. So, many screened phenotyped units are finally given to non-alloimmunized patients.
Summary and Conclusions: Screening random units to identify a specific extended phenotype for alloimmunized patients is a non cost-effective approach. For non-urgent cases, the best solution is to call donors with the phenotype needed. Keeping an updated registry of phenotyped donors, is of great help especially when RBC units with rare phenotypes are needed. Blood Centers –like ours- that routinely have to perform extended RBC phenotyping, should create electronic registries and share them with others.

PB1984

HUMAN PLATELETS STORED AT MINUS 196°C RETAIN NORMAL CALCIUM-SIGNALLING AND ACTIVATION IN RESPONSE TO STRONG AGONISTS

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Background: Human platelet concentrates (hPC) can be stored at 20 – 24°C for 5 days. This limiting storage time might be extended to several years by cryopreservation in liquid nitrogen.
Aims: The aim of the present work was to evaluate intracellular calcium-signaling and surface activation end-points of cryopreserved platelets.
Methods: Four hPC were frozen by computer-controlled rate freezing in 10% DMSO with human albumin, stored at minus 196°C for 4-6 weeks, thawed in a water-bath at 37°C and analysed by flow-cytometry as published (Daskalakis *et al.* Cytometry B. 2014).
Results: We observe two populations of cryopreserved and thawed platelets. One population (median ca. 40%) expresses negatively charged phospholipids, has a decreased surface density of glycoprotein (GP) Ib, and cannot be activated. The other platelet population (median ca. 60%) has surface densities of GP Ib, IIb, and IIIa similar to control fresh platelets, does not bind annexin-V (detecting the expression of negatively charged phospholipids) nor PAC-1 (recognizing an activation-induced epitope of the fibrinogen-receptor), is able to mobilize intracellular calcium in response to strong agonists, such as convulxin (a specific agonist of the collagen receptor GPVI) or thrombin, and behaves as fresh control platelets for secretion of alpha- and dense-granules, activation of the fibrinogen-receptor, and development of procoagulant activity.
Summary and Conclusions: We show that cryopreservation and thawing of human platelets results in a fraction of platelets (about 60%) maintaining normal calcium mobilisation, granule-secretion, activation of the fibrinogen-receptor, and development of procoagulant activity in response to strong platelet agonists. According to these findings cryopreserved platelets presumably could have normal haemostatic effects *in vivo*.

PB1985

CONSENT FOR BLOOD TRANSFUSION IN MAJOR ELECTIVE VASCULAR SURGERY IN THE UK - CURRENT REGIONAL PRACTICE

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Background: Providing patients receive the "right blood, to the right patient at the right time", risks of transfusion are low, but potentially fatal. Major vascular surgical procedures have a high incidence of transfusion requirements. The UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) has produced guidelines on the standards for seeking consent for blood transfusion. We sought to audit our practice against these standards.
Aims: To audit the current practice on consent for blood transfusion, amongst vascular surgeons and anaesthetists in one region of the UK.
Methods: All Consultant Vascular Surgeons, Consultant Vascular Anaesthetists and higher vascular surgical trainees in one geographical region of the UK were invited by email to complete an on-line questionnaire. Data was analysed using Microsoft Excel software.
Results: 38 responses were received (9 consultant surgeons, 18 consultant anaesthetists, 11 surgical trainees). 63% were not aware of the SaBTO guidelines; awareness was lowest amongst surgical trainees. Only 53% stated they "always" take consent for transfusion. 86% of those taking consent documented this somewhere in the clinical notes, but patient information leaflets were rarely provided. Retrospective notification that a patient has received a transfusion was given only "sometimes" by 55%, or "never" by 21%.
Summary and Conclusions: Awareness of SaBTo guidelines was low in our region. Areas identified for change in our practice following this audit include: provision of patient information leaflets to be part of pre-operative assessment; consent for transfusion to be documented on the surgical consent form; patients receiving transfusion during surgery are to be retrospectively informed and provided with patient information leaflet. This will be documented in the clinical notes.

PB1986

ANTI-JKA AUTO-ANTIBODY CAUSING HEMOLYSIS IN A PATIENT WITH IMMUNE THROMBOCYTOPENIA: AN INTERESTING CASE REPORT

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Background: Bibliography about anti-jka auto-antibodies is not rich. Rare reports exist about anti-jka auto-antibodies that caused significant hemolysis. Most patients involved, had either an autoimmune disease (ulcerative colitis, lupus erythematosus, autoimmune thrombocytopenia) or drug intake (chlorpropamide, methyldopa etc).
Aims: We report an interesting case of a patient with autoimmune thrombocytopenia who presented hemolysis caused by anti-jka autoantibodies.
Methods: The patient was a 63 year old male with a history of immune thrombocytopenia diagnosed 15 years ago, drug-resistant tuberculosis and mild anemia (Hb 11.7 g/dl, Ht 34% at steady state). The patient had not been transfused within the last 9 years.
Results: In May 2013, he presented deteriorating anemia which was attributed to both hemolysis (LDH increase to 652 IU/dl and unconjugated bilirubin 3,2 gr/dl) and gastric blood loss (melena). Cross-matched blood units were found incompatible. The direct antiglobulin test (DAT) was positive (3+) with polyspecific antiserum, weakly positive with monospecific anti-IgG (+ weak), and strongly positive with anti-C3d (4+). The DAT was negative with anti-IgA, anti-IgM and anti-C3c monospecific antisera. The indirect antiglobulin test (IAT) was positive. For identification of the antibody in the serum, we used a commercial panel (BIORAD's) of eleven cells. Elution of the antibodies coating the red cells was performed with the acid elution technique and commercial reagents (DiaCidel's Elu-Kit). The IAT was positive with the gel test LISS-Coombs technique at 37 º C (low ionic strength saline as enhancing agent, incubation at 37º C) and clearly indicated anti-jka specificity. The same antibody specificity was identified in the eluate. The results were negative with the 11 enzyme-treated cells of the panel. The patient's red blood cells were positive for both the jka and the jkb antigen of the Kidd system. All jka-negative red blood cell units were found compatible with the patient's serum. All the above indicate that the patient had autoantibodies showing anti-jka specificity. The patient was transfused with 2 compatible units of jka-negative red cell concentrates and was administered high dose corticosteroid treatment with satisfactory response (the Hb level stabilized and LDH normalized). Two months later all tests were repeated and gave the same results, but additionally the panel of enzyme-treated cells gave positive results indicating anti-jka, too.
Summary and Conclusions: Anti-jka autoantibodies were formed in a patient with immune thrombocytopenia and drug-resistant tuberculosis. The antibodies activated complement and caused moderate hemolysis. Strange enough, at the time of the initial identification, the antibodies did not give a positive reaction with enzyme-treated panel cells, which happened upon control, later on. The DAT and IAT were still positive two months later while the hemoglobin was stable and the patient was transfusion-independent. In this case it is unclear whether the autoantibody formation was related solely to the autoimmune disorder or to a drug received for the tuberculosis.

PB1987

WHICH PATIENTS CAN BENEFIT FROM PREOPERATIVE AUTOLOGOUS BLOOD DONATION?

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Background: Preoperative autologous blood donation (PABD) brings benefits for recipient, transfusion services and hospitals. Advantages of PABD are reduction of infectious disease transmission, decrease of alloimmunization, transfusion-asociated reactions, post-operative thrombotic events and other immunologic efects such as transfusion-related-immuno-modulation (TRIM), transfusion-asociated acute lung injury (TRALI), graft-*versus*-host disease, transfusion refractoriness, among others. Other benefits of PABD are blood availability for patients with unusual blood group or immunohematologic problems, diminution in global blood reserves, hospital costs, length of in-hospital stay and even in judicial complaint. Nevertheless, alternatives of allogenic blood transfusion are not innocuos and they not always accomplish cost-effective criterion
Aims: In accordance with the abovementioned it is neccesary to evaluate programs of PABD and proceed to create correction and/or improvement strategies to apply more effective protocols having into account patient´s, surgery and even surgeon characteristics.
Methods: We retrospectively revised PABD experience (2007-2012) of the Tranfusion Service at Baracaldo Universitary Hospital. We defined *effectiveness* as the percentage of patients who exclusively received autologous blood transfusion with respect to the total patients transfused, and *Yield* as the ratio between transfused units/removed units in patients included in the program. The PABD program at this center consists on the extraction of one unit per week, beginning 30 to 35 days before the surgery and finalizing at least one week before it. We prescribe oral iron since the first appointment and until two months after surgery.
Results: 193 patients (71♀ and 122♂) were sent to PABD consultation. Global effectiveness was 74,2%. Twenty three (11,9%) patients were excluded from

PABD, eleven of them required allogenic transfusion. Despite there are no important differences in global transfusion rates among included and excluded cases (50,6% *vs.* 47,8%), these latest patients were transfused with minor values of hemoglobin (7,8 g/dL *vs.* 8,9 g/dL, *p <0,001)*. 86 (50,5%) patients from the 170 patients included at PABD, required transfusion and 14 (8,2%) of them needed allogenic blood. Global yield was 44%. Patients who showed statistically greatest yield were: 50 years old or younger (p=*0,048*), hemoglobin values at pre-anesthetic evaluation between 14.4 -15.6 g/dL (p=*0,005*) and maxillofacial surgery (p=*0,043*). There is a direct negative correlation between hemoglobin values at pre-anesthetic evaluation and yield in patients of knee surgery (*Spearman Rho: -0,478,* p=*0,002*), woman (*Spearman Rho: -0,362,* p=*0,004*) or older than 50 years old (*Spearman Rho: -0,232,* p=*0,025*). Overtransfusion risk were observed in patients included at PABD program, men (38,2% *vs* 11,9% in women, p=*0,004)*, younger than 50 years old (39,5% *vs* 16,7% in older than 50 yeras old, p=*0,012)* or maxillofacial surgery. The total quantity of removed units during the period reviewed were 338 units, and we observed adverse effects related with extraction in 6 patients, none of these events were severe and the most frecuent was hypotension (*n*=4).

Summary and Conclusions: PABD program at this center is a secure alternative of allogenic blood transfusion in most of patients without increasing postoperative morbi-mortality. Likewise, it is a usefull strategy in specific cases.

PB1988

TRANSFUSION INDICATION PREDICTIVE SCORE [TIPS]: A PROPOSED RISK STRATIFICATION SCORE FOR PERIOPERATIVE RED BLOOD CELL TRANSFUSION IN CARDIAC SURGERY

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Background: Allogeneic packed red blood cell (PRBC) transfusion is known to be associated with increased morbidity and mortality in cardiac surgery. A number of scores have been developed to predict the risk of perioperative blood transfusions.

Aims: This study was performed to derive a score to predict the risk in our patients.

Methods: Clinical, surgical and laboratory details of all patients underwent cardiac surgery at the Sultan Qaboos University Hospital (SQUH) over five years were reviewed. We used univariable and multivariable logistic regression to develop the score, the Hosmer-Lemeshow test for calibration, the receiver operator curve for discrimination, and the bootstrap procedure for internal validation of the derived score.

Results: The studied sample included 413 patients with mean age of 58±12 (16-87) years. Seventy-four percent of patients underwent coronary artery bypass, 20% value surgery and 6% had other procedures. The following were found to be statistically significant transfusion predictors (score given): cerebrovascular disease (4), use of aspirin or clopidogril within seven days of surgery (3), non elective surgery (2), hematocrit (Hct)<35% (2), underlying Glucose-6-phosphate dehydrogenase (G6PD) deficiency (2), use of cardiopulmonary bypass (CPB) (2), age > 60 years (1), diabetes mellitus (1) and male gender (-2). We classified the observations into three groups: group 1 including observations with total score of <2, group 2 including observations with total score of 2-5 and group 3 including observations with total score of >5. The calculated probabilities of receiving transfusion were 42%, 63% and 91% for groups 1, 2 and 3 respectively.

Summary and Conclusions: We derived a simple score that can be utilized to assess the need of blood transfusion in patients undergoing cardiac surgery. We are the first to report G6PD deficiency and history of cerebrovascular disease as predictors. We recommend prospective external validation of the proposed score on a larger cohort of patients.

PB1989

FACTORS AFFECTING POST-TRANSFUSION PLATELET INCREMENTS AND REFRACTORINESS IN CHILDREN WITH HEMATOLOGIC AND ONCOLOGICAL DISEASES

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Background: Children with aplastic anemia and cancer receive frequent platelets transfusion but most of them became refractory to transfusion during their treatment course

Aims: to study the frequency of platelet refractoriness among the hematologic and oncologic patients in an Egyptian University center, to identify the determinants of platelet refractoriness including the immune causes by evaluating the antiplatelets antibodies and the non-immune causes.

Methods: A cross sectional study was performed including 60 children with onco-hematologic diseases necessitating platelets transfusions and admitted at Ain-Shams university pediatrics hospital ,hematology-oncology unit . They were divided into 2 groups; thrombocytopenia secondary to aplastic anemia and secondary to childhood cancer. Revision of hospital records for diagnosis and its date, history of platelet transfusion , bleeding, its site and severity, number of platelets transfusions prior to testing, drugs used with emphasis on amphotericin B/vancomycin/ ciprofloxacin. Laboratory investigations including complete blood picture prior to platelet transfusion, 1 hour post transfusion, and 18-24 hours after transfusion with calculation of platelets increments and corrected count increment .Poor response to platelets transfusion was considered if CCI is less than 5000. Screening for antiplatelet antibodies was done by platelets immunofluorescence test using flowcytometry.

Results: Oncology group were 26 children with mean age ±SD 7.67±4.59 years , 14 females (53.8%) and 12 males (46.2%) and aplastic group were 32 children with mean age 10.03±4.65 years, 13 females (40.6%) and 19 males (59.4%).52.9% of the oncohematological patients have CCI <5000 .There was no significant difference between patients with CCI above and below 5000 as regard gender, diagnosis, type of platelet transfused, blood group, age , frequency of transfusion, frequency of fever or use of concurrent use of ciprofloxacin or amphotericin use .CCI was lower in patients using vancomycin (P=0.029) and with different blood groups (P=0.012).We had a mean platelets increment of 27.04 and 22.77 in the one and 18-24 hours in the oncology group and 13.28 and 9.07 x 10 ^{9}/L in the aplastic group (P=. Aplastic group had higher frequency of transfusion (P=0.010)and longer duration since diagnosis (P=0.013) and lower use of single donor platelets(P<0.001).No significant correlation found between Ab% and CCI at 1h post transfusion and at 18-24 hours post transfusion in oncology & aplastic patients. The best cut off point for antibody levels between positive and negative patients was found > 11.3 with a sensitivity of 96.55% and specificity of 100%.

Summary and Conclusions: Conclusion : we had a frequency of poor platelets response to transfusion of 52%. using non-leuco-reduced platelets products. Platelets antibodies were not correlated with platelets increments. Patients with aplastic anemia had a higher levels of antibodies compared to children with cancer probably due to higher use of random platelets preparation and higher frequency of previous transfusions. CCI was lower in children using vancomycin.

PB1990

OUTCOMES OF THERAPEUTIC LEUKAPHERESIS FOR HYPERLEUKOCYTOSIS IN ACUTE MYELOID LEUKEMIA VERSUS OTHER HEMATOLOGIC NEOPLASMS

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Background: Hyperleukocytosis is arbitrarily defined as a leukocyte count greater than 100 x 109/L, and typically appears in some hematological neoplasms. Hyperleukocytosis implies a higher morbimortality related to the possible development of leukostasis, tumor lysis syndrome and/or disseminated intravascular coagulation. The number of leukocytes necessary for leukostasis changes with each pathology, owing to, among other factors, different morphological, molecular and plasticity characteristics of the blast cell, as well as the capacity of the endothelial cell to release cytokines. Leukoreduction is a measure to prevent adverse events and death owing to hyperleukocytosis, since it influences cell differentiation in the bone marrow by increasing the proportion of blast cells in S-phase, a fact that translates into increased efficiency of certain antineoplastic agents. Although some scientific societies support leucocytapheresis (LCP) implementation in specific cases, there is insufficient evidence about its use in the early treatment of leukemia with hyperleukocytosis. In addition, the efficiency of LCP remains controversial. The invasive and risky nature of this technique, the need for experienced staff and, in some cases, central venous access, as well as the additional costs and limited scientific evidence proving its effectiveness in global long-term survival are reasons why some centers prefer more conventional treatment in asymptomatic and pediatric patients.

Aims: To analyze the safety and effectiveness of therapeutic LCP as a leukoreduction strategy and its influence on early mortality in our case series, adjusted to the independent mortality risk factors described in the literature. And to compare results obtained between patients with Acute Myeloid Leukemia (AML) *versus* other hematologic neoplasms.

Methods: We did a retrospective review of LCP procedures carried out over nine years (June 2003 to June 2012) for the treatment of hyperleukocytosis at the Hematology and Hemotherapy service of Miguel Servet Universitary Hospital. Data was obtained from patient clinical histories and electronic medical records (Intranet, Modulab, Netbank, Izasa®). Registration during each LCP session in the transfusion unit was performed in a prospective way, to ensure there was no loss of data. At our center, we considered starting LCP

with a leukocyte count greater than 100 x 109/L, or at presentation of leukostasis symptoms. Demographic, clinical, analytical and technical variables were reviewed. Tumor lysis syndrome was defined according to the following criteria: hyperkalemia, hyperuricemia, hyperphosphatemia, hypocalcemia and uremia13. Early mortality was defined as death within the first 14 days after diagnosis.
Results: Thirteen patients underwent a total of 27 leukocytapheresis procedures. After an average of 2 sessions, a statistically significant drop in the initial leukocyte counts was observed ($p<0.01$), as well as a relevant drop (>50%) in lactate dehydrogenase levels. The only analytical value in the global series statistically related to early mortality in univariate analysis was initial creatinine levels greater than 1.2 mg/dL ($p=0.012$, OR=2.5). Patients with acute myeloid leukemia had worse outcomes within 6 months of having finished leukocytapheresis sessions, and in terms of mean global survival and mean time of mortality. However, global mortality rates were similar in patients with and without AML.
Summary and Conclusions: Despite the limited and only slightly homogeneous casuistry of our case series, we can conclude that leukocytapheresis is a safe and effective therapeutic measure for leukoreduction in hematological pathologies of any lineage, primarily in patients without acute myeloid leukemia.

PB1991

THE EFFECTIVENESS OF PLATELETS TRANSFUSIONS AND OXIDATIVE STRESS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA DURING INDUCTION CHEMOTHERAPY

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Background: The effectiveness of platelets transfusions is dependent on immune and nonimmune factors. Nonimmune factors are fever, sepsis, splenomegaly, disseminated intravascular coagulation (DIC), venoocclusive disease, graft *versus* host disease and others. Development of many nonimmune factors are associated with reactive oxygen species (ROS) formation and disturbance of antioxidant status. So one may suppose relationship between ROS formation and/or antioxidant status imbalance and effectiveness of platelets transfusion.
Aims: The aim was to study the interface of platelets transfusion effectiveness with ROS formation and antioxidant enzymes' activity in patients with acute myeloid leukemia (AML) during induction chemotherapy (ICT) scheme "7+3".
Methods: Data of 15 patients with de novo AML with median age 42 years (31-63) were analyzed. AML was diagnosed according to WHO 2008 criteria. ICT scheme included Ara-C 100 mg/m^2 IV every 12 h 1-7 days and idarubicine 12 mg/m^2 IV 1-3 days. Conditions to start platelets transfusions were platelets level less than $10x10^9$/l or hemorrhagic syndrome irrespective of platelets level or DIC. The effectiveness of platelets transfusions were evaluated by corrected count increment 24 hours (CCI_{24h}). Level of malondialdehyde (MDA) in serum was used to characterize the ROS formation. Antioxidant status was evaluated by activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ceruloplasmine (CP). Collection of peripheral blood (PB) samples was done before and after ICT, during the lowest parameters of PB and after restoration of hematopoiesis. The treatment was begun and PB samples were collected after the signing of the informed consent.
Results: Complete remission was diagnosed in 10 patients (66.6%). Median number of platelets transfusions was 8 doses. Two groups of patients were formed): with effectiveness of ≥50% platelets transfusions (8 patients) and with effectiveness of <50% platelets transfusions (7 patients). In group of patients with effectiveness of ≥50% platelets transfusions gradual decreasing of enzymes with maximal low levels during bone marrow aplasia was found. In a subsequent there was the increasing of enzymes level with maximal activity of SOD. This dynamics of antioxidant enzymes activity was accompanied by absence of MDA level changing significantly. In group of patients with effectiveness of <50% platelets transfusions gradual decreasing of SOD level was found after ICT and in a subsequent period its level remained reduced. Opposite situation was found for 2 other enzymes: there was the trend to increasing level of CP and CAT in most of CP. This dynamics was accompanied by increased level of MDA which did not decrease to initial level after restoration of hematopoiesis although there was some kind of trend.
Summary and Conclusions: Dynamic changes of MDA level and activity of antioxidant enzymes was found in AML patients during ICT. The changes of separate enzymes activity were different in patients with different effectiveness of platelet transfusions. The relationship is unknown. It is possible to suppose that MDA level's excess continuing during all ICT period may contribute to development of such serious complications as infection or damage of vessel endothelium with subsequent formation of consumption syndrome and ineffectiveness of platelets transfusions.

PB1992

SEARCH ROR EVENTUAL BACTERIAL CONTAMINATION OF BLOOD COMPONENTS PRESENTING VISIBLE HEMOLYSIS OR IMPLICATED IN ADVERSE TRANSFUSION REACTIONS

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Background: The risk of bacterial contamination of blood products still exists despite efforts to conform with all rules of hygiene and safety at each step of the collection and preparation of blood components. Possible mechanisms include: donor bacteremia at the time of donation or contamination during the collection procedure (use of a contaminated bag, poor skin decontamination, entry of air) contamination during the blood processing procedure or during storage. Transfusion of blood components heavily contaminated will result in sepsis and occasionally death, especially in immunocompromised recipients. Contaminated platelets are more commonly associated with adverse reactions than packed red cells, because the storage temperature of platelet units (20-24°C) favors bacterial growth.
Aims: Since issue of relatively fresh blood and the administration of antibiotics to several transfused patients for their underlying disease, might prevent the appearance of fully blown symptoms of bacteremia, we decided to screen for possible bacterial contamination every unit of packed red cells or platelets implicated in a febrile transfusion reaction –both hemolytic and non-hemolytic- and every unit of packed RBCs with visible hemolysis.
Methods: During one year (2013), the Microbiology Department performed bacterial culture of samples from a) 39 units of packed RBCs presenting visible hemolysis (4 collected and processed in our Center and 35 in other Blood Banks) and b) 3 units of platelets and 24 units of packed RBCs, implicated in transfusion reactions characterized by chills, rigor, fever, with or without vomiting, diarrhea etc. Leukocyte depletion had been carried out with post storage and bedside filters in 7 and 6 units respectively. The samples were inoculated into appropriate nutritious materials (Blood Brain Heart Infusion agar, MacConkey agar, Chocolate agar, Sabouraud dextrose agar, Phenylethyl alcohol agar) that allow fast bacteria growth and incubated for 5 days under aerobic and anaerobic conditions. Enrichment broth techniques were also used in order to exclude false negative results.
Results: Of the 66 blood samples cultured, none grew positive.
Summary and Conclusions: Our reassuring results probably reflect the efficacy of: detailed interview and questioning of donors to identify symptoms that might indicate any underlying infectious process; not accepting donors during digestion to avoid transient intestinal bacteremia; meticulous skin disinfection and diversion of the first 30 ml of donated blood; optimal blood component processing and storage. The febrile reaction in the case of non-leukodepleted units (11/24) could be attributed to anti-leukocyte antibodies. The techniques used for bacterial culture were sensitive enough for all microorganisms except for *Y. entercolitica*. However, the absence of severe clinical picture and the excellent outcome is not in favor of such contamination.

PB1993

THERAPEUTICAL LIPID APHERISIS IN CHILDREN:A SINGLE CENTER EXPERIENCE

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Background: Familial hypercholestrolemia (FH) is a genetic disorder which is characterized by high cholesterol levels, spesically very high levels of low density lipoproteins (LDL). Most of FH patients generally resistant drug teratment and diet. Untreated pediatric patients with homozygous familial hypercholesterolemia usually have myocardial infarctions, heart failure, or death by the teenage years. For the last 30 years or so, LDL apheresis has been used and progressively become a mainstay in the management of FH.
Aims: Therapeutical Lipid Apherisis (TLA) decrease LDL and cholesterol. This procedure aim to prevention high risk cardiovasculer disease. We evaluated our TLA procedures retrospectively.
Methods: 180 TLA procedures have succesfully applied 6 FH patients in our center between August 2012 - February 2014. Left ventricular function and valvular status was checked every apheresis therapy. Central veneous cathaters used to intravenous acces for all patients. We used DALI (Direct absorbpsion of Lipoproteins, Fresenius) and Liposorber D-System (Kaneka) for TLA. Blood samples were taken directly before and after each therapy. Blood count, electrolytes, albumin, total cholesterol, LDL, high density lipoprotein (HDL), triglycerides and fibrinogen were measured. Adverse events were documented weekly.

Results: Four of the cases were boys and two were girls. The age range was between 10-15 years (median age 12.5). LDL pre-treatment value was 222 - 900 (mean 444 mg/dl) and the post-treatment value was 64-685 (mean 192 mg/dl). This corresponded to a%57 acute reduction of LDL. Cholesterol pre-treatment value was 267-1014 (mean 514 mg/dl), and the post-treatment value was 88-830 (mean 240 mg/dl). This corresponded to a%53 acute reduction of cholesterol. The resulting data showed a significant decrease in the amount of trigliserid (22%), HDL (24%), albumin (12%), fibrinogen (20%), hemoglobin (9%), platelet (8%) and leukocyte (0.2%).
Summary and Conclusions: Low-density lipoprotein apheresis effectively lowers LDL cholesterol in the short term, but there is little published information on the long-term safety and efficacy of this treatment in children.

PB1994

KNOWLEDGE,ATTITUDE AND PRACTICE OF BLOOD CONSERVATION STRATEGIES AMONG CLINICIANS IN TWO TERTIARY HOSPITALS IN NIGERIA

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Background: Millions of lives are saved each year through blood transfusions, yet the quality and safety of blood transfusion is still a concern particularly in the developing countries. The need for sophisticated blood processing, storage and cross-matching, coupled with increasing public concerns about the safety of blood products, have fuelled the need for blood conservation strategies to minimize transfusion
Aims: To assess the extent of available knowledge, as well as the attitudes and the utilization of blood conservation strategies amongst medical doctors, who are the implementers of blood transfusion and conservation in clinical practice.
Methods: A cross-sectional study was conducted among doctors in public and private tertiary hospitals. A pre-tested, self-administered questionnaire was devised to collect data. Data was entered and analyzed descriptively and qualitatively.
Results: A total of 104 doctors participated in the study consisting of 57 males (54.80%) and 47 females (45.29%). Almost all participants had transfused blood in their practice and all participants were aware that blood transfusions have complications. Majority 80 (76.9%) had heard about blood conservation techniques, about 51 (49%) had used any of the strategies.
If available, 93 (89.4%) would use any of the strategies, only 1 (1%) would not.
Summary and Conclusions: This study has revealed the need for an effective transfusion committee in our hospitals that will actively educate and increase the drive for utilization of blood conservation methods amongst doctors, so as to increase its practice. More studies will also be required to assess the effectiveness of blood conservation strategies in our environment, with the aim of developing more effective and efficient ways of blood conservation.

PB1995

RED CELL ANTIBODIES IN PATIENTS WITH HEMOGLOBINOPATHIES. REPORT OF A CENTRE

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Background: Delayed hemolytic reactions and incompatibility problems are sometimes encounterd in blood banks. Such problems arise mainly in heavily transfused patients.
Aims: Patients with hemoglobinopathies (thalassemia major and intermedia, sickle cell syndromes) need regular transfusions. This study is to determine the incidence of red cell alloantibodies and autoantibodies in this group of patients in our centre as well as clinical significance of these antibodies.
Methods: This is a retrospective study of the last decade (2003-2013) that includes data from the blood bank and hematology department involved in the care of patients with hemoglobinopathies. Eighty five (85) patients with thalassemia major and intermedia and 33 patients with sickle cell syndromes are in a regular follow up and transfusion program. Leukodepleted Packed red cell units are transfused. All units are checked for ABO and Rh (D) antigens. Prior to every transfusion sera of the patients are tested for the presence of alloantibodies by indirect antiglobulin test (IAT) and after a positive result antibody identification is performed by a RBC panel Direct antiglobulin test (DAT) is performed every 12 months and in any case of incompatibility. DAT is performed with Dianed ID-Gel and IT with ID –Gel and Capture R assays.
Results: Eighty five patients with thalassemia major and intermedia are in a regular follow-up. Thirty eight (44.7%) of them had positive DAT and IAT tests during the previous ten years. This group includes 17 females age (26-60) and 21 males age (19_66)Most of them had splenectomy dyring their childhood (24/38). DAT was positive in 33 patients (86.8%). IgG was the predominant antibody .Reactivity was 1+-3+, but only two patients had 3+. IAT was positive in 20 patients (52.6%) with specific antibodies identified in 17 patients (anti Fyb,anti-S, anti-Kell, anti-D, anti-e, anti-Kell, anti-kpa, , anti-Cw, anti- Fya, anti-Jka, anti- Leb, anti-Lea, anti-C) Cross matching was impossible in 4 cases and prednisone was given .Three of these patients had more than two antibodies.In the group of sickle cell syndromes patients (5F, 5M) 10 patients (10/33, 30%) had IAT positive test and only one had also DAT positive. Antibodies that were identified include the following : anti-Kell, anti-kpa, anti- Jka, anti-e, anti-Jkb, anti-E,anti- A1, anti-Cw, anti-Fya, anti-D, anti-C. One of these patients had delayed transfusion hemolysis, received prednisone and fully compatible blood transfusions and finally had splenectomy.
Summary and Conclusions: Patients with hemoglobinopathies are in need for regular transfusion. This important therapeutic approach makes them vulnerable in developing auto and alloantibodies against red cell .Pathogenesis of antibodies formation is not fully understood, it seems that patients with sickle cell syndromes who are not regularly transfused are prone for developing alloantibodies more frequently compared to thalassemia patients who acquire autoantibodies and alloantibodies as well.Although most of the times these antibodies have no clinical significance, regular testing with DAT and IAT as well as extended red cell cross matching in some cases is essential for preventing delayed hemolytic reactions and difficulty in identifying compatible red cell units.

PB1996

ACTIVITY OF OXIDATIVE STRESS DURING EFFECTIVE IRON CHELATION THERAPY

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Background: The toxic effect of posttransfusional iron overload on cardiac and liver tissue is the result of damage caused by reactive oxygen species (ROS). ROS are the consequence of nontransferrin bound iron formation. Previously different positive effects of therapy with deferasirox (DFX) were demonstrated including decreased serum ferritin (SF) level. On the other hand it was recommended to take care with the DFX dose after decreasing SF level till ≤1.000 ng/L. But it is really unclear whether it is necessary to modify the dose of DFX if the SF level reached the specified borderline.
Aims: The aim of the study was to determine the activity of oxidative stress during iron chelation therapy (ICT) with DFX by measurement the concentration of malondialdehyde (MDA) and activity of such antioxidant enzymes like ceruloplasmin (CP) and katalase.
Methods: The data of 8 patients who were treated with DFX during at least 1 year was collected and analyzed retrospectively. The diagnoses were like myelodysplastic syndromes (5 patients), mixed myeloid neoplasm (1 patients), aplastic anemia (1 patient) and pure red cell aplasia (1 patient).
Levels of SF, MDA, and antioxidant enzymes were determined before ICT and then every 3 months after the beginning of treatment with DFX.
Results: The median age of patients was 62 years (33-71). Indications to start ICT were at least 2 of the next conditions: regular RBC transfusions, number of RBC transfused >20 units, SF level >1.000 µg/L and/or transferring saturation >80%. The pretreatment SF level was 3733±1761 µg /L (1037 – 7353). The initial dose of DFX was 10-20 mg/kg/day according to the intensity of RBC transfusion and the aim of ICT. The dose of DFX was reduced temporary in 2 patients because of gastrointestinal toxicities of grade 2-3. Daily dose of DFX did not exceed 20 mg/kg/day. After 12 months of ICT the SF level was decreased on 1544±1063 µg /L (from +182 to -5013); p=*0.026*. In 2 patients the SF was decreased to the level <1.000 µg/L and in 1 patient SF level was not changed after 12 months of ICT. In spite of the biochemical effects of DFX all patients continued to be transfusion dependent. At the same time the concentration of MDA was not changed significantly even in patients with decreased SF level <1.000 µg /L. After 1 year of ICT the activity of CP was decreased from 0,83±0,3 till 0,69±0,1 g/L and activity of catalase was increased from 2,9±1,7 till 4,1±1,5 U/ml but the differences were not significant.
Summary and Conclusions: Treatment with DFX may decline posttransfusional SF level after 12 months of ICT effectively. The remaining high concentration of MDA is a marker of persistent oxidative stress as a consequence of continuing RBC transfusion dependence. So we conclude that ICT with DFX has to be continued all the period when patients transfused with RBC even if SF level is decreased successfully.

PB1997

TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE IN AN IMMUNOCOMPROMISED PATIENT AFTER AUTOLOGUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION - A CASE REPORT

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Background: Transfusion-associated graft-vs-host-disease (TA-GVHD) is rare but usually fatal complication of transfused cellular blood components. The incidence of TA-GVHD is uncertain. The three primary risk factors for developing TA-GVHD are: degree of recipient immunodeficiency, number of viable T lymphocytes transfused and genetic diversity in HLA expression between donor and recipient. Diagnosis of TA-GVHD may be difficult at a time due to rarity in occurrence and overlapping clinical features with infections and drug reactions. TA-GVHD present with fever, rasch, abdominal pain and diarrhoea 2-30 days after transfusion. Laboratory data revealed pancytopenia and liver disfunction. Mortality rate is >90%. Death occurs on average 51 days following the transfusion.
Aims: We describe a fatal case of TA-GVHD that occurred after transfusion of blood in an immunocompromised patient who has conducted autologous stem cell transplantation.
Methods: Case report: In a 49 year old men patient (Pt), diagnosis of Myeloma Multiplex was established in 2008. Pt received six cycles of chemotherapy (VAD) prior to autologus SCT. The maintenance therapy was applied after transplantation.One year after the transplantation relapse occured and secondary SCT had been done in pt (in decembar 2009.). In his medical history was partial resection of the sigmoid with coloprotectomiam and J Pouch for familial polopoze sixteen years ago. Posttransplanted autologous hematopoietic reconstitution was done timely. Two weeks after transfusion he presented with fever, nausea,vomiting and diarrhea with normal biochemistry. Infection were excluded. Histopathologic analysis of biopsy Pouch confirmed Pouchitis chronica. But twenty-three days after transfusion on physical examination noted skin rash and patechie all over the body. The raches were diffuse confluent erythematous maculopapular rash over trunk and extremities, which blanches on pressure.His respiratory, cardiovascular and central nervous system examination was normal. Laboratory data revealed pancytopenia (Hb: 8,5g/dL, WBC counts: $2,3x10^9$/L; platelat count $75x10^9$/L). Biochemistry parametar revalead high value SGPT (196 U/L), SGOT (455U/L), LDH (421U/L), serum alkaline phosphatase (276U/L), creatinine (232umol/L). X-ray chest and ultrasonography of abdomen were normal. Histology of bone marow was normal. Histology of skin confirmed TA-GVHD.
Results: He was treated with supportive care therapy with broad spectrum antibiotics and antifungal agents as appropriate. After the evidence TA-GVHD was treated with pulse steroids, then the triple immunosuppressive therapy (Cyclosporin A, Mycophenolate mofetil,steroids). During treatment leads to the development of infection (blood culture grew Staphilococcus aureus). The effect of treatment is reflected in the loss of fever, diarrhea and skin rash with recovery of renal function, of liver function and of blood count parameters. Six months after transfusion leads to deterioration of general condition and development of severe infectious syndromes, pancytopenia, heart and liver failure. Blood culture grew Pseudomonas aeriginosa. Demonstrated reactivation of cytomegalovirus infection. The therapy is turned off CsA, continued implementation Mycophenolate mofetil and again implementation steroids. Eight months after transfusion leads to worsening TA-GVHD and death.
Summary and Conclusions: As in our case, fever, nausea,vomiting and diarrhea, skin rash and patechie all over the body, renal and liver dysfunction were suggestive, but the diagnosis was confirmed at skin biopsy. He is treated with triple immunosuppressive therapy (Cyclosporin A, Mycophenolate mofetil,steroids) complicated with severe infectious syndromes.The reason for pt death is TA-GVHD, but he lived four times longer that the average. Due to its rarity, TA-GVHD requires further investigation in order to define uniform approach and develop treatment and transfusion guidelines.

PB1998

ABO-INCOMPATIBLE IN ALLOGENIC BONE MARROW TRANSPLANTATION

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Background: Practically, the totality of patientes undergoing hematopoietic stem cell transplantation (HSCT) need transfusion support at some point along its evolution. In the HSCT in which there is no existing identity between donor and recipient in the ABO system, it can occur that the recipient presents antibodies which react to the donor's antigens (major incompatibility) , or that the donor presents antibodies against recipient's antigens (minor incompatibility) . If both situations occur simultaneously , it is called mixed incompatibility. The barrier group does not affect the future of TPH , but it can cause more or less severe hemolytic reactions during the recovery period.
It is important to correctly choose the blood group component to transfuse until a stable chimera is achieved, with a complete group change and a negative Direct Coombs Test. In case of major incompatibility, the transfused homoderivatives should be those compatible with the recipient's group. On the contrary, in the case of a minor incompatibility, the transfused homoderivatives should be compatible with the donor's group. In case of mixed incompatibility, will have to transfuse red blood cells group O. The type of immunohaematological complication will depend on the degree of incompatibility . Major incompatibility: delayed or immediate hemolysis, delayed red cell production, pure red cell aplasia . In those patients with minor or mixed incompatibility, we differentiate immediate or delayed hemolysis.
Aims: We collected all ABO-incompatible allogeneic transplants performed at our center in the last 2 years
Methods: It has been analyzed the degree of incompatibility, hemolytic complications and the type and quantity of reed blood cells transfused within 4 weeks after the infusion of progenitors.
Results: A total of 25 allogeneic HLA-identical sibling transplants (17 men / 8 women), 20 of them with myeloablative conditioning and 5 with a reduced-intensity conditioning, were performed. According to pathology: 10 patients with acute myeloid leukemia , 3 with acute lymphoblastic leukemia , 1 with acute leukemia of ambiguous lineage , 3 with myelodysplastic syndrome, 1 with myelofibrosis, 1 with chronic myeloid leukemia, 2 with non-Hodgkin's lymphoma, 1 Hodgkin lymphoma , 2 with bone-marrow aplasia and 1 for multiple myeloma. 5 patients presented ABO incompatibility: 3 with minor incompatibility, 1 with mayor incompatibility and 1 displayed mixed incompatibility. In none of these cases, hemolisys parameters were observed.
The average of red blood cells transfused were 5.2 units (2↔10): 2 in major incompatibility, 6 in minor incompatibility and 1 in mixed incompatibility. The average recovery time for the red series was 31.4 days (12↔74): 14 days in major incompatibility, 27.7 in minor incompatibility and 74 days in the patient with mixed incompatibility.
Summary and Conclusions: The average recovery time of the red series in our cases was 31.4 day , in comparison to those patients who had no ABO incompatibility which was of 21 days approximately.
The patient with mixed incompatibility, there was not an increase of the transfusional requeriments, in spite of a more long time of recovery of the red series.

PB1999

THE INFLUENCE OF ANTI HUMAN LEUKOCYTES ANTIBODIES I AND II CLASS ON PLATELETS TRANSFUSIONS EFFECTIVENESS IN PATIENTS WITH ONCOHEMATOLOGICAL DISORDERS

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Background: It is supposed that development of human anti-platelet (anti-HPA) and anti-leukocyte (anti-HLA I and II class antibodies – HLA I and HLA II Ab) is the main mechanism of ineffectiveness of platelet transfusions in patients with oncohematological diseases. There are different methods to prevent Ab production including blood products filtration or irradiation. It has been shown that chemotherapy (CT) by itself may inhibit immune system activity and reduce Ab production significantly. However the immune mechanism may lead to the absence of expected increment of platelet number in peripheral blood after transfusions.
Aims: The aim of the study was to evaluate the concentration of HLA I/II Ab during the course of intensive CT, and thus to determine the role of immune mechanisms in development of platelet transfusions ineffectiveness.
Methods: We have analyzed data of 37 patients including 24 with acute myeloid leukemia (AML) and 17 with multiple myeloma (MM). AML patients were treated with induction scheme "7+3" (7 patients) or consolidation CT including high dose Ara-C (17 patients). All MM patients were treated with melphalan 200 mg/m^2 as a conditioning regimen before autologous stem cell transplantation. Corrected count increment 24 hours (CCI_{24h}) was used to determine the effectiveness of platelet transfusions. Solid phase ELISA method (GEN-PROBE: LIFECODES QuickScreen and LIFECODES B-Screen) was used to determine HLA Ab persistence in peripheral blood.
Results: Median age of patients with AML and MM was 42 yrs (23-60 yrs) and 52 yrs (34-65 yrs), respectively. HLA I and/or II class Ab were detected in 34.6% of patients before intensive CT (of them 83.3% – female). Additionally, there were 5 (13.5%) and 3 (8.1%) patients in whom we determined HLA I and HLA II Ab during dynamic monitoring, respectively. The concentration of HLA I and HLA II Ab decreased in 7 (53.8%) and 9 (75.0%) patients, increased in 4 (30.8%) and 3 (25.0%) patients, and was without any changes in 2 (15.4%) and 0 patients, respectively. Different scenarios of platelet concentration have been found. All patients (AML+MM) were distributed in 2 groups according to effectiveness of platelet transfusions. HLA I and HLA II Ab were determined in 1 (3.8%) and 5 (20.8%) patients with ≥50% platelet transfusions effectiveness, and in 12 (92.3%) and 8 (61.5%) patients with <50% platelet transfusions effectiveness. The inverse relationship between effectiveness of platelet

transfusion and presence of HLA I and HLA II Ab was found: *r=-0.881;* p=*0.000* and *r=-0.336;* p=*0.042,* accordingly.
Summary and Conclusions: In spite of the fact that HLA-A I and II class Ab are rare events in patients with oncohematological diseases, our data show that immune mechanisms participate in the development of platelet transfusions ineffectiveness during CT. At the same time, decrease of HLA Ab concentration even during the short period of dynamic monitoring allowed us to suppose that intensive CT may reduce the immune system activity by lowering Ab production and thus, at some degree, prevent the rate of platelet transfusions ineffectiveness. But as there is high incidence of platelet transfusions ineffectiveness, we may conclude that non-immune mechanisms play a principal role.

Non-malignant hematopoietic disorders

PB2000

VITAMIN B12 DEFICIENCY: CORRELATION BETWEEN MTHFR POLYMORPHISMS AND CLINICAL AND LABORATORY FINDINGS

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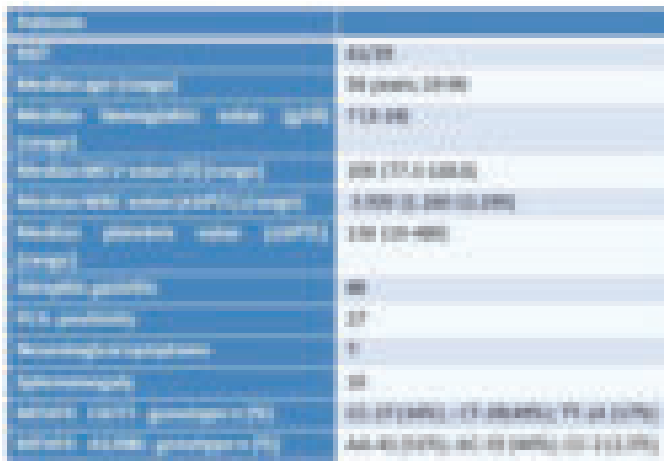
Background: Vitamin B_{12} deficiency is a common condition, particularly among the elderly. The classical manifestations include megaloblastic anemia, due to ineffective erythropoiesis, and neurological complication such as peripheral neuropathy, depression, cognitive disturbances and dementia, due to demyelination of the cervical and thoracic dorsal and lateral columns of the spinal cord. Most common causes are pernicious anemia, gastrectomy, ileal resection, atrophic gastritis, long-term vegetarian or vegan dietary. The interaction between folate and B_{12} is responsible for the megaloblastic anemia seen in both vitamin deficiency. Folate metabolism plays an essential role in DNA synthesis and methylation processes. Methylfolate trap hypothesis is based on the assumption that 5-methyltetrahydrofolate (5-MTHF) cannot be transformed back to its precursor 5,10-methylenetetrahydrofolate (5,10-MTHF), because the reaction catalyzed by MTHFR is irreversible. In cobalamin deficiency, methionine synthase is inactive, causing accumulation of 5-MTHF. So trapped 5-MTHF flows out of the cells, leading a progressive cellular loss of plyglutamated folates. The C677T and A1298C MTHFR gene polymorphisms are associated with a decreased enzyme activity. So homozygous genotype may protect patients with reduced methionine synthase activity from defective DNA synthesis because folate metabolism tends to be shifted to thymidylate synthesis.
Aims: Our aim is to examine cobalamin-deficient patients with reduced MTHFR activity, according to the polymorphisms mentioned, in order to evaluate their predisposition to develop anemia, neurological symptom and atrophic gastritis.
Methods: We studied 80 Caucasian patients with a diagnosis of megaloblastic anemia consecutively admitted to our Hematology Division from 2006 to December 2013. All patients were tested for C677T and A1298C SNPS by polymerase chain reaction. By univariate analysis we correlate hemoglobin, MCV, white blood count (WBC), neutrophil count, platelets count, folate and B12 levels, neurological symptoms and presence of atrophic gastritis and positivity of parietal cells antibody (PCA) at diagnosis with the distribution of C677T and A1298C genotypes (Table 1). Statistical analysis was performed using Fisher's exact test and x^2 test.
Results: We found a correlation statistically significant between patients carrying MTHFR C677T homozygous genotype and lower hemoglobin value (P= 0.022), higher MCV value (P=0.05), lower WBC (P=0.03) and lower platelet count (P=0.05). Forty of the 80 patients had a diagnosis of atrophic gastritis documented by istological examination; twentyseven patients were positive for PCA. We found a significant association between patients carrying MTHFR C677T homozygous genotype and patients with a diagnosis of atrophic gastritis and the absence of antiparietal cell anytibodies (P=0.03). MTHFR A1298C polymorphism showed no correlation with all the variables previously analyzed. Moreover the analysis of plasma levels of folate and vitamin B12 showed the presence of low folate levels in patients with gastric biopsy negative (p=0.02) and antibody negative (P= 0.04).

Table 1.



Summary and Conclusions: On the basis of results it can be assumed that MTHFR C677T polymorphism, which shunt folate towards thymidylate

synthesis and away from methionine synthesis, do not protect against macrocytic anemia and it is associated with a lower WBC and a lower platelet count. Moreover we can speculate that impaired MTHFR function could increase susceptibility to atrophic gastritis in cobalamin deficient-patient with PCA negativity.

PB2001

DELTA BETA THALASSEMIA PATIENTS WITH HOMOZYGOUS XMN-1 POLYMORPHISM THAT ARE CHARACTERIZED BY A MILDER PHENOTYPE

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Background: Thalassemia intermedia is an inherited group of disorders with clinical presentations varying between the thalassemia carrier state and thalassemia major phenotype. This variability depends on the heterogeneous molecular mechanism of the disease. db-Thalassemia is a heterogeneous disorder characterized by elevated levels of Hb F in adult life. It is a rare disorder in Pakistan. The homozygous variety Gg (Ag db)^o are rare , present with a wide range of clinical phenotypes, and belong to the group of thalassemia intermedia.
Aims: We determined clinical, haematological and genetic features of Inv/Del G_g ($A_g$db)^o
Methods: Blood samples were first screened for common beta chain mutation found in Pakistani population and then screened for Inv/Del G_g ($A_g$db)^o db, Xmn-1 polymorphism and alpha chains deletions.
Results: We determined clinical, haematological and genetic features of Inv/Del G_g ($A_g$db)^o db in 100 thalassemia intermedia patients, six of which had Inv/Del G_g ($A_g$db)^o mutations, all were homozygous (+/+) for Xmn-1 polymorphism. Among pathological features, high levels of hemoglobin F, late clinical presentation, hepatosplenomegaly, variable transfusion dependence and an absence of alpha chain deletions were observed. This study explains strong link between the Xmn-1 Gg-polymorphism and the Asian-Indian Inv/Del.
Summary and Conclusions: These findings explain the determined capacity of patients to produce fetal hemoglobin in delta beta-thalassemia. These patients lack alpha chain deletions and may potentiate the qualification of factors favouring HbF production as a prominent source of the thalassemia intermedia phenotype.

PB2002

CHALLENGES IN THE MANAGEMENT OF CHRONIC REFRACTORY AUTOIMMUNE CYTOPENIAS IN CHILDREN: SINGLE CENTRE EXPERIENCE

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Background: Autoimmune cytopenias are a group of heterogeneous primary or secondary conditions (mainly Autoimmune Lymphoproliferative Syndrome, ALPS) characterized by immune-mediated destruction of one or more hematopoietic cell lineages. Steroids and intravenous immunoglobulin (IVIgG) alone or in combination represent the drugs of choice. Refractoriness, lack of compliance and risk of early and late side effects may be reasons to change strategy and use other treatment. Immunosuppressors like mycophenolate mofetil (MMF) and sirolimus seem particularly efficacious and safe therapeutic alternatives.
Aims: To evaluate long term efficacy of immunosuppressive agents in chronic refractory autoimmune cytopenias
Methods: Medical records of patients treated at the Unit of Hematology from 1989 to January 2014 have been revised. For the scope of this study definitive and probable ALPS were stated according to the 2009 revised criteria and ALPS related diagnosis was defined if at least one absolute or primary additional criteria was present. Primary autoimmune cytopenia was considered if none of the ALPS criteria was present. Chronicity and refractoriness were defined by disease duration (> 6 months) and by lack of remission or dependence on steroids and/or IVIgG. Alternative therapies used when failure or dependence on first line option occurred were: cyclosporine A, azathioprine, rituximab,methotrexate, 6-mercaptopurine, cyclophosphamide, fludarabine, MMF, sirolimus and splenectomy. The choice of treatment sequence was done according to availability of drugs in the past and more recently according to an institutional protocol, designed according to the current literature. Chi-square test was applied to assess significance in populations'comparison.
Results: A total of 58 patients were considered eligible for the study. 36 (62%) were females and median age at diagnosis was 5,3 years (0.1-15.3y) while median age at last follow up was 13,5 years (1.7-48.5y). Within the whole group, 11/58 subjects (19%) were diagnosed with definitive and probable ALPS, 25/58 (43%) subjects were defined as ALPS related syndrome, while the remaining 22/58 (38%) were classified as primary autoimmune cytopenia. Thrombocytopenia was documented, both isolated or combined with other cell lineage cytopenia, in the majority of subjects (81%). In the whole group, only 4/58 (7%) patients didn't receive any therapy. Among 54/58 (93%) who needed treatment, steroids and immunoglobulin alone or in combination were able to control cytopenia in 16/54(30%); in 38/54 patients (70%) other immunosuppressive regimens were necessary. Nineteen out of 38 patients receiving more than one line therapy (50%) showed complete or partial response to MMF, 13/38 (34%) to sirolimus, 1/38 (3%) to cyclosporine; in 5/38 of cases (13%) the response was not defined because contemporary treatment or the follow up is too short. Treatment failure or transient response was observed using other immunosuppressive agents, monoclonal antibodies and chemotherapies. In one case even splenectomy alone was not able to reach stable remission. Response to immunosuppressive treatment according to subtype of disease is shown in the following Table 1. The efficacy of MMF in ALPS compared to ALPS related and primary autoimmune cytopenia showed a statistical significance (P=0.002, chi square test). MMF and sirolimus have been well tolerated by the patients except transient abdominal pain, nausea, headache and dermatitis in 15% of patients assuming MMF.

Table 1. Response to immunosuppressive treatment according to diagnostic subtype.

Summary and Conclusions: In our experience more than 2/3 of the cohort required more than one line of therapy. MMF and sirolimus allowed to obtain partial or complete, and sustained remission in 82% of patients refractory to other treatment or dependent to corticosteroids. Moreover in patients affected with ALPS, MMF controlled cytopenia in almost all cases. Use of MMF and sirolimus may be considered in primary and secondary autoimmune cytopenia, mainly in resistant diseases thus sparing corticosteroids, cytotoxic chemotherapies and monoclonal antibodies burdened by known side effects.

PB2003

HYDROXYUREA AND CD47 EXPRESSION IN RED BLOOD CELLS IN ADULTS WITH SICKLE CELL DISEASE

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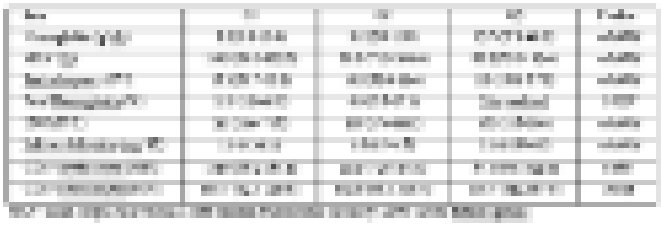
Background: Senescent or abnormal erythrocytes are removed by a particular apoptotic phenomenon called eryptosis. Sickle cell disease (SCD) is known as presenting excessive eryptosis. CD47, a membrane antigen, is an important self-recognition marker and its reduced expression is a trigger to red blood cells (RBCs) phagocytosis. In addition, RBCs are known by their abnormal adhesion to endothelial cells and CD47 has been identified among the molecules involved in these interactions. Hydroxyurea (HU) is the only approved drug for treatment of SCD, since HU improves laboratorial and clinical manifestations, such as hemoglobin level, fetal hemoglobin, and frequency of vaso-occlusive crisis. However, the overall mechanisms involved in the HU action are not fully elucidated. Early studies have shown that HU may increase the expression of membrane proteins, including CD47.
Aims: Due to the increasing interest on the role of CD47 in SCD, we aimed to analyze the expression of CD47 in sickle RBCs and its relationship with HU treatment.
Methods: Fourty patients [70% female, median age 30 years old (range: 18-70y)] in steady state SCD followed at Anemia-Out-Patient Clinic from Escola Paulista de Medicina/UNIFESP were studied. Of those, 20 were in use of maximum tolerated doses (MTD) of HU for more than six months (G1) and 20 without HU (G2). As controls, 10 healthy individuals (G3) were also included. Ethical Committee approved this study, and all patients and control individuals gave their informed consent. RBCs from patients and controls were stained using the Monoclonal Antibodies CD47-FITC (clone B6H12, BD Bioscience) and CD71-PE (clone YDJ1.22, Beckman Coulter®), according to the manufacturer instructions. Data acquisition (100.000 events) and analysis were done using FACS Calibur flow cytometer and CellQuest software (Becton Dickinson, San Jose, CA). Expression of CD47 was evaluated on total RBCs (SSCxFSC) and on reticulocytes (CD71 positive cells) and the results expressed in median fluorescence intensity (MFI). Data were analyzed by non-parametric tests, with α=5%.
Results: The laboratorial data showing the positive effect of HU are presented in Table 1. CD47 expression in patients reticulocytes was higher than in controls

(p=0.043). No difference was seen between G1 and G2 (p=0.291). On total RBCs no difference on CD47 expression among the three groups was observed (p=0.188) (Table 1).

Table 1. Laboratorial data (median, range) of the three groups analyzed: G1) patients in use of HU; G2) patients without HU and G3) normal individuals.



Summary and Conclusions: There are few studies regarding the CD47 expression in SCD, all of them in children. Those studies showed an overexpression of CD47 in erythrocytes and reticulocytes of patients undergoing HU treatment, suggesting that HU acts modulating the adhesion receptor expression. We observed high expression of CD47 in patients' reticulocytes probably due to the high number of immature RBCs. Similar to previous studies, our patients in use of HU presented higher CD47 expression than patients without HU, but without significance. It is important to point out that we studied only adult patients.

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PB2004

TEN YEARS OF EXPERIENCE WITH MIGLUSTAT IN TREATMENT OF TYPE 1 GAUCHER DISEASE: THE SPANISH ZAGAL PROJECT

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Background: The most common clinical variant of Gaucher disease is type 1, which is characterized by anemia, thrombocytopenia, hepatosplenomegaly and
skeletal manifestations. Real-world clinical experience with the substrate reduction therapy (SRT), miglustat (Zavesca®; Actelion Pharmaceuticals), in type1, Gaucher disease (GD1) has developed in Spain since2, 004.

Aims: To report updated follow-up data from a prospective, open-label investigational study that evaluated miglustat in terms of efficacy and safety in naïve GD1 patients and as maintenance in GD1 previously stabilized with enzymatic replacement therapy (ERT)

Methods: The ZAGAL study as a prospective observational study that was initiated in 2004 to establish a set of recommendations for the collection of efficacy, safety and quality of life data in a structured longitudinal manner, and to coordinate the use of miglustat for the treatment of GD1 in real-life settings. Guidelines for miglustat therapy in GD1 were devised by the Spanish Foundation for the Study and Therapy of Gaucher Disease (FEETEG) in order to optimize and standardize miglustat use. All patients received 100 mg miglustat t.i.d. with dietary recommendations to exclude carbohydrates during the first weeks of starting therapy.

Results: We have included in treatment of 53 patients with GD1 who were attending routine clinic visits; 15 naive patients and a further 38 patients who had been switched from previous enzyme replacement therapy (ERT). Long-term changes in organ size, blood counts, disease biomarkers, bone marrow infiltration, bone mineral density (BMD), and overall clinical status were analyzed. Safety and tolerability were also evaluated. Assessments were performed every 6 or 12 months. Approximately 69.8% of patients achieved and maintained therapeutic goals. The plasma biomarkers, chitotriosidase activity and CCL-18/PARC concentration, showed a trend toward progressive increases but did not show a good correlation with clinical activity. Bone marrow infiltration was reduced, as evidenced by a statistically significant decrease in S-MRI scores. Improvements in BMD were also observed after long-term monotherapy with miglustat. In safety and tolerability assessments, 47.1% of patients showed gastrointestinal disturbances and 30% discontinued miglustat therapy for this reason. Nevertheless, gastrointestinal adverse effects were reversible, and symptoms could be improved with a controlled diet, a disaccharidase supplement, and probiotic foods.

Summary and Conclusions: After ten years on follow-up miglustat shown as effective therapy as in naïve with mild or moderate GD1 Spanish patients as in a long-term maintenance therapy in patients previously stabilized with ERT

PB2005

EVALUATION OF ALPHA THALASSEMIA IN CASES WITH HYPOCHROMIC MICROCYTIC ANEMIA: ISTANBUL EXPERIENCE

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Background: Hypochromic microcytic anemia is a common condition in clinical practice and alpha-thalassemia has to be considered as a differential diagnosis. Alpha thalassemia is not rare in Turkey, carrier frequencies a represented as 2.9-4.1%. Alpha-thalassemia syndromes are caused by defects on one or more of the four α-globin genes. The α-globin gen mutations could be either more commonly deletional or non-deletional. The clinical course is correlated with number of affected genes. Single gen defect causes silent carrier status, while four gen defects cause Hb-Barts and hidrops fetalis.

Aims: The aims of this study were to determine the frequency of alpha-gene mutations in subjects with unexplained hypochromic microcytic anemias and to evaluate genotype-phenotype correlation.

Methods: In this study, 180 children (109 male, 71 female) with hypochromic microcytic anemia not responding to iron therapy were eligible. They were tested for alpha thalassemia after evaluated with Hb electrophoresis for beta thalassemia. Two ml venous blood sample was drawn from each patient into the EDTA tubes for DNA isolation. *in vitro* amplification was made with PCR multiplex method using Biotin marked primers belonging to alpha globin encoding gene zones. Products of the amplification process were investigated for mutations of the alpha globulin genes using the Vienna Lab α-Globlin StripAssay TM commercial kit that includes 21 alpha thalassemia mutations.

Results: Thirteen different mutations were determined in 75 (41.6%) of the 180 patients. The most common mutation was 3.7 single gene deletion (n:34 patients, 45.3%). Others were 20,5 kb double gen deletion (n:12, 16%), MED double gen deletion (n:11, 14.6%), α2 IVS1 (n:8, 10.6%), α2 cd142 Hb Koya Dora (n:6, 8%), α2 poly-A1(Saudi type) (n:5, 6.7%), and α1 cd 14 (n:2, 3%), respectively. Hb Adana, Hb Ikaria, 4.2 single gen deletion, α2 poly-A2 (Turkish type) and FIL mutation were found in 1.3% (n:1) of the patients. Seven patients (9.3%) had alpha thalassemia triplication. Some deletions (-SEA, -THAI) and some mutations (α2 cd19, α2 init cd, α2 cd59, α2 cd125, Hb Paksé and Hb Constant Spring) weren't determined in our patients. The clinical forms were mild hypochromic anemia (silent carrier, n:26,%34.6), moderate hypochromic anemia (alpha thalassemia trait (n:30, 40%), alpha thalassemia triplication, n:5, 6.7%) and non-transfusion dependent thalassemia (NTDT) (Hb H (n:12, 16%) and triplication with Beta thalassemia trait (n:2, 2.6%). The mean Hb values were 11.1 g/dl, 10.8 g/dl and 9.5 g/dl among silent carriers, alpha thalassemia traits and patients with Hb H disease, respectively.

Summary and Conclusions: Triplication causes thalassemia intermedia phenotype when it was together with beta thalassemia trait. The most common clinical form of alpha thalassemia is alpha thalassemia trait among patients with hypochromic microcytic anemia. Alpha thalassemia should be considered in the differential diagnosis of hypochromic microcytic anemia especially in cases without iron deficiency and β-thalassemia carrier state. We were able to diagnose 41.6% of unexplained cases of hypochromic microcytic anemia as a-thalassemia carriers. Alpha thalassemia genetic testing appears cost-effective in an otherwise unexplained, longstanding hypochromic microcytic anemia in Turkey similar to Mediterranean and the Middle East countries.

PB2006

BONE INVOLVEMENT IN SPLENECTOMIZED AND NON-SPLENECTOMIZED PATIENTS WITH TYPE I GAUCHER DISEASE

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Background: Gaucher disease is caused by a deficiency in the lysosomal enzyme glucocerebrosidase which leads to accumulation of glucocerebroside in the body, predominantly in the liver, spleen, and bone marrow. Skeletal involvement occurs in 70% to 100% of patients with Gaucher disease and includes a diverse array of symptomatic and radiological findings. Skeletal manifestations do not consistently correlate with the severity of systemic manifestations and contribute much of the morbidity and disability associated with Gaucher disease. Before the era of enzyme replacement therapy splenectomy has been the only treatment option. At present, when effective treatment is available, senvsitive assessment and monitoring of skeletal involvement are critical to timely intervention before permanent disability is incurred.
Aims: The aim of this study was to assess bone involvement in treatment-naïve adult patients with type I Gaucher disease and compare the severity of bone involvement in splenectomized and non-splenectomized Gaucher patients.
Methods: The group included 100 treatment-naïve adult patients with Gaucher disease type I aged from 16 to 79 years (median age 30 years): 36 males and 64 females. Splenectomy was performed in 39 patients in the past when it had been the only treatment option. Standard radiographs and MRI of femurs, hip and knee joints were used to assess the bone involvement. The following criteria were used to characterize bone involvement: bone marrow infiltration with Gaucher cells; osteonecroses in diaphysis and/or metaphyses of femurs; avascular necroses of femoral heads; pathological fractures. Statistical analysis included descriptive statistics, frequency analysis and analysis of variance. All statistical analyses were performed with the SAS software. Statistical significance was defined as a p value <0,05.
Results: Four degrees of bone involvement were established: 1. Mild bone involvement – 14% of patients: bone marrow infiltration with Gaucher cell; 2. Moderate bone involvement – 58% of patients: bone marrow infiltration with Gaucher cells; osteonecroses in diaphysis and/or metaphyses of femure; 3. Severe bone involvement – 25% of patients: bone marrow infiltration with Gaucher cells; osteonecroses in diaphysis and/or metaphyses of femurs; avascular necroses of 1 or both femoral heads; 4. Extremely severe bone involvement – 3% of patients: bone marrow infiltration with Gaucher cells; osteonecroses in diaphysis and/or metaphyses of femurs; avascular necroses of femoral heads; > 2 pathological fractures. Statistical analysis showed that splenectomy was a strong risk factor for severe bone involvement (p<0,0001, OR=6,97 (CI 95% 2,64-18,45). The age when splenectomy had been performed was revealed to contribute: the mean age of splenectomy in patients with severe and extremely severe bone involvement was 11,6 years whereas in patients with mild and moderate bone involvement the mean age of splenectomy was 19,6 years (p=0,03).
Summary and Conclusions: The simple and convenient system for the assessment of bone involvement in Gaucher patients was developed. Splenectomy, especially performed in the early age, is a risk factor for severe bone involvement in patients with Gaucher disease. Hence splenectomy in Gaucher patients might be performed only in urgent cases (*e.g.*, traumatic rupture of the spleen).

PB2007

CORRELATION BETWEEN CEREBRAL AND CARDIO-PULMONARY VASCULOPATHY IN CHILDREN WITH SICKLE CELL DISEASE

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Background: Sickle Cell Disease (SCD) is a systemic disease with heterogenic clinical manifestations. Vasculopathy leads to progressive organ damage in SCD, mainly in brain and lungs. Cerebrovascular disease is evident since childhood while cardio-pulmonary complications manifest in adulthood. Relationship between cerebral and cardiopulmonary organ damage is not clear. In literature there are only few studies and results are contrasting.
Aims: (1) To evaluate prevalence of cerebral and cardio-pulmonary vasculopathy through instrumental examinations in children with SCD; (2) To explore correlation between cerebral and cardio-pulmonary vasculopathy.
Methods: Data were retrospective and systematically collected. We included children with SCD with neurosonology, neuroimaging and echocardiography examinations performed in a maximum 6 month interval. We applied the Stroke Prevention Trials (STOP) criteria to detect altered cerebral velocity by Transcranial Doppler and Transcranial Doppler Imaging (TCD and TCDi); Magnetic Resonance Imaging (MRI) is classified abnormal if there is at least one lesion measuring at least 3 mm visible in at least 2 planes of T2-weighted images. To evaluated vascular lesions at Magnetic Resonance Angiography (MRA) we used a modified Houkin score[4]: when score is > 1, MRA is considered abnormal. To evaluate echocardiography we considerate TRV ≥ 2.5 m/s as threshold for suspect of pulmonary hypertension and functional parameters measured by Tissue Doppler Imaging (TDI).
Results: 38 children (F 18, M 20), mean age 8.67±3.8 years, were included. 95% were Africans. Cerebral Vasculopathy: TCD/TCDi were conditional or abnormal respectively in 16% and 21%. Ischemic silent infarcts on MRI were observed in 42% (bilateral 95%). Intracranial stenosis were detected on MRA in 68% (bilateral 73%). 34% had abnormalities at MRI and MRA, suggesting both small and large vessel vasculopathy. Cardio-pulmonary vasculopathy: The left ventricle, whose mean end-diastolic and end-systolic diameters were increased, showed signs of early diastolic dysfunction in 33%, TRV ≥ 2.5 m/sec in 24%. Statistical analysis showed: (1) correlation between blood velocity at TCDi and stenosis at MRA. (2) linear correlation between volume lesion at MRI and grade of stenosis at MRA in patients with abnormalities at MRI and MRA. (3) correlation between some echocardiographic parameters and cerebral velocity at TCD.
Summary and Conclusions: The systematic assessment of cerebral and cardio-pulmonary vasculopathy shows high prevalence of organ damage since young age. The limited correlation between cerebral and cardio-pulmonary organ damage could be explained by the different ages of onset of vasculopathy in the two vascular beds and needs further prospective evaluation.

PB2008

EVALUATION OF BONE DISEASE BY IMAGING TECHNIQUES AND THEIR CORRELATION WITH PROINFLAMMATORY CYTOKINE PROFILE AND GENETIC VARIABILITY RELATED TO BONE REMODELING IN TYPE 1 GAUCHER DISEASE

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Background: Bone disease and bone complications are a major cause of morbidity in type 1 Gaucher disease (GD1), and more than 80% of patients have some type of bone involvement (bone infarcts, osteonecrosis, osteoporosis, spine collapse). The bones in GD may be affected by one of several mechanisms, infiltration by Gaucher cells, secretion of cytokines by these cells which enhance the osteoclastic activity that leads to bone resorption. In addition there are genetic component related to bone remodeling, which may explain 70% of bone mass variability. The combination of bone marrow MRI, bone plain X-ray and the measure of bone density permit to assess the extension and complications of bone disease in GD.
Aims: To evaluate the relation to bone disease indicators and proinflammatory cytokine profiles and some of the relevant polymorphisms related with osteoporosis in GD1.
Methods: Data from 93 adult GD1 patients from the Spanish GD Registry, diagnosed between 1995-2008 and follow-up during at least 5 years, stratified by age and gender, were analysed. Bone marrow changes were evaluated by a semi-quantitative score [S-MRI], bone density was evaluated using calcaneal ultrasound CUBA CLINICAL BONE DENSITOMETER. Analytical study were performed in frozen stored DNA and plasma quantifieation of 9 cytokines (IL4, IL6, IL7, IL10, IL13, MIP1α, MIP1β, TNFα). In addition, the genotype, clinical characteristics, surrogate biomarkers profile were collected. Data were analyzed using Kolmogorov-Smirnov analysis, t-test or Mann-Whitney-U-test, one-Way ANOVA or Kruskall-Wallis test and correlations using the Spearman test.
Results: 43 males (46.2%) mean age: 34.7 years (18-70) and 50 females (53.8%) mean age: 39.3 years (18-68). Concerning S-MRI score the patients were classified as normal 20.4%, mild infiltration12.9%, moderate 29.0% and severe with complications 30.1%. Related to BMD 15.1% had osteoporosis, 17.2% osteopenia and 21.5% normal density. Significant differences were observed among *VDR* TaqI genotypes of normal and bone affected patients (p=0.031) and in MIP-1α concentration between affected and non-affected patients (p=0.016) but with overlap. *VDR* gene codifies vitamin D receptor. Significant positive correlations were found in non-BD group between IL10/IL13/IL4/IL6/IL7; MIP1α/MIP1β/IL6/IL7/TNFα and in bone affected group between IL10/IL4/IL7/TNFα and IL6/IL13
Summary and Conclusions: According our study the splenectomised patients have more severe indicators of bone disease. The VDR TaqI genotype and MIP-1α concentration have a predictive value in GD bone involvement.

PB2009

EARLY DIAGNOSIS OF BONE MARROW NECROSIS (BMN): CORRELATION BETWEEN MRI AND HISTO-PATHOLOGIC FINDINGS

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Background: Bone marrow necrosis (BMN) is defined as necrosis of myeloid tissue and medullary stroma of the hematopoietic bone marrow (BM) and frequently manifests with bone pain, fever, and peripheral cytopenia. MRI is being used increasingly in evaluation of disease of the bone marrow because it is a noninvasive method of imaging large portions of the marrow in a short time. The underlying diseases of BMN are diverse, with the most common being hematological malignancies, followed by non-malignant causes such as sickle cell disease, infections, drugs, and others.
Aims: The purpose of this study is to correlate between the MRI and histo-pathologic findings in BMN, assess the MRI features that distinguish BMN from similar conditions such as avascular necrosis (AVN) and to illustrate the role of MRI in early diagnosis of BMN in cases where hidden hematological malignancy is suspected.
Methods: We retrospectively reviewed the clinical, laboratory and MRI findings of eight (8) cases of histo-pathologically proven Bone marrow necrosis (BMN). The diagnosis was based on the results of BM aspirate and trephine biopsy. MRI was done using a 1.5-T unit (Signa, GE Healthcare). Sagittal T1-,T2-, STIR sequences of the whole spine were performed in all cases. Gadolinium was injected in 6 cases. The histologic findings in each case were reviewed by two consultant hematopathologist and the MR images were reviewed by two consultant radiologists. Informed consent was obtained from patients.
Results: In our study, 4 patients had hematological malignancies with proliferative features (2 cases of acute leukaemia and 2 cases of lymphoma), 2 patients were post-chemotherapy and two patients were known sickle cell disease. In all cases, MRI of the spine showed extensive, diffuse, geographic pattern of signal abnormality consisting of a central area of variable signal intensity surrounded by a distinct peripheral enhancing rim which was serpiginous in 6 cases. Bones other than the spine like iliac bones, sternum and sacrum were involved in 5 patients. Early extraosseous intraspinal anterior epidural extension of disease was present in 4 cases. Marked compression on the spinal cord and cauda equine was detected in 3 patients. Bone marrow aspirate showed blurred, faint cellular outlines (necrotic cells) that appeared as a mixture of smeared cells, bare degenerating nuclei, and cell debris with increased amounts of amorphous pink background material. The BM biopsy revealed that hematopoietic tissue replaced by amorphous eosinophilic material and the trabecular bone had been preserved (Figure 1).

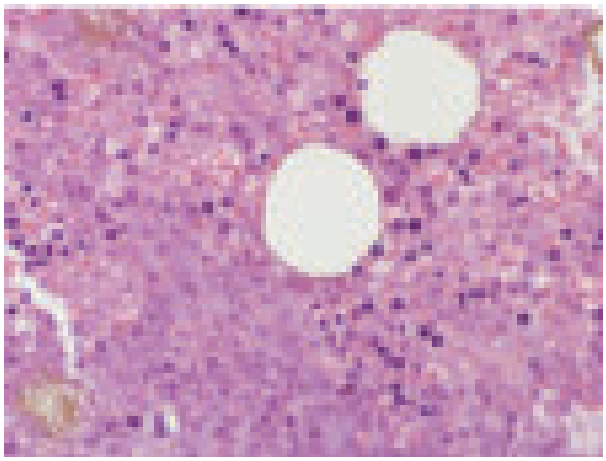

Figure 1. High power of bone marrow trephine biopsy showing areas of necrosis. Note the necrotic cells appearing as eosinophilic cell ghosts.

Summary and Conclusions: Early diagnosis of BMN and its possible underlying hidden malignancy can prevent clinical deterioration and death. MRI plays an increasingly important role in the evaluation of bone marrow disease. Unlike most of the marrow disorders, which often have nonspecific findings, BMN which is a rare clinico-pathologic entity has a distinctive MRI appearance. The lesions in BMN can be differentiated from those of AVN on MRI on the basis of the site, extensive distribution, and natural history of the lesions.

PB2010

AUTOIMMUNE HEMOLYTIC ANEMIA: DESCRIPTIVE STUDY OF 91 CASES

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Background: Autoimmune hemolytic anemia (AIHA) is caused by autoantibody-induced hemolysis (the premature destruction of circulating red blood cells); usually idiopathic, it is also associated with infection, lymphoproliferative disorders, autoimmune diseases, and some drugs.
Aims: Study the epidemiological, clinical, biological, etiological and therapeutic aspects of AIHA.
Methods: This is a retrospective and analytic study about 91 cases of autoimmune hemolytic anemia (AIHA) observed in the hematology and the two internal medicine departments of Sousse, over a period of 14 years. In this work, we have tried to describe the clinical aspects of these AIHA and evaluate the contribution of different diagnosis exploration and therapeutic means used.
Results: There were 33 men and 58 women (sex ratio = 0.56) with a mean age of 51 years [14 – 87]. Regarding the medical history, 19 patients were hypertensive, of whom 8 were receiving Methyldopa, 9 patients were diabetic, 7 had thyroid dysfunction and 18 had a history of autoimmune disease. The circumstances of discovery were an anemic syndrome in 68 of patients, mainly due to paleness and asthenia found in 68 and 60 patient respectively. Physical examination revealed icterus in 42 cases, splenomegaly in 41 cases, hepatomegaly in 8 cases, lymph node in 16 cases and fever in 21 cases. Concerning biology, regenerative anemia was normocytic in 43 cases and macrocytic in 48 cases, thrombocytopenia below 100000/mm3 was observed in 13 patients. There were also biological signs of hemolysis : hyperbilirubinemia in 58 patients, high LDH rate in 60 patients. Direct Coombs test was positive for IgG in 50cases, C 3 in 10cases, Ig G + C 3 in 21cases, IgA in 1 case, IgG+C 3+IgM in 3 cases and cold agglutinins search returned positive in 6cases. There was Evans syndrome in 13 patients. AIHA was idiopathic in 39 cases including 3 cases of pregnancy. In the other cases, it was secondary : lymphoproliferative desorders in 16 cases, autoimmune disorders in 26 cases, 8 cases were secondary to Methyldopa, 1 case was associated with a myelodysplastic syndrome and 1case of CMV infection. The therapeutic consisted of transfusion in 37 cases and all patients underwent a corticosteroid treatment in addition to folic acid therapy in 65 cases and etiological treatment in the non idiopathic cases. We were thus able get a complete remission in 55 cases. In severe cases of chronicity or relapse, immunosuppressive therapy was prescribed in 14 patients, anti-CD20 monoclonalantibody were prescribed in 4 patients and splenectomy was performed in 3 patients.
Summary and Conclusions: Glucocorticoids and/or intravenous immunoglobulins are the mainstay of the treatment in the majority of patients with warm AIHA. When these treatments fail, patients often require cytotoxic drugs or splenectomy. The current research in many other autoimmune diseases that can sometimes be associated with AIHA should still allow a better understanding of the mechanisms involved in the occurrence of these diseases and to refine treatments whose essential aim is to improve the effectiveness of both new and already available treatments (including rituximab) in order to limit the use of corticosteroids.

PB2011

EVANS SYNDROME MANAGEMENT: A MONOCENTRIC RETROSPECTIVE STUDY OF 11 CASES

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Background: Evans syndrome (ES) is a rare auto-immune cytopenia disease, characterised by positive direct Coombs auto-immune haemolytic anemia (AHAI) and immune thrombocytopenia (ITP). Lacking of prospective clinical trials, actual Evans Syndrome management is based on empirical data and indirect clues from ITP and AHAI treatment-guidelines. Glucocorticoids and immunoglobulins are usualy the first-line treatments. The efficacy of second-line therapies, as Thrombopoietin-receptor agonist (TPO-ar), splenectomy or rituximab, is not actually well known.
Aims: Describe clinical characteristics, response to treatment and outcome of adult patients with ES.
Methods: Medical records and biologic data of patients with autoimmune cytopenia diagnosis or both anemia and thrombocytopenia in first diagnosis were reviewed for inclusion criteria assessment.
Results: Eleven of the 308 eligible patients fulfilled the inclusion criteria, including 6 women and 5 men. The mean age at diagnosis was 53.8 - +/- 23 years for autoimmune cytopenia and 54.6 + / - 24.1 for Evans syndrome. ITP preceded the onset of AHAI in 6 (54.5%) patients whereas cytopenia occurred simultaneously in 3 cases. A need for transfusion was observed in 4 cases and ITP related bleeding symptoms in 54.5% of patients, with nor life threatening haemorrhage. ES was considered as “primary” in 3 cases and “associated” in others cases (72.7%). Aetiologies were Systemic Lupus Erythematosus (=1), Primary Antiphospholipids Syndrome with venous and arterial thrombosis (=1), Coeliac disease (=1) Chronic Lymphocytic Leukemia (=1), angioimmunoblastic T-cell Lymphoma (=1), IgG1 subclass primary immunodefiency (=1) and 2

patients with undetermined disease (1 atypic lymphoproliferative disorder and 1 lung fibrosis with non granulomatous mediastinal lymphadenopathies). Concerning the management, all patients received oral or intravenous glucocorticoids as first-line treatment and achieved complete response (CR) in 9 cases or and partial response (PR) in 1. Simultaneously intravenous immunoglobulins were used in 5 cases. Second-line treatment was necessary for 5 patients: four patients underwent splenectomy, with relapse in 3 cases and 4 patients were treated with rituximab with constant CR but relapse in 3 cases after a mean follow-up of 15 months for both treatmenst. Others second-line treatments included azathioprine (=3), hydroxychloroquine (=1) and vinka-alcaloids (=1). One patient with CLL benefited from alemtuzumab. Finally one patient was treated with TPO-ar for a severe corticoresistant ITP, relapsing after splenectomy and rituximab, firstly with romiplostim and secondly with eltrombopag, he maintains CR under eltrombopag, allowing oral glucocorticoids withdrawal. Mean follow-up was 5.2 years +/- 5.8. At end of follow-up, 80% of patients were in prolonged response included 6 in CR, 2 in PR. Three patients remain in CR off treatment. Four patients had died during follow-up, at mean age of 72 years +/- 15.8, from underlying lymphoid malignancies.

Summary and Conclusions: ES is rare chronic autoimmune disorder, with recurrent relapses and currently refractory to glucocorticoids. Splenectomy and rituximab seem to be effective as second-line therapies, but with frequent relapse. TPO-ar may be effective for refractory ES-related.

PB2012

INFLUENCE OF THE PROTEIN KINASES INHIBITOR MALEIMIDE DERIVATIVE ON BLOOD CELLS PARAMETERS OF RATS WITH 1,2-DIMETHYLHYDRAZINE-INDUCED COLON CARCINOGENESIS

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Background: Maleimide derivative (MI-1, 1-(4-Cl-benzyl)-3-Cl-4-(CF_3-phenylamino)-1H-pyrrole-2,5-dione synthesized in Taras Shevchenko National University of Kyiv, Ukraine) is a competitive inhibitor of PDK1, VEGF-R1,2,3, Src(h), Syk(h) and other protein kinases. MI-1 inhibits the proliferation of the tumor cells (HCT-116, SW-620 – Colon Cancer and others).

Aims: Histological studies revealed MI-1 decreases the number of colon tumours in rat with 1,2-dimethylhydrazine(DMH)-induced colon carcinogenesis. The aim of the study was evaluation of MI-1 effects in these rat on blood cells parameters.

Methods: The Wistar male rats were divided into 8 groups (8-15 rats per group): I, V (control) – was treated 0.1 ml saline subcutaneous once a week for 20 weeks and/or 0.1 ml sunflower oil *per os* daily for 20 and 26 weeks; II, VI – 20 mg/kg DMH dissolved in 0.1 ml saline subcutaneous once a week for 20 weeks; III, VII – 20 mg/kg DMH for 20 weeks and MI-1 *per os* daily at the dose of 0.027 mg/kg dissolved in 0.1 ml of the sunflower oil for 20 and 26 weeks; IV, VIII – 20 mg/kg DMH for 20 weeks and 2,7 mg/kg MI-1 for 20 and 26 weeks, respectively. The dose of 0.027 mg/kg corresponds to a concentration that caused 50% inhibition of tumor cells proliferation *in vitro*, and the dose of 2.7 mg/kg – 90%. The data were statistically processed using Kruskal-Wallis and Mann-Whitney U-test using SPSS 16.0 for Windows.

Results: Administration of MI-1 at the dose 2.7 mg/kg for 20 weeks to rat with DMH-induced carcinogenesis decreases in the number of reticulocytes (median (25;75 percentile) *0,19 (0,15;0,21);* p=0,006), and trend to increases in MCH (*18,02 (17,44;19,03)* p=0,074) and MCHC (*309,42 (292,38;318,27);* p=0,036) in erythrocytes *vs.* DMH group *(0,28 (0,24;0,39); 17,50 (17,00;17,96)*; *288,10 (284,71;303,73),* respectively). These parameters in group 2,7 mg/kg MI-1+DMH do not differ from the control group *(0,17 (0,15;0,19); 18,31 (17,95;18,45); 310,78 (306,25;316,18),* respectively*)* at the same time in DMH group reticulocytes is increased (p=0,0001), MCH (p=0.002) and MCHC (p=0.007) are decreased. At 26 weeks of DMH-induced carcinogenesis in DMH group hemoglobin concentration (*135,04 (127,16;138,33)* and erythrocytes count (*7,54 (7,46;7,69)*) trend to decrease (p=0,014 and p=0,048) *vs.* control group (*141,02 (135,14;144,32)**;** 7,89 (7,49;8,01),* respectively) and do not differ in 2,7 mg/kg MI-1+DMH group (*141,01 (132,85;146,21); 7,69 (7,46;7,82),* respectively). Administration of MI-1 at the dose 2.7 mg/kg for 26 weeks to rat with DMH-induced carcinogenesis decreases in the number of monocytes *(1,40 (0,95;2,50)* and platelets (*646,32 (575,23;700,50)*) that do not differ from the control group (1,23 (0,94;1,68); *629,34 (590,19;711,48),* respectively) at the same time are increased in DMH group (*1,97 (1,52;2,58)* p=0.008; (*783,90 (687,64;922,27),* p=0.006, respectively).

Summary and Conclusions: MI-1 prevents anemia in rat with DMH-induced colon carcinogenesis, as evidenced by a decrease in the number of reticulocytes and restore mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in erythrocytes. MI-1 normalizes the number of monocytes and platelets in the blood after 26 weeks of DMH-induced carcinogenesis, reduces the involvement of these cells in the tumors progression. Reduction of the monocytosis and thrombocytosis may be mediated by: 1) decrease in the number and size of tumors and, consequently, the influence of their cytokines on hematopoietic tissue; 2) maleimide oppression of proliferation and differentiation of hematopoietic progenitor cells through inhibiting of VEGFR-kinase and of non-receptor PDK1-, Src- and Syk-kinases, that are involved in hematopoiesis and carcinogenesis.

PB2013

UNUSUAL ASSOCIATION OF CD8+ T-CELL LYMPHOCYTOSIS WITH INVASIVE THYMOMA

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Background: T-cell lymphocytosis in association with invasive thymoma is a rare finding. In the majority of previous cases, the lymphocytosis consisted of a mixed population of T cells expressing CD4, CD8, or neither marker and showed a negative result for T-cell receptor (TCR) gene rearrangement.

Aims: We report an unusual case of a patient with a previous history of invasive thymoma and myasthenia gravis who was subsequently found to have pure red cell anemia as well as peripheral blood and bone marrow CD8+ T-cell lymphocytosis.

Results: A 38-year-old woman presented to the emergency department with abdominal pain. She had received a diagnosis of invasive thymoma and myasthenia gravis 9 years prior. The thymoma was treated surgically and followed by chemotherapy and radiation therapy until 1 year prior. A computed tomography scan of the abdomen and chest showed multiple thymoma metastasis involving the lung, pleura, pericardium, liver, both ovaries, and peritoneal seeding. The patient's CBC showed the following: white blood cell count, 44,400/μL (58.9% neutrophils, 34.9% lymphocytes); hemoglobin, 9.5 g/dL; and platelet count, 170,000/μL. Progressive anemia (6.5 g/dL) and persistent lymphocytosis (7,700–31,000/μL) were noted over 3 months. Analysis of a peripheral blood smear showed an increased number of small lymphocytes with dense chromatin, no nucleoli, and scanty agranular cytoplasm. Flow cytometry of the peripheral blood revealed that 78.4% of lymphocytes were T cells with CD3+ and CD8+. The ratio of CD4+/CD8+ cells was 0.13. A bone marrow study was performed, which showed hypocellular marrow with erythroid aplasia and diffuse interstitial infiltration of small lymphocytes. Immunophenotyping by flow cytometry showed proliferation of T cells with expression of CD2, CD3, CD5, CD7, and CD8. The result of TCR gene rearrangement was negative.

Summary and Conclusions: In most reported cases of T-cell lymphocytosis associated with thymoma, lymphocytes consisted of a mixed mature population of T cells, and patients showed only peripheral blood lymphocytosis with absence of extensive bone marrow lymphocytosis. Chronic peripheral T-cell lymphocytosis with bone marrow lymphocytosis generally reflects the proliferation of a neoplastic T-cell clone. The differential diagnosis between reactive lymphocytosis and lymphoma/leukemia is of chief importance in patients with thymoma. Flow cytometric studies and TCR gene rearrangement analysis can establish the monoclonality or polyclonality of lymphocytes and thereby provide the appropriate diagnosis. Our case was diagnosed as T-cell lymphocytosis associated with thymoma, which was confirmed by a combination of lymphocyte morphology, immunophenotyping, and TCR gene rearrangement.

PB2014

ANALYSIS OF CHIMERISM BY QUANTITATIVE REAL-TIME PCR IN PATIENTS RECEIVING ALLOGENEIC STEM CELL TRANSPLANTATION FOR NON MALIGNANT DISEASES

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is a procedure to reestablish hematopoietic function in patients whose bone marrow or immune system is damaged or defective, constituting a therapeutic option not only in hematological malignancies but also in non-neoplastic diseases. Chimerism monitoring has become an important tool for assessment and follow up in these patients. New techniques for analysis of hematopoietic chimerism by quantitative real-time PCR (qrt-PCR) amplification of null alleles or insertion/deletion polymorphisms (indels), can detect up to 0.01% of host cells in the post- HSCT period.

Aims: To evaluate the sensitivity of the technique and the relationship between hematopoietic chimerism and the evolution of non-malignant diseases in the post- HSCT period

Methods: We studied 8 patients diagnosed with nonmalignant disorders (1 familial hemophagocytic lymphohistiocytosis, 2 severe aplastic anemias, 1 osteopetrosis, 1 adrenoleukodystrophy, 1 Chediak-Higashi syndrome and 2 immunodeficiencies) diagnosed between January 2007 and 2012 at our center. The median age at diagnosis was 3.1 years (r, 1-9 years) . Matched unrelated donors were used in 7 (87%) cases and 100% (8) were HLA identical. 7 patients

(88%) were infused with bone marrow (BM) and 1 (2%) with cord blood . The conditioning regimens used were 3 busulfan-cyclophosphamide (BUCY), 4 fludarabine-melphalan + antithymocyte globulin (ATG) and 1 fludarabine + cyclophosphamide + Alemtuzumab . Chimerism was determined in DNA extracted from whole peripheral blood samples on days +21, +60, +90, +120, +150 and +180 post- HSCT, by quantitative real-time PCR (qrt-PCR) amplification of insertion/deletion polymorphisms. Qualitative variables were analyzed using chi -square and non-parametric tests were used for quantitative variables.

Results: The median time to neutrophil recovery was 15 days (r, 11-29 days) and platelet recovery, 25 days (r, 15-44 days). 25% (2) developed acute GVHD grade II -IV. No patients developed chronic GVHD. Primary graft failure was observed in 1 patient (12%) and 12% (1) presented secondary graft failure. The median recipient chimerism in patients with acute GVHD was 3.5% at day +21, 1.15% and 1.35 at days +60 and +90, while those without GVHD presented 23%, 18% and 16% on days +21, +60 and +90, respectively. Recipient chimerism was significantly associated with implant failure and subsequent recurrence of underlying disease, showing lower levels of chimerism in those who maintained the graft *versus* those that did not. Mortality was 37% (3) 2 of infectious cause and 1 GVHD.

Summary and Conclusions: New qrt-PCR techniques have proven to be a valuable diagnostic tool for monitoring residual host hematopoiesis in hematological malignancies, however there are no studies that validate its usefulness in non-malignant diseases. In our experience we find that this technique could be used for monitoring these patients with adequate sensitivity to predict implant failure and relapse. However we believe multicenter analysis with larger series are necessary to validate theses results.

Platelets disorders

PB2015

A COLLECTIVE DESCRIPTIVE STUDY OF MANAGEMENT OF IMMUNE THROMBOCYTOPENIC PURPURA (ITP) IN THREE HOSPITALS

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Background: Adult idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder caused by antiplatelet autoantibodies that cause platelet destruction by the reticuloendothelial system. The disease has been well-documented in Sri Lanka and the rest of the south East Asia. Since the disease itself gives rise to minimum morbidity and mortality, we often find that the patient suffers from complications of overtreatment rather than the disease itself. Therefore it is vital to have an understanding of "when to treat the patient" which is mostly decided on an individualized patient basis. Countries where expensive treatments such as rituximab and thrombopoietine receptor agonists are not a freely available option, management has to be optimized at an "individual basis". We studied 72 patients with chronic ITP followed up over a minimum period of 2yr to 8yr in three tertiary care hospitals in Sri Lanka. We looked at the age groups and gender, modes of presentation and time taken for response to initial therapy and the current options of therapy with their side effects. Our study evaluates the relationship between the severity of the thrombocytopenia and the incidence of bleeding and thereby to come to a consensus on the best approach to management options.

Aims: To study the characteristics of immune thrombocytopenic purpura(ITP), response and side effects of standard therapy, other possible options of management in three centers in Sri Lanka.

Methods: Retrospective data was collected from 72 patients with diagnosed ITP and followed up for a minimum of 2 years from January 2007, at three tertiary care hospitals in Sri Lanka. Department of Haematology, University of Sri Jayawardenapura. General Hospital, Ratnapura. General Hospital, Gampaha.

Results: The platelet count was below $10x10^9$/l in 40(55%) at presentation.Skin bleeding was the commonest presentation and seen in 37(51%). In 17(23.6%)it was an incidental finding. Out of the total 72 patients, 69 were considered for treatment either due to the degree of thrombocytopaenia (< $30x10^9$/l) or the presence of significant bleeding manifestations. All of them were treated with steroids initially and 67(97%) achieved a platelet count of more than 50 x 10^9/l during the first course.But only 12(17%) patients managed to retain this response and achieve a remission. Out of the patients who relapsed, 6 underwent splenectomy which led to a remission in 3(50%). In the 54(78%) who did not achieve remission, medical second line therapies were not effective. Out of the 69 patients who were treated only 15 (22%) went in to complete remission. Out of the patients who were considered for treatment, 11(16%) had platelets counts less than $30x10^9$/l but were asymptomatic and were managed by a 'watch and wait' policy. Only 1 (9%) of them had bleeding episodes.

Summary and Conclusions: Our study revealed that majority of patients was middle aged females with muco cutaneous bleeding. Most of the study subjects continued to have low counts througout the disease and remained corticosteroid dependent which caused significant long term side effects due to treatment. ITP has a very tumultuous clinical course and assessing each patient and tailoring the management on an individual basis is important. In this study16% of patients with a platelet count below 30 x 10^9/l were safely managed without treatment.

PB2016

PLATELET KINETIC EARLY NORMALIZATION IS A STRONG PREDICTOR OF SUBSTAINED RESPONSE AFTER DISCONTINUATION OF TREATMENT WITH ROMIPLOSTIM IN IMMUNE THROMBOCYTOPENIC PATIENTS.4 YEARS FOLLOW-UP

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Background: Background: (TPO)-receptor agonists (romiplostim and eltrombopag) are new therapeutic modalities in the treatment of ITP. Romiplostim treatment was associated with a number of benefits compared with the standard of care in non splenectomized ITP patients: higher platelet response rate, lower rates of treatment failure and splenectomy, fewer bleeding events, and fewer blood transfusions. As reported in different case series only 15% of patients achieve a durable response after treatment discontinuation

Furthermore, there are no criteria to identify patients potentially cured and that could stop therapy. For all these reasons we have carried out a study of platelet kinetics in all patients treated with TPO- receptor agonists and we have identified a subset who achieved a normal platelet kinetics and that is no longer relapsed 4 years after discontinuation of the drug.

Aims: To validate the study of platelet kinetics as a predictor of response to therapy with romiplostim and if the its early normalization can identify a subset of patients who may discontinue the drug .

Methods: A total of 18 adult patients, female (63%),median (range) age 55 (31 – 78) years, median (range) baseline platelet count 19 (3 – 32) x 109/L.,median of 4 (1 – 7) prior ITP therapies, received romiplostim administered once weekly sc, with dose adjustments to maintain platelet counts in the target range of 50–150x109/L. The median time since ITP diagnosis was 6,8 years (range, 0.6–12.8 years) and 10% had undergone a splenectomy. Patients received romiplostim for a median of 98 weeks (range, 18–104); taking the average weekly dose of all patients, the median was 4 mcg/kg. Home administration was started by 16% of patients (3/18) but 2/3 patients discontinued home administration and resumed weekly outpatient injection. All patients achieved a platelet count 50x10^9/L.

Results: Results: 3 out of 18 experienced thrombocytosis and rebound thrombocytopenia. A PKS with (111)In oxine-labeled autologous platelets was performed in all patients failing steroid treatment, before romiplostim was started. A gamma function was used for the calculation of platelet mean life span (MLS) that was greatly reduced in 100% of patients.3 patients, who had achieved early a stable platelet count 150x109/L. despite the discontinuation of romiplostim maintain normal platelet count after 48 months of follow-up. Stricking a second PKS six months after starting the treatment showed in these subset, a normal platelet half-life with normal uptake on the spleen and liver.

Summary and Conclusions: In our opinion early PKS normalization is a strong predictor of sustained responses after discontinuing romiplostim without additional therapy and may easily detect patients cured by the drug.

PB2017

THROMBOEMBOLISM RISK IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Patients with immune thrombocytopenia (ITP) may be at increased risk of thromboembolism because of the abnormal platelet function.

Aims: This study aimed to characterize risk of thromboembolic events in ITP patients.

Methods: We retrospectively evaluated 239 patients (107 men, 132 women; median age: 61 years) diagnosed between January 1997 and August 2011. Every patient received steroid treatment according to the platelet count and the extent of bleeding. Logistic regression analysis was used to identify risk factors associated with the development of thromboembolic event after ITP diagnosis.

Results: Sixty-four (26.7%) patients had operation after ITP diagnosis. Among those who had operation, most of them were treatment-responsive (complete response 63.7%, response 18.9%) and had manageable postoperative blood loss (<250ml) (89.1%). Ten patients (4.2%) developed a thromboembolic event after diagnosis. Multivariate analysis revealed that operation (HR 9.998, 95% CI 1.999~50.004, p=0.005) and higher platelet count (>30.0 ×10^9/l) at diagnosis (HR 4.636, 95%CI 1.157~18.574, p=0.03) were independent risk factor for thromboembolism (Table 1).

Table 1. Demographic and clinical characteristics of patients with immune thrombocytopenia.

Summary and Conclusions: ITP patients who had operation have increased risk of thromboembolism, although clinical bleeding was minimal and manageable. Patient who had higher platelet count (>30.0 ×10^9/l) at diagnosis also have increased risk of having thromboembolic event.

PB2018

THROMBOPOIETIN RECEPTOR AGONISTS (TPO-RA) IN REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA (ITP): ABSENCE OF CROSS-RESISTANCE BETWEEN ELTROMBOPAG AND ROMIPLOSTIM IN TWO PATIENTS.

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Background: The recent availability in clinical practice of thrombopoietin (TPO) receptor agonists (TPO-RA), such as eltrombopag and romiplostim, which are effective in increasing the platelet count by stimulating TPO receptor on progenitors of megakaryocytic and inducing their proliferation and maturation with relatively low toxicity, has lead to important advances in the therapeutic management of ITP.

Aims: The potential absence of cross-resistance between the two drugs, likely due to their different mechanisms of action, is illustrated by the following description of two cases, recently observed by us, with therapeutic response symmetrically opposite to these thrombomimetic drugs, having these two patients failed previous treatment with one or the other agents.

Results: The first case regarded a 54-year old female (Patient A, Figure 1) with an ITP which was refractory to steroids and intravenous immunoglobulins (IVIG); the patient was also unresponsive to romiplostim, which dosage was appropriately escalated and maintained at the maximum weekly dose of 10 mcg/kg for about 4 weeks. So that, eltrombopag (50 mg/day) was started (Figure 1). A prompt platelet response (platelet count: 41 × 10^9/L) was observed 1 week after the start of treatment (Figure 1). A complete response was achieved after other 4 weeks of treatment, being the patient on 50 mg/day of eltrombopag without any adverse events. However, at that time, after four additional weeks of eltombopag, the platelet count exceeded 350 × 10^9/L. Therefore, eltrombopag was withdrawn. Since then, 19 months after treatment discontinuation (Figure 1), her platelet count was maintained between 189 and 367 × 10^9/L; the last platelet count performed in February 2014 was of 312 × 10^9/L. The second case (Patient B, Figure 1) was a 73 years old woman with a 5-years history of steroid-refractory ITP and occasional IVIG requirement. She refused splenectomy so that eltombopag was started at the dose of 25 mg/day and then escalated to 50 mg/day and finally 75 mg/day; the last dose was maintained for 4 weeks without any benefit (Figure 1). Therefore, she was started on gradually increasing subcutaneously doses (from 1, 3, 7 to 10 mcg/kg/week) of romiplostim for a total of eight weeks, achieving platelet counts higher than 20 and 80 x 10^9/L after 4 and 8 weeks respectively. To date, about 29 months after the first dose (Figure 1), a normal platelet count is maintained by romiplostim without any side effects.

Figure 1.

Summary and Conclusions: In conclusion, we suggest that further confirmation of a distinct pattern of response and the absence of cross-resistance between these two thrombomimetic drugs should be explored by prospective studies by directly comparing the two TPO-RAs.

PB2019

EFFICACY AND SAFETY OF ROMIPLOSTIM FOR TREATMENT OF INMUNE THROMBOCITOPENIA (ITP): EXPERIENCE OF A SINGLE CENTER

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Background: ITP is an autoimmune disease characterized by increased platelet destruction and suboptimal platelet production. Thrombopoietin receptor agonists (TRA) romiplostim and eltrombopag have changed in the last years the treatment of ITP. Both can increase platelet production without acting on the immune system, and they are approved for treatment of chronic ITP in adults.
Aims: To describe the efficacy and safety of romiplostim use for ITP in our center.
Methods: We retrospectively analyzed a total of 20 adult patients at our institution, who were treated with romiplostim for ITP. Clinical and biological parameters were recorded as well as treatment response and tolerability.
Results: Our series comprises 9 men and 11 women with a mean age of 59 years (range 19-85). All the patients received steroids as the first line treatment. After that, 65% received rituximab, 65% received anti-D immunoglobulin and in only 25% splenectomy was performed before starting romiplostim. None of them had been previously treated with eltrombopag. The mean number of previous lines before romiplostim was 2.7 (range 1-4), and only 3 patients (15%) received romiplostim as second line. Romiplostim was effective, increasing platelet count over 50.000/mm^3, in 75% (15/20 cases) of our adult ITP patients. However, only six of these patients (30%) are still on treatment. For the remaining 9 patients in whom romiplostim was successful, the evolution was as follows: four patients achieved a resolution of ITP and they are currently without treatment; two patients stopped the treatment after 4 and 43 months, because of poor adherence; finally, three patients died while they were on treatment with normal platelet count. The average dose among patients in which romiplostim was effective was 2.9 µg/kg/week. In five patients (25%) romiplostim was not effective, and the maximum dose they received ranged from 5 to 10 µg/kg/week before stopping the treatment. Two of these patients died while they were on treatment without response. Change of TRA from romiplostim to eltrombopag took place in 5 patients due to three circumstances: lack of efficacy (2 cases), platelet count fluctuations (1 case), and poor adherence (2 cases). In this setting, eltrombopag was effective in 4 of these 5 cases (80%). We have analyzed if age, duration of the disease, number of previous lines of therapy, the previous use of rituximab or anti-D immunoglobulin, or previous splenectomy, may be associated with efficacy of romiplostim. We found statistically significant association only with the number of prior lines, with a higher probability of efficacy in patients with fewer prior lines (p=0.033). In terms of drug tolerability, our results were: no adverse effect in 12 patients (60%); a mild adverse event (grade <2) which did not require decrease or suspension of any dose, mostly headache, joint and muscle pain, in 5 cases (25%); an adverse event grade 3 (headache) requiring dose reduction was observed in two patients (10%); finally, only one patient (5%) presented a severe adverse event, grade 4 (deep venous thrombosis and pulmonary embolism) which required temporary suspension of the treatment. Five deaths occurred on treatment, but none of them seems to be related to romiplostim; three of them (60%) had an infectious etiology, in which previous immunosuppressive treatments may have played some role.
Summary and Conclusions: In our series, romiplostim was effective in 75% of ITP adult patients, including ITP resolution in 20% of cases. The number of prior lines of therapy seems to be related to the efficacy of treatment, achieving a better response those patients with fewer prior lines. In our experience, tolerability to romiplostin is quite good and only a dose reduction or a temporary suspension of the drug is occasionally required.

PB2020

RITUXIMAB IN HAEMATOLOGICAL AUTOIMMUNE DISEASES: A SINGLE CENTRE EXPERIENCE

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Background: Rituximab is a anti-CD20 monoclonal antibody broadly used in the treatment of lymphomas CD20+ that have shown efficacy also in the treatment of autoimmune disorders.
Aims: We report the outcome results of 21 consecutive adult patients (14 females and 7 males) with autoimmune diseases treated with Rituximab in our Hematology Department. Sixteen of them had primary immune thrombocytemia (pITP), 1 ITP secondary to HCV infection, 1 cyclic ITP, 2 suffered from autoimmune hemolytic anemia (AHA), 1 from Evans syndrome (ES). All the patients received Rituximab after failure of at least one previous line of therapy (always including also steroids) or after relapse. Tirthteen had a standard dose (SD) of Rituximab (375 mg/m^2 weekly for four administrations) and 8 low dose (LD) (100 mg flat dose weekly for four administrations); all patients but 2 completed the planned 4 doses of drug: 1 of these, a pITP patient, had 3 administrations and the other (the ES patient) had only 2 doses because of sudden hearing loss considered probably related to the drug.
During the therapy with monoclonal antibody all of our patients continued low doses steroids that stopped when the response occurred.The median age at infusion of Rituximab was 55 years (range 26-80 y).The median time between diagnosis and Rituximab therapy was 43 months (range 1-345).
Results: The overall response was 61.9% (13/21: 11 females (78%) and 2 males (28%)), particularly 9 out of 16 pITP patients had a complete response (CR) (56%), the HCV associated ITP had a partial response (PR) (100%), the 2 AHA had a CR as the Evans syndrome patient (100%). Only 2 of the responders relapsed (15%), they had both pITP (relapse rate: 22%) and their recurrence of disease occurred after 6 months in one case and 71 months in the other. Tirthteen patients were less than 60 years old (62%) and 8 more than 60 y (38%) at the time of anti-CD20 immunotherapy; in the first age group 6 responded (46%), in the older one 7 (87%) (p 0.058). Considering only pITP: 4 (25%) received Rituximab in the first year of disease, 12 (75%) after 1 y and between those 9 (56%) after 5 y; the responses were 75%, 37%, 44% respectively (p 0.38). The median time to response was 1.4 months (range: 5 days-8 months) and its median duration 17.6 months (1-70). Nine of 11 patients still in response had a follow up (FU) shorter than 2 y (81%), 2 (19%) had a longer FU time. Considering the dose, 7 of the recipients of SD responded (54%) , so did 6 of those had LD (75%). P(0.058). Considering sex of responders 11 were females and 2 males (p 0.026). The side effects were 4, reported in 3 patients: we observed a grade 1 neutropenia, a grade 1 abnormal liver function test , 1 reversible hearing loss and 1 relapse of a sarcoma.
Summary and Conclusions: Our experience confirms that Rituximab is an effective and safe therapy option in the management of autoimmune diseases and, although the small number of people analyzed could represent a limitation, does show better response in females but there was not significant differences according the age, the dose of the drug and the duration of the disease.

PB2021

A SURVEY OF THE ETIOLOGY OF IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Immune Thrombocytopenia Purpura (ITP) is an autoimmune disorder characterized by accelerated platelet destruction and suboptimal platelet production. Patients with ITP have variable disease progression and response to treatment indicating that ITP has a heterogeneous etiology. Factors contributing to this heterogeneity are not well understood.
Aims: To determine patterns relating socioeconomic, environmental and lifestyle factors associated with treatment response in ITP patients.
Methods: A self-reported prospective questionnaire-based study was created based on literature searches and 45 ITP patient interviews. The survey was reviewed by Platelet Disorder Support Association (PDSA) members and hematologists, revised and then administered to ITP patients at Weill Cornell Medical College-New York Presbyterian Hospital. The survey comprising of 83 multi-step questions relating to socioeconomic, environmental and other patient characterization factors was given to 110 patients or the patients' parents if the patient was <18 years old. 95 subjects were analyzed for the treatment responses (15 did not receive treatment) and all 110 subjects for overall patient characteristics. Associations between responses to rituximab and thrombopoietic (TPO) agents (Eltrombopag and/or Romiplostim), and potential factors influencing ITP were assessed using chi-square and fisher's exact test. All analyses were performed using SAS version 9.3 (Cary, NC).
Results: Patients were between 1 - 89 years old (median age: 37 years). Of 110 patients, 19 had ITP for<6 months, 80 had ITP for > 6 months and 11 did not specify duration. Rituximab treatment outcomes: 63 subjects received Rituximab, of which 21 had complete response (CR), 10 partial response (PR), and 32 no response (NR). Factors significantly associated with poor response to Rituximab included: more stress prior to diagnosis, specifically having a relative pass away (p=0.001 for CR/PR *vs.* NR and p=0.007 for CR *vs.* NR); easy bruising/increased petechiae (p=0.027 for CR *vs.* NR) and exercise infrequency prior to diagnosis (p=0.025 for CR *vs.* NR). TPO agent treatment outcomes: 56 subjects received TPO agents, of which 45 had CR, 8 PR, and 3 NR. Factors significantly associated with poor response to TPO agents included subjects experiencing fatigue prior to diagnosis (P=0.02 for CR *vs.* PR *vs.* NR); frequent drinking of cranberry juice prior to diagnosis (p=0.005 for CR *vs.* PR *vs.* NR) and having diabetes (P=0.007 for CR *vs.* PR *vs.* NR).
Summary and Conclusions: Preliminary analysis in this pilot study indicates potential associations between unexpected epidemiologic factors and treatment response. Subjects who did not experience stress from a relative passing away,

exercised more prior to the diagnosis of ITP, and who did not have easy bruising/increased petechiae responded better to rituximab treatment. Subjects who did not experience fatigue, did not frequently drink cranberry juice prior to the diagnosis of ITP, and those who did not have diabetes appeared to respond better to TPO agents. Surprisingly, these results did not identify environmental, chemical or toxic exposures to be related to response to treatment (outcome from). IVIG and steroid responses were too historical to be analyzable and too few patients had had a splenectomy. Based on the initial analysis, an updated, simplified version of the questionnaire will be used in a larger study to identify sub-groups of patients in whom onset of ITP and other characteristics can be related to outcomes including but not limited to treatment response.

PB2022

PREDICTORS OF PLATELETS COUNT IN PEDIATRIC PATIENTS WITH CONGENITAL CYANOTIC HEART DISEASE

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Background: Congenital heart defects are the commonest non-infectious causes of mortality in newborns in the western world. Erythrocytosis, thrombocytopenia, platelet function defects, coagulation factors deficiencies are the main hematologic disorders described in cyanotic congenital heart disease.
Aims: to assess the immature platelets fraction in pediatric patients with congenital heart disease and thrombocytopenia compared to non thrombocytopenic , detecting the risk factors that may predispose patients with congenital heart disease to low platelet count
Methods: The study included 60 pediatric patients with congenital cyanotic heart diseases attending at pre-catheter visit at Ain-Shams University-cardiology department between October 2012 and July 2013.Patients were subjected to history taking including data for age , gender , type of congenital cyanotic heart disease, age of onset of cyanosis , cyanotic episodes and their frequencies , drugs and family history. Physical examination included anthropometric measures, oxygen saturation by pulse-oximetry and vital signs. Laboratory investigation included *complete blood count (CBC)* using Coulter Counter GEN-S (Coulter Corporation, USA), *C-reactive protein (CRP)* by latex agglutination test using spectrum kits latex (Fortress, England), reticulated platelet count as a marker of platelet production using Sysmex XE-2100 (Sysmex, Japan), *Prothrombin Time* (*PT*, Pro Time) test, *Partial thromboplastin time* (*PTT*) and D-dimer for patients with thrombocytopenia.
Results: Our patients had a median age of 18.5 months and interquartile range of 51 months 30, (65.2%) males and 16 (34.8%) females. Nine patients had transposition of great arteries, 30 patients had tetralogy of Fallot, 6 patients had double outlet right ventricle and one of them had single ventricle. There was no significant difference between patients with thrombocytopenia and patients with normal platelet count as regard demographic data or clinical presentation, type of congenital cyanotic heart disease or C-reactive protein status. Immature platelets fraction (IPF) was higher in patients with thrombocytopenia 14.15±5.2 compared to non-thrombocytopenic 6.68± 3.39(P=0.003), and PT was longer (17.08±3.37, 13.99±1.41 sec, P= 0.01 respectively) with no difference in PTT between both groups (P=0.272). A significant positive correlation was found between platelets count with D-Dimer and a significant negative correlation with PT and IPF. A significant positive correlation was also found between IPF and hemoglobin, RBC count , Hematocrit, PT and reticulocytic count, and a significant negative correlation between IPF and MCHC, D Dimer, platelets count, reticulocyte hemoglobin content and platelets large fraction. Patients with different types of CCHD had no significant difference as regard platelets count. There was significant higher RDW and D Dimer in patients with TOF. There was significant higher mean platelet volume and PT in patients with CRP positive compared to those with CRP negative.
Summary and Conclusions: thrombocytopenia was detected in 13% of the screened pediatric patients with congenital cyanotic heart disease. Immature platelets fraction as a marker for platelets production; together with D-dimer were elevated in patients with thrombocytopenia suggesting peripheral platelets destruction as an underlying mechanism for thrombocytopenia. RBC count ,hemoglobin level, hematocrit and reticulocyte hemoglobin content as a marker for iron deficiency were not significant determinants for platelet count.

PB2023

THROMBOTIC THROMBOCYTOPENIC PURPURA AND SJOGREN SYNDROME, A RARE ASSOCIATION

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and serious disease that is potencially lethal without prompt recognition and treatment. The classic pentad of symptoms consists of microangiopathic hemolytic anemia, thrombocytopenia, renal impairment, central nervous system involvement and fever. However, virtually any system may be affected. Sjögren's syndrome (SS) is a common autoimmune disease with exocrine gland damage, with special salivary and lacrimal envolvement. TTP is frequently associated with connective tissue diseases (1-6% in general population), but association with Sjogren syndrome (SS) is rare. The etiology remains unclear in about 37% of the cases of TTP and can be associated with autoimmune diseases in 13% of the cases. The association of SS and PTT is a rare condition, with high death rate, only 14 cases reported to date, at our knowledge. Measuring of ADAMTS13 activity is helpful for a correct diagnosis of PTT which has a specific treatment, differentiating it from another thrombotic microangiopathies.
Aims: Report of two cases of TTP in context of a SS, with different clinical outcomes.
Methods: Review of clinical data about two patient admitted in 2013 in our unit with diagnosis of TTP associated with SS. Abnormal levels of ADAMTS13 activity were considered under 40% and abnormal titer of ADAMTS13 antibody above 15 U/l, according to our lab.
Results: Case 1: A 40 year old healthy woman presented to the emergency service (ES) with subit aphasia, hemiparesia and confusion. Images studies were normal. Laboratory data showed Hb 10,1 g/dL, platelets 6 G/L, schystocyte on blood smear, LDH 709 U/l and indirect bilirrubin 1,4 mg/dL. Creatinine and hemodinamic status were normal. Patient started immediately plasma ex-change (PEX), after collecting blood samples for study of an eventual TTP. Extreme low activity (0%) of ADAMTS13 and high level of anti-ADAMTS13 confirmed the diagnosis of acquired TTP. Patient was furtherly studied and autoimmune panel showed strong positivity for anti-SSA60 and anti-SSB what was suggestive of SS, besides lacking classic symptoms of this autoimmune disease. After 6 sessions of daily PEX and corticotherapy, patient was in complete response (>48h of platelets > 150 G/L and no symptoms) and remains stable. Case 2: A 59 year old woman, with know history of SS and already under corticotherapy (5mg prednisolone daily) presented to the ES with epistaxis, gingivorrhagia and mild abdominal pain. Blood analytics showed Hb 12,1 g/dL, platelets 6 G/L, creatinine 1,49 mg/dL (acute renal insufficiency), LDH 1683 U/L, and indirect bilirrubin of 3,2 mg/dL. No schizocytes reported at presentation. Corticotherapy was intensified, but clinical situation deteriorated with appearance of paresthesia and general muscle weakness. Blood smear showed schizocytes and the possible diagnosis of TTP was assumed, with beggining of PEX. Patient became hemodynamic unstable, with neurologic aggravation and elevation of cardiac enzymes. The situation had a quick and inexorable evolution to death. Low levels of ADAMTS13 activity (0%) and very high antibody titer (>99 U/L) confirmed the diagnosis of acquired PTT.
Summary and Conclusions: Either TTP or SS are rare entities. Association of both is far rarer. The combination of SS and TTP has particular high death rate, so high suspicion is essential to quick start of therapy.

PB2024

THE ROLE OF THROMBOPOIETIN RECEPTOR AGONISTS (TPO-R) IN THE TREATMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA OF ADULT-THE EXPERIENCE OF FUNDENI CLINIC OF HEMATOLOGY

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Background: TPO-R agonists represent an available therapeutical option for those patients with chronic immune thrombocytopenia (ITP) refractory or with contraindication for splenectomy. The two agents are : Eltrombopag(Revolade) with oral ,daily administration and Romiplostim(N'Plate), with subcutaneously, weekly administration. Clinical trials showed their efficacy in order to maintain a safe number of platelets ~50000/mmc ,prevent fatal bleeding and offer to the patients a good quality of life with few adverse reactions.
Aims: To evaluate indications for TPO-R treatment of patients diagnosed with ITP and clinical evolution after the treatment.
Methods: a clinical epidemiological retrospective study of 33 patients with ITP, treated with TPO-R agonists in Fundeni Clinic of Hematology between 2011-2013.
Results: therapeutical indications were – third line therapy (failure after splenectomy and corticosteroids):6 patients; bridging through splenectomy -11 patients; second line therapy- 13 patients(4 patients with hepatitis C and B active infection, who undergone concomitant antiviral therapy, maintaining normal level of platelets).The age was variable, between 21-68 years old. 27 patients received Eltrombopag and 9 patients Romiplostim. 2 patients received both Eltrombopag and Romiplostim. 29 patients responded with platelets above 50000/mmc,9 of them had a sustained response,maintained after stopped medication :4 after Romiplostim therapy and 5 after Revolade treatment . 2 patients were lost from evidence. During treatment were reported as adverse reactions headache, artralgias and one female patient had a transient episode of hepatocytolysis and cholestasis, but without clinical evidence.
Summary and Conclusions: the TPO-R agonists are a good option for the treatment of ITP refractory patients , for splenectomy bridging or for those with antiHCV/HVB therapy. The terapy is safe, with minimum adverse events. Because of the financial reasons the period of the treatment is limited.

PB2025

NOVEL MUTATIONS IN THE GPIIB GENE IN TURKİSH CHILDREN WITH GLANZMANN THROMBASTHENIA

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Background: Glanzmann thrombasthenia (GT) is an inherited disorder of platelet aggregation, characterized by qualitative and quantitative defect on platelet αIIb-β3 integrin (GpIIb/IIIa), resulting in lifelong bleeding tendency due to defective platelet plug formation.
Aims: In this study, we examined 20 patients with Glanzmann thrombasthenia for clinical findings and molecular genetic analysis to determine whether there was any mutation in the GPIIb gene, and a correlation between clinical phenotype and genotype. This is the first genetic study conducted in Turkey about GT.
Methods: There were twelve females and eight males with a median age of 15.25 years. The most common bleeding type was epistaxis (85%). Life threatening bleedings were seen in five patients.
Results: Seven (35%) patients showed various mutations (five novel and two previously described mutations) in the GPIIb gene. Novel mutations including 9079 T>A (p. S416G), 9092 G>T (p. V420L) and 9179 T>A (p. P448T) were located in exon 13. The others including 4466 G>T (p.G159V) and 11453A>G (p.T646A) were located in exon 4 and exon 19, respectively. Previously described mutations including 4420 T>G (p.V147G) and 9607 T>G were located in exon 4 and exon 13, respectively. No correlation was found between clinical phenotype and genotype.
Summary and Conclusions: In conclusion, our findings showed that exon 13 was an important region for platelet aggregation; therefore, we suggest that 13 patients without diagnosis of mutation may have mutations in β3 integrin gene. Furthermore, molecular genetic analysis should be performed in these patients.

PB2026

THROMBOPOIETIN LEVEL IN HCV EGYPTIAN PATIENTS

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Background: Thrombocytopenia is a common finding in patients with chronic hepatitis. A variety of pathogenic mechanisms are reported to be implicated in thrombocytopenia related to chronic HCV infection.
Aims: The aim of the present study is to evaluate the role of thrombopoitin (TPO) in pathogenesis of thrombocytopenia in HCV infected patients and to demonstrate if there is a correlation between TPO level and viral load, degree of inflammation and degree of fibrosis.
Methods: This study was carried out on a group of 30 HCV infected patients recruited from Kasr El-Eini hospital. Liver function tests, polymerase chain reaction (PCR) for HCV, complete blood counts, serum TPO levels and abdominal ultrasonography were done to all case. Patients were classified according to their viral load into 3 groups *Group I* (6 cases) with low viral load *Group II* (14 cases) with moderate viral load and *Group III* (10 cases) with high viral load.
Results: showed that platelets count and serum TPO are significantly higher in patients with mild viremia compared with groups with moderate or high viremia . No statistically significant differences were found between group II and III. Liver biopsies were done for 17 patients. It was found that the platelets count is significantly higher in patients with mild inflammation (*Group A)* when compared with patients with moderate inflammation (*Group B)*. Again TPO was found to be significantly higher in *Group A* when compared with *Group B.* When those patients were classified according to degree of fibrosis, the platelet count showed significant relationship with degree of fibrosis being higher in patients with less fibrosis. A positive correlation between platelets counts and serum TPO was found.
Summary and Conclusions: several contributing factors may lead to HCV related thrombocytopenia , of which TPO level which is affected by viral load , grade of inflammation , stage of fibrosis ,being the higher viral load and the more inflammation and fibrosis ,the lower level of TPO and accordingly the lower platelets counts.

PB2027

BONE MARROW EXAMINATION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA: IS THERE ANYTHING DIFFERENT IN OLDER PATIENTS?

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Background: Immune thrombocytopenia (ITP) is a disorder characterized by immune-mediated accelerated platelet destruction and supressed platelet production. In the bone marrow examinations of patients with ITP, some investigators found megakaryocyte numbers to be increased while others have found them to be normal. Similarly,abnormal megakaryocyte morphology including diminished granularity and decreased nuclear ploidy among other findings were observed by some investigators but not by others. Although recent guidelines recommend against bone marrow examinations in typical ITP patients the recent introduction of thrombopoietin receptor agonists as an effective treatment for ITP has refocused attention on abnormalities of bone marrow megakaryocytes.
Aims: In this sudy, we retrospectively analysed the bone marrow aspiration, flow cytometry-CD45 sidescatter (SSC) and biopsy results of our patients with ITP by dividing them into two groups according to age (<60 years and ≥60 years) and tried to determine the differences between two groups.
Methods: Ninety eight newly diagnosed ITP patients who were admitted between 2010-2013 and who had available bone marrow aspiration, CD45 SSC and biopsy results are included in the study. ITP was diagnosed according to American Society of Hematology criteria with a platelet count below $100x10^9$/L and no cytopenias except iron deficiency anemia. Since all patients with thrombocytopenia (<$100x10^9$/L) undergo bone marrow biopsy regardless of age in our clinical practice, patients are divided into two groups: <60 years old (group 1) and ≥60 years old (group 2). All bone marrow aspirates and biopsies were performed using the same techniqueand the same method of slide preparation. CD45 SSC results were recorded as percentages of normoblasts, granulocytes, lymphoctes, monocytes and myeloid/erythroid ratio. Bone marrow biopsies were evaluated by experienced pathologist. Length of the specimen, cellularity, presence of dysplasia or fibrosis with number, morphology and distributionof megakaryocytes were recorded. Informed consent was obtained from patients.
Results: In group 1 there were 49 patients. Mean age was 41.31±12.77 years, 31 (63.3%) female and 18 (36.7%) male. In group 2 there were 49 patients. Mean age was 70.78±7.88 years, 26 (53.1%) female and 23 (46.9%) male. All blood and bone marrow samples were obtained before starting therapy for ITP. Unfortunately, megakaryocyte numbers on bone marrow aspirates were not recorded in most patients so we could not comment on this point. However, flow cytometry results and bone marrow findings were not different between two groups.
Summary and Conclusions: In conclusion, we could find no difference between bone marrow examinations of young (<60 years) and older (≥60 years) patients with ITP and our study supports that biopsy should not be recommended in typical ITP patients as already mentioned in guidelines.

PB2028

IMMUNE THROMBOCYTOPENIA IN THE ELDERLY: CLINICAL COURSE AND TREATMENT

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Background: Immune thrombocytopenia (ITP), which is often diagnosed in the elderly, is a hematologic disorder characterized by thrombocytopenia induced by autoimmune mechanism.
Aims: In this study, we evaluated the clinical features, the risk of bleeding and the response to various treatment options in elderly ITP patients (age≥60 yr) at our center from 1980 to 2012.
Methods: A retrospective study for 210 ITP patients was performed in Department of Hematology, County Hospital, Timisoara, Romania. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 12 months prior to their most recent visit.
Results: The median age was 65 years with 61% women and 39% men. The median platelet count was 22×109/L (range 1-75×10^9/L) at diagnosis. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Prior to the observational period, 28% of patients had been splenectomized and the most reported treatment was corticosteroids. During the observational period, 65% of all patients were treated. The most frequent reasons given for treatment were platelet count (58%), followed by bleeding symptoms (49%). Corticosteroids represented 63% of treatments, followed by IVIg (20%), azathioprine (8%) and rituximab (3%). Splenectomies (4% of patients) and platelet transfusions (38% of patients) were performed during the observational period. For monitoring the platelet levels, 70% of patients visited their hematologist 1 to 6 times during the observation. Main reasons for a visit were a low platelet count (42% of visits) and bleeding (34% of visits).
In the evaluable 15 patients who died during follow up, eight deaths were considered. To be directly attributable to hemorrhage induced by thrombocytopenia.
Summary and Conclusions: There are more females in the ITP patients over 60 years of age. The risk of bleeding is quite high in elderly ITP patients, but life-threatening bleeding events rarely occur. This study provides the results of treatment practices in our country.

PB2029

CHANGES IN PLATELET AGGREGATION DURING PREGNANCY AND IMMEDIATE POSTNATAL PERIOD

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Background: Platelet dysfunction is implicated in uteroplacental disorders. During the early stages of gestation platelets have important roles in the process of placentation. Platelet function contributes to enhanced haemostasis at delivery. However, there is limited data on the changes of platelet function during normal pregnancy. Understanding physiological changes of platelet aggregation during different stages of pregnancy is helpful for better understanding of pathophysiology of abnormal placentation.
Aims: To assess platelet aggregation during three trimesters of pregnancy and immediate postnatal period in normal healthy women compared to control non-pregnant group.
Methods: Cross-sectional cohort study including a total of 46 women: 10 participants for each trimester, 10 postnatal cases and 6 control non-pregnant women. Case selection was based on specific inclusion criteria. 30mL of venous blood was obtained from each participant following consent. Light transmission aggregometry was performed with Dual channel Payton 600B aggregometer using six platelet aggregating agonist (epinephrine, adenosine triphosphate, collagen, ristocetin, arachidonic acid and U46619).
Results: Platelet aggregation in response to ADP and epinephrine was decreased in 4/10 and 3/10 women in the first trimester, respectively. A significant difference (p=0.0075) was detected in percentage aggregation at 3 minutes induced by epinephrine 2 μM in the first trimester group compared to non-pregnant controls (47% *vs* 82%). In addition, mean aggregation at 3 minutes induced by epinephrine 3μM was reduced in the first trimester (48% *vs* 82%) (p=0.0042). 6/10 and 4/10 women in the third trimester had abnormal aggregatory response to ADP 2 μM and ADP 3 μM respectively. A significant difference (p=0.0005) was detected in percentage aggregation at 3 minutes with ADP 2 μM in the third trimester compared to non-pregnant controls (45% *vs* 81%). One woman had abnormal aggregation response in the third trimester including prolonged lag time and reduced maximal aggregation with collagen, and reduced maximum aggregation with ristocetin 1.5 mg/ml, arachidonic acid and U46619.
Summary and Conclusions: Pregnancy is associated with reduced platelet reactivity in response to epinephrine during early pregnancy and an abnormal aggregation response to ADP in the third trimester. These changes may have important role in the process of placentation in early pregnancy as well as haemostasis at delivery.

PB2030

THE ANTIOXIDANT TREATMENT IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Oxidative stress (OS) plays a role in the pathophysiology of immune thrombocytopenia (ITP). Several studies revealed increased reactive oxygen species (ROS) levels and a decreased antioxidant capacity in ITP, suggesting a possible antioxidant mechanism of therapy in chronic ITP.
Aims: To evaluate the response of treatment in patients with ITP, with and without antioxidants.
Methods: We studied 24 patients with chronic immune thrombocytopenia hospitalised in the Clinic of Hematology from Craiova (Romania) between 2011 and 2013, with high levels of reactive oxygen species (FORT test) and low antioxidant capacity (FORD test). The patients were distributed in two groups: 12 patients (group A) received only corticosteroids as first line therapy and 12 patients (group B) received corticosteroids and antioxidant supplementation (informed consent obtained).
Results: In group A, seven patients responded to corticosteroids and the non-responders were treated, as second line therapy, with Vinca alkaloid regimens (one), laparoscopic splenectomy (two) or thrombopoietin receptor agonists (two). In group B, eleven patients responded to corticosteroids plus antioxidant supplementation; at one there was necessary a second line therapy (thrombopoietin receptor agonist). Follow-up at one year revealed that all eleven patients who received antioxidant supplementation and had a healthy diet were in clinical complete remission, whereas only seven patients from group A were in clinical remission.
Summary and Conclusions: In our small groups of patients, the antioxidant supplementation had a beneficial role in the response to therapy and the evolution of patients with ITP.

PB2031

DEMOGRAPHICAL AND CLINICAL CHARACTERISTICS OF ELDERLY PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA: A RETROSPECTIVE MULTICENTER STUDY

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Background: Idiopathic thrombocytopenic purpura (ITP) is a chronic, immune-mediated disease characterized by a temporary or sustained decrease in platelet counts. ITP is associated with various clinical consequences which may be serious, especially for the elderly, when it's not properly treated.
Aims: We aimed to present our experience about 50 elderly patients with ITP from 4 different centers.
Methods: Data from 4 centers in Anatolia were collected and analyzed in order to put forward the demographical and clinical characteristics of elderly patients with ITP over the age of 65.
Results: Total of 50 patients who were above the age of 65 at the time of ITP diagnosis were evaluated retrospectively. Sixty-two percent of the patients were female. The mean age was 71,5±6 (SD) years. Among the comorbidities, hypertension was the most common disease with a percentage of 24%, followed by coronary artery disease and diabetes mellitus, both with a rate of 8% and chronic obstructive pulmonary disease with a rate of 3%. Ninety-two percent of the patients had no organomegaly, while 4% had mild hepatomegaly and 2% had mild splenomegaly and 2% had mild hepatosplenomegaly. Mean followup duration was 31,6 ±25 (SD) months. At the time of evaluation, 94% of the patients were alive, 6% were deceased, and reasons of death were not related to ITP. Mean hemoglobin value was 13,13±1,9(SD) gr/dl. Female patients had statistically significant lower hemoglobin levels, compared to male patients. Mean platelet count was 12.760±10.000(SD)/μl . There was no significant difference between two genders in terms of platelet count. As first line therapy, 86% of the patients were given methylprednisolone, 8% were given dexamethasone and 6% were followed up without therapy. Mean remission duration after first line therapy was 27,9±22,8(SD) months. As second line therapy, 45% of the patients were given methylprednisolone, 35% were given dexamethasone, 10% were performed splenectomy and 10% were treated with other modalities. Ninety percent of the patients responded well to the second line therapy. Mean remission duration after second line therapy was 12,9±8,5(SD) months. Twelve percent of the patients needed third line therapy, where 50% of these were given eltrombopag, 33% had splenectomy and 17% were given rituximab. All patients responded well to the third line therapy. Mean remission duration after third line therapy was 17,9±18,9(SD) months.
Summary and Conclusions: Female and male patients had similar platelet counts, where female patients tend to have lower hemoglobin levels. Female patients had longer remission durations at first line therapy, compared to the male patients and the difference was statistically significant. Studies with larger patient groups should be performed in order to see if there's a difference between genders in terms of response to therapy.

PB2032

HERMANSKY-PUDLACK SYNDROME DURING PREGNANCY: A SYSTEMATIC REVIEW

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Background: Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder, it is characterised by oculocutaneous albinism, bleeding diathesis and decreased visual acuity. HPS can also cause pulmonary fibrosis, inflammatory bowel disease and renal diseases. The prevalence of HPS is 1 in 500000 – 1000000 and in Puerto Rico is 1 in 1800 with carrier rated 1 in 21.
Aims: To see how does Hermansky-Pudlack syndrome affect the pregnancy, labour/delivery and postpartum period.
Methods: Review of literature of HPS during pregnancy wa performed for the period of 1970 to 2010 which is revealed 7 articles including 11 pregnancies in 8 women (4 were from Puerto Rico).
Results: The commonest bleeding symptoms in these women were menorrhagia, easy bruising and nose bleeding. There was no reported ante partum haemorrhage. Haemostatic coverage for labour and delivery was given in 7 pregnancies, platelet transfusion in 2 and DDAVP in 5 pregnancies. Mode of delivery was emergency caesarean section in 3 pregnancies (all for obstetric indications) and remaining had vaginal delivery. Regional analgesia used in one woman without haemostatic cover and no complications. Postpartum haemorrhage reported in 6 pregnancies (including one who had DDAVP as

prophylactic measure during delivery), 2 required blood and platelet transfusion, 2 received blood alone and one only platelet transfusion. Pregnancy outcome was 10 alive babies without any complications.
Summary and Conclusions: This review revealed a high risk of PPH in women with HPS. Close collaboration between obstetric and haemophilia team is essential to reduce this risk.

Bleeding disorders (congenital and acquired)

PB2033

CIRCUMCISION IN CHILDREN WITH CONGENITAL FACTOR DEFICIENCY
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Background: Circumcision is the most frequently performed surgical prosedure for religious reasons in Turkey.
Aims: Examination of hemostasis tests carefully before circumcision leads to the recognition of patients with bleeding diathesis. We evaluated 158 records of patients diagnosed as congenital factor deficiency (CFD), underwent circumcision in our clinic between 1990-2014, retrospectively.
Methods: Information of patients were retrieved from patient records and from the records contained in the data-processing environment introduced in 2005. Circumcisions performed with an open method, with dorsal slit technique. Fibrin glue was not used during circumcision procedures. All of the factor preparations were administered as bolus via the peripheral vein. No catheters were used.
Results: Of the patients, 84 (53.2%) had Hemophilia A, 45 (28.5%) FVII deficiency, 11 (6.9%) Hemophilia B, 4 (2.5%) vWF deficiency, while 4 (2.5%), 3 (1.9%), 3 (1.9%), 2 (1.3%), 2 (1.3%) had fibrinogen, FV, FXI, FX, FXIII deficiencies retrospectively. Age of patients ranged from 2 to 24 years (mean 14.4 years). Thirty-four asymptomatic patients were diagnosed before circumcision (21.5%). In 35 patients circumcision were applied without any replacement therapy (22.2%). We used FVIII in 80 patients, FIX in 11 patients, vWF+FVIII in 4 patients, fresh frozen plasma in 13 patients, rFVIIa in 10 patients, PCC in 2 patients and fibrinogen in 3 patients during surgical interventions. Replacement therapy was achieved with single dose in 36 patients for circumcision (29.3%). Also in 87 patients 2-10 doses replacement therapy was needed (70.7%). In 35 patients antifibrinolytic agents were used also (28.5%). Bleeding during and after surgical procedures were observed in nine patients (5.6%). Transfusion was needed in two patients (1.3%). Thrombotic events were not observed. Antibody occurrence was not detected in these patients. No delay in surgical wound healing was noted.
Summary and Conclusions: Children with CFD can be safely circumcised in a center with experience In our study, we evaluated preparation before circumcision factor replacement prior to surgery and postoperative processes in patients.

PB2034

ACQUIRED HEMOPHILIA A WITH IMMUNE THROMBOCYTOPENIA IN ADOLESCENT PATIENT
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Background: Acquired hemophilia A (AHA) is rare autoimmune disorder caused by autoantibodies against coagulation factor VII (FVIII) in the nonhemophilic population. The age distribution of autoantibodies typically biphasic with small peak between 20 and 30 years (mainly postpartum inhibitors) and a major peak in patients aged 70 to 80 years. The incidence of AHA in children is very rare. Indeed, the incidence in children under 16 years has been estimated to be 0.045 per million/year compared with 14.7 per million/year in the elderly over 85 years. Also, immune thrombocytopenia is an acquired immune-mediated disorder caused by increased destruction of platelets opsonized by anti-platelet autoantibodies.
Aims: We experienced a case of AHA with thrombocytopenia caused by autoantibody in 18 years old boy.
Methods: He without any previous bleeding history presented easy bruise on lower extremities and he had diagnosed AHA. A initial activated partial thromboplastin time (APTT) was 136 seconds, the level of FVIII was 0.6% and FVIII inhibitor was 13.1 Bethesda Units (BU). Moreover, platelet counts were low (34,000/uL), and we proved platelet-associated autoantibody. Other autoimmune diseases were ruled out. He was instantly treated with oral prednisolone (1mg/kg/d) and added to oral cyclophosphamide (2mg/kg/d) one week later due to elevation of FVIII inhibitor (14.7 BU).
Results: About seven weeks later after treatment, FVIII inhibitor was disappeared and APTT was normalized, we started to tapering off the medication. During treatment of inhibitor eradication, platelet counts were also increased and normalized.
Summary and Conclusions: We report our experience as first successful antibody eradication for FVIII inhibitor and platelet-associated autoantibody simultaneously in adolescent AHA patient.

PB2035

RADIOISOTOPE SYNOVECTOMY IN PATIENTS WITH HAEMOPFILIA: SINGLE CENTER EXPERIENCE

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Background: Haemophilic arthropathy represents the most common clinical manifestation of haemophilia, related to recurrent haemarthoses and chronic synovitis. Haemophilic arthropathy is radiologically characterized by destructed articulation, bone deformities, complete closure of intra-articular spaces. Surgical synovectomies are not cost-effective procedures whereas radioisotope synovectomy (RS) is both less invasive and inexpensive procedures. It is accepted that RS is the gold-standart therapy before surgical synovectomy
Aims: We aimed to investigate the efficacy and complications of RS in the patients with haemophilia.
Methods: Twenty five RS were performed in 12 patients with haemophilia A and B, age ranging 6-21 mean (11 years) at the time of RS,for last five years. We preferred to use Yttrium ^{90} (Y^{90}) for knees and ankles, and Erbium ^{169} (Er^{169}) for elbows. Radioisotopes such as Y^{90} and Er^{169} were injected intra-articularly for treating target joints and chronical synovitis.
Results: We have evaluated our experience for knees (n:12), for ankles (n:9) and for elbows (n:4) in total 25 RS procedures for 12 patients. After RS joint bleedings were decreased for all patients. Radioisotop injections were three times repeated in right ankle of one patient. We have observed local hematoma after RS in three patients. For elbows, RS with Er 169 seems to be safe treatment method.
Summary and Conclusions: We had observed that RS was a safe and efficient therapeutic strategy.
Key Words: Haemophilia A and B, Haemophilic arthropathy, radioisotope synovectomy.

PB2036

LUPUS ANTICOAGULANT HYPOPROTHROMBINEMIA SYNDROME WITH SEVERE BLEEDING IN A 4-YEAR-OLD CHILD

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Background: Lupus anticoagulant is an acquired inhibitor which is commonly associated with thromboembolic manifestations. But in some rare cases, the antibody has affinity to prothrombin and patients have hemorrhagic tendency; sometimes they may present with severe bleedings, depending on their factor II level. The association of acquired factor II deficiency and lupus anticoagulant is recognized as "Lupus Anticoagulant Hypoprothrombinemia Syndrome" (LAHS).
Aims: We report a case of a 4-year-old boy with severe bleeding due to LAHS, with spontaneous recovery.
Results: A 4-year-old boy was admitted in pediatric emergency for macroscopic hematuria. He had no other symptom. Physical examination was normal. Initial laboratory evaluation consisted of a urine examination that confirmed hematuria (32833 red cells/µL and 75 leucocytes/µL, without infection) and blood tests revealed a decreased PT (40%) and a prolonged APTT (69.9 sec (24.0 – 35.0 sec)). Platelet count was normal (279 G/L). The measure of clotting factors showed an isolated factor II deficiency (20%). Lupus anticoagulant was positive. C-reactive protein was 10 mg/L (<10 mg/L). An abdominal echography was performed to exclude other hemorrhagic sites and showed signs of gastro-intestinal infection. The patient was diagnosed with LAHS related to gastro-intestinal infection. Two days after his admission, hematuria disappeared. Ten weeks later, a control blood evaluation showed that all parameters became normal. We performed a specific research of antiprothrombin antibodies (INOVA Quantalite) what demonstrated increased rates of IgG and IgM antiphosphatidylserine antiprothrombin (Table 1).

Table 1.

	Patient	Normal values
antiphosphatidyl serine IgG	50,78 U/ml	<11 U/ml
antiphosphatidyl serine IgM	17,49 U/ml	<25 U/ml
antiphosphatidyl serine IgA	2,82 U/ml	<20 U/ml
antiphosphatidylserine/prothrombine IgG	>150 U/ml	30 U/ml
antiphosphatidylserine/prothrombine IgM	>150 U/ml	<30 U/ml

Summary and Conclusions: LAHS was first described in 1960. In 2011, the 74 cases reported between 1960 and 2011 had been reviewed (Mazodier K, Arnaud L, Mathian A *et al.* Lupus Anticoagulant Hypoprothrombinemia Syndrome, report of 8 Cases and Review of the Literature. Medicine 2012; 91: 251-260). LAHS mostly affects young women. But sometimes it occurs in children. It is mainly related to autoimmune diseases, in particular systemic lupus erythematosus, or to acute viral infection. However, few cases of drug-induced LAHS or lymphoma-associated are reported. Clinical features and the severity of bleeding are correlated to the factor II level. According to the review of the 74 cases reported in the literature, some patients were asymptomatic, but 89% of them had hemorrhagic manifestations with severe bleedings in 51%. Macroscopic hematuria was present in 15% of patients. In children, it is generally a transient situation which does not need any treatment, and clinical and biological features usually normalize in few days except when the LAHS is revealing an autoimmune disease or when there are severe complications in relation to the bleedings.

PB2037

DIRECT APTT RATIO (PLATELINLS / ACTINFS) PERMITS TO QUICKLY AND RELIABLY DETECT BLEEDING RELATED FACTOR DEFICIENCY WHEN ISOLATED PROLONGATION OF APTT IS FOUND IN PEDIATRIC PRE-OPERATIVE SC

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Background: Isolated prolongation of activated partial thromboplastin time (aPTT) found during paediatric pre-operative screening could be due to coagulation factor deficiency or more frequently to nonspecific inhibitor like lupus anticoagulant (LA).
Aims: The aim of our study is to evaluate the clinical values of an additional aPTT with ActinFS and/or a mixing aPTT study to identify the bleeding related factor deficiency (BRFD) like Factor VIII, Factor IX or Factor XI deficiency.
Methods: During 4 years, an aPTT with ActinFS and a mixing aPTT were added for 308 paediatric patients in which an isolated prolongation of aPTT with PlatelinLS was found among 9048 pre-operative screening. A direct aPTT ratio between PlatelinLS and ActinFS was calculated. For 156 patients (50.6%), this prolonged aPTT was confirmed in a second sample. Measurements of bleeding related factors (FVIII, FIX and FXI), Factor XII and the LA research were performed respectively in 141,131 and 88 samples.
Results: We found 17 BRFD, 26 FXII deficiency and 64 positive LA. A prolonged ActinFS had a significant association with BRFD ($P<0.0001$) while a corrected mixing study had not. The direct aPTT ratio had a significant association not only with BRFD ($P<0.0001$) but also with positive LA ($P<0.05$). In applying a cut-off value of this ratio as <1,29, the sensitivity and specificity for BRFD were 82% and 76% respectively, while they were 59% and 93% using ActinFS alone. Normal ActinFS was found in 7 mild BRFD cases with factors levels superior to 30%, five of 7 had a direct ratio <1,29.
Summary and Conclusions: The direct aPTT ratio between PlatelinLS and ActinFS without mixing study permits to identify quickly and reliably a BRFD for an isolated prolongation of aPTT found in paediatric pre-operative screening.

PB2038

SINGLE CENTER REGISTRY OF RARE BLEEDING DISORDERS FROM TURKEY

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Background: Deficiencies of coagulation factors (except factor VIII and factor IX) are generally much rarer than the hemophilias.
Aims: The purpose of this poster presentation is to review the disorders in terms of clinical manifestations and complications from hemorrhage.
Methods: Records at Erciyes University Medical School, department of pediatric hematology, were reviewed from 1998 to 2012 and 31 patients with rare bleeding disorders (RBD) were evaluated. The data were collected based on age at diagnosis, familiy history of disease, bleeding sites, treatment strategies and complications of disease.
Results: Ten patients had afibrinogenemia (plasma fibrinogen level<100 mg/dl), five had factor V deficiency, three had factor VII deficiency, five had factor X deficiency, three had factor XI deficiency, and the last five had factor XIII deficiency, respectively. The mean age of the patients was 6.41 years (ranging from 3 months to 13 years) at diagnosis. Twenty two out of 31 patients were male, and nine were female. Out of 31 patients, three with afibrinogenemia, two with factor V deficiency, two with factor X deficiency, and four with factor XIII deficiency coming from two diferrent families were siblings. Among all RBDs, the most common sites of bleeding were skin and mucus membranes. Furthermore, 11 patients had intracranial hemorrhage (ICH), one patient had

gastrointestinal hemorrhage, one patient had hematuria. Six patients with afibrinogenemia and three patients with factor XIII deficiency had ICH. Four patients diagnosed preoperatively and three patients postoperatively after circumcision and tonsillectomy as RBD. The patients were administered fresh frozen plasma, cryoprecipitate and fibrinogen concentrates. All patients survived, but four of them with ICH had sequelae such as decrease in intellectual capacity and motor deficit.
Summary and Conclusions: This registry provides a comprehensive information and could be an important resource for hematologists.
Key Words: Rare bleeding disorders, registry.

PB2039

STUDY OF THROMBIN GENERATION IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA
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Background: Although thrombocytopenic, patients with primary immune thrombocytopenia rarely have severe bleeding episodes, mainly because their blood platelets are hyperfunctional.
Aims: We aimed to study if the level of thrombin generation is another factor that may explain the rarity of bleeding in patients with primary immune thrombocytopenia.
Methods: We made a pilot study on a group of 10 patients with primary immune thrombocytopenia found in the records of the Hematology Service of Emergency County Clinical Hospital Sibiu, who presented consecutively to clinical and biological control in January 2014. We retained age, peak of thrombin generation (obtained wiht alpha Ceveron device), the platelet count, blood hemoglobin level, the hematocrit, white blood cells number, international normalised ratio (INR), and the activated partial thromboplastin time. The peak of thrombin generation of these patients was compared with that of a control group - 10 voluntarily subjects without any known disease. The results were analyzed with arithmetic mean, standard deviation, Student's t test, and Pearson test, and they have allowed conclusions that we hope to have implications for clinical practice.
Results: The mean age of surveyed patients was 59.11+/-15.14 years. The average peak of thrombin generation in patients with ITP was 166.57+/48.88, and in the witnesses - 123.29+/-20.64; the difference is statistically significant ($p < 0.05$). The thrombin generation peak in patients with ITP was inversely correlated with age (r=-0.316), platelet count (r=-0.672), hemoglobin (r=-0.756), hematocrit (r=-0.433) and INR (r=-0.294); it did not correlate with the number of leukocytes and activated partial thromboplastin time.
Summary and Conclusions: Patients with ITP have an average peak of thrombin generation significantly higher than that of witnesses. The lower the age of patients with ITP, the higher the peak of thrombin generation. The higher the peak of thrombin generation in patients with ITP, the smaller the hemoglobin, hematocrit and INR values. The smaller the platelet count in patients with ITP, the higher the peak of thrombin generation - another reason for them to present bleeding rarely.

PB2040

HAEMORRHAGIC SYNDROME DUE TO PRIMARY HYPERFIBRINOLYSIS AS THE FIRST CLINICAL SIGN OF PROSTATE CANCER
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Background: Hematologic disorders are commonly seen in patients with prostate cancer (PCa). The most frequently observed disorder in PCa patients is acute or chronic disseminated intravascular coagulation (DIC). The incidence of DIC complication in PCa rages from 13% to 30%, and can be presented as catastrophic bleeding or various thrombotic events. In contrast, bleeding symptoms in the setting of primary hyperfibrinolysis in this malignancy are seen in only 0.40% to 1.65% patients, usually provoked by surgical procedures. It is supposed that metastatic PCa cells produce high levels of urokinase type plasminogen activator. Laboratory diagnosis of hyperfibrinolysis is based on the increase of biomarkers like D-Dimer; fibrinogen split products, plasminogen, and euglobulin lysis test (ELT). But, these tests are not specific for primary hyperfibrinolysis and they are also elevated in other pathological condition.
Aims: We reported the diagnosis and treatment of a rare case of haemorrhagic syndrome caused by primary hyperfibrinolysis, which was the first clinical symptom of metastatic prostate cancer.
Methods: The patient's blood was collected in vacuum tubes containing 3.2% sodium citrate and lithium heparin.Thrombelastometry analysis was performed with the Rotem® analyzer. Routine coagulation test was performed with IL ELITE PRO®and ACL 300® analyzer.
Results: A 64-year-old male was admitted to our hospital with large haematomas: in the right pectoral and axillary area, size 20x7 cm, right hemiabdomen size, 30x30 cm and left lumbal area, size 25x5 cm. Hematomas developed two days before hospitalization. Patient denied neither subjective symptoms nor any medication. Performed labory analises showed mild normocytic anaemia of chronic disease (Hb 108g/L, MCV 90fL, RBC $3.72x10^{12}$/L, Hct 0.331, Plt $419x10^{9}$/L, WBC $9.5x10^{9}$/L, Neutrophils 60%; serum iron 12,9µmol/L, ferritin 7820µ/L), highly elevated alkaline phosphatase (1390U/L), lactate dehydrogenase (1740U/L) and prostate specificic antigen (above 150ng/mL). Viral test vere negative (HBs Ag, anti HCV and anti HIV). Initial coagulation testing: protrombin time and activated partial thromboplastin time were within the normal range (14.4s and 31.9s, respectively), as well as anti-thrombin III (86.6%), while fibrinogen level was very low (1.068 g/l) and D-Dimer assay result was 1122 ng/mL. Results obtained by rotation thrombelastometry have pointed on primary fibrinolysis. First line treatment for patient was combined administration of tranexamic acid (3x500 mg intravenous) and transfusion of ten units of cryoprecipitate (400 ml). Next day, fibrinolytic function measurements by rotation thrombelastometry were within the normal ranges. APTEM test wasn't registered pathological fibrinolysis. Fibrinogen level was normalized within two days (2.4 g/l). There were no newly developed hematomas. Transrectal biopsy of prostate was successfully performed without any haemorrhagic complications, revealing prostate adenocarcinoma-G3, Gleason score 9. Further examination confirmed the laboratory findings of metastatic bone disease. Afterwards, the patient was referred to the oncologist for further treatment with androgen deprivation therapy together with oral tranexamic acid.
Summary and Conclusions: This case represent primary hiperfibrinolysis with bleeding diathesis as the first clinical sign of previousy undiagnosed metastatic PCa. Rotation thrombelastometry in this severe complication helps to achieve prompt and proper diagnosis.The management of hyperfibrinolysis was done within a short period of time thanks to adequate diagnostic procedure.

PB2041

INTRACRANIAL HEMORRHAGE IN HEMOPHILIA PATIENTS
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Background: Intracranial hemorrhage (ICH) in hemophilia patients is the most common cause of death in Korea. Early suspicion and prompt treatment of ICH is a very important for saving their life and minimizing neurologic sequelae.
Aims: We investigate the prognosis of the hemophilia patients with ICH who registered in Daegu & Kyungpook area.
Methods: We evaluated the clinical courses, laboratory findings, brain image, effect of treatment and prognosis of ICH. Nine of 161 patients registered in our Department of Pediatrics suffered 16 episodes of ICH for 10 years.
Results: All were male hemophilia A patients (severe; 5, moderate; 3 and mild; 1) with median age of 48 months at diagnosis of ICH. Two patients who had inhibitor became negative and one patient who had found inhibitor on annual routine follow-up has had inhibitor until now. The median time interval from first symptom to hospital visit was 7 hours. Chief complaints were vomiting in 6 patients, headache in 4, seizure in 3 and mental change in 1. All patients except one were initially given factor VIII concentrate, 50 units/kg and then continuous infusion, 2-3 units/kg/hour. One patient who had factor VIII inhibitor was given factor IX concentrates, 100 units/kg with activated prothrombin complex (FEIBA), 75 units/kg at every 12 hours. All except one with hematoma in cerebellar vermis and the third ventricle are alive without any neurologic sequela
Summary and Conclusions: It is desirable to have early treatment, prophylaxis, regular follow-up and patient education about abnormal symptoms to reduce the complications of them.

PB2042

ACQUIRED HEMOPHILIA IN ELDERLY PATIENTS WITH NON HODGKIN'S LYMPHOMA
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Background: Acquired hemophilia is a rare disorder characterized by autoantibodies against circulating coagulation factors. Patients often have not history of bleeding disorder and present spontaneous hemorrhage, an isolated prolonged aPTT and PT and antibodies against coagulation factors. It has an incidence of 0,2-1 cases/million/year. This condition may be associated in 50% of cases with autoimmune disease, solid tumor, lymphoproliferative disorders and pregnancy.

Aims: In this work, we describe our experience with three cases of indolent NHL with isolated prolonged aPTT and PT.
Methods: Case 1 : A 72 year-old man referred to our Institution because of recurrent epistaxis and abnormalities of coagulation tests PT INR 2.5, aPTT ratio 2.73. No history of bleeding disorders, recent surgery or new drug intake were reported. Spleen enlargement, with a large focal lesion, and pancytopenia (Leukocytes 2800/μL; Haemoglobin 9 g/dL; Platelets 82,000/μL) were observed. Moreover, it was present a reduction of Factor VIII, Factor II, Factor V, Factor VII, Factor IX, Factor X and Factor XI activity (FVIII:C 16%; FII: 44%; FV:8%; FVII: 11%; FIX: 10%; FX:30%; FXI:27%) and positivity of antibodies against coagulation factors. Lymphoid infiltrates were observed in bone marrow examination. Moreover, increased PET/CT splenic uptake and in a supraclavear lymphonode was observed. FNAB in the supraclavear lymphonode, performed after an injection of 60 μg/Kg recombinant Factor VIIa enabled the NHL CD5-CD22+lambda+ diagnosis. The patient underwent to six administrations of chemotherapy with CEOP and achieved complete remission with coagulation parameters and factor activity normalization. Then, during followup, 11 months after the end of frontline treatment, the patient came to our Department for the onset of abdominal bleeding and relapse of the lymphoproliferative disease. After surgical treatment, the patient underwent to R-FN chemotheapy treatment (6 courses), with complete remission of disease. After the end of treatment, he underwent to Rituximab-based maintenance treatment with persistence of normality of coagulation and factor activity parameters. Case 2 : A 62 year-old woman came to our observation for lymphoadenopathy, hepatoplenomegaly, anemia and lymphocitosis. She didn't present any personal or familial history of bleeding disorders and didn't take any drug. Laboratory tests showed: PT INR 3.26, aPTT ratio 4.92; there was also a reduction of factor VIII activity, Factor II activity, Factor VII activity, Factor IX activity, Factor activity X and Factor activity XI (FVIII:C: 2.3%; FII: 32%; FVII: 47%; FIX: 1%; FX:43%; FXI:1%) and appearance of antibodies against many coagulation factors. PET/CT revealed increased uptake at axillary and inguinal lymphonodes and spleen. Bone marrow analysis showed a lymphoid infiltrate and enabled the diagnosis of NHL CD5-CD22+lambda+. The patient underwent to six courses of R-Fludarabine, and after the second administration it was observed a PT and aPTT normalization. Case 3 : A 80 year-old man came to our observation because of pancytopenia and abnormalitites of coagulation tests: PT INR 2.28, aPTT ratio 3.2. No story of previous bleeding disorders. The activity of various coagulation factors was reduced : FII 54% FV 68% FX 60%. PET/CT documented splenomegaly with increased splenic uptake. Bone marrow examination enabled the NHL CD20+CD19+CD5-CD22+CD23-lambda+ diagnosis. The patient underwent to R-Chlorambucil courses, after which a normalization of PT and PTT was observed.
Results: All patients of this study underwent to Rituximab-based maintenance treatment to prevent the emergence of lymphoproliferative clone.
Summary and Conclusions: The onset of an acquired coagulation disorder can be an useful diagnostic and progonostic marker of immunological impairment due to an underlying lymphoprolipherative disease. In particular, maintenance treatment may actively control the neoplastic clone reducing the risk of bleeding.

Thrombosis and vascular biology

PB2043

PULMONARY EMBOLISM – RETROSPECTIVE STUDY OF A PORTUGUESE ONCOLOGY CENTER

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Background: The association between cancer and venous thromboembolism (VTE) is well-established, and about 20% of oncologic patients will develop it. Pulmonary embolism (PE) is an important cause of morbidity and mortality in this group of patients.
Aims: Characterization of patients with the diagnose of PE in a Portuguese Oncology Center.
Methods: Retrospective study of all episodes (n=234) of PE referenced to Clinical Board Administration in the last five years (from January/2009 to December/2013). Demographics, past medical history, risk stratification of PE (Caprini and Khorana) and probability of mortality at 30 days (PESI - Pulmonary Embolism Severity Index) were analyzed.
Results: A median age of 63 years (15-87 years) was encountered, with a female prevalence (58.5%). The most prevalent malignancies was colorectal cancer (20.9%), followed by lung (18.4%) and breast cancer (18.4%). Metastatic disease was found in 60.3%. The following risk factors for PE were identified: chemotherapy (62.8%); central venous catheter (31.6%); recent surgery (8.5%); hormonal therapy (6.4%); and at least one cardiovascular risk factor (54.7%). Of note, 3 patients (1.3%) had a previous diagnose of VTE, and were under anticoagulation therapy. In patients treated with chemotherapy, Khorana's risk scoring was: 37.1% low risk, 51.5% intermediate risk and 11.4% high risk. According to Caprini's risk stratification all hospitalized patients had a moderate to high risk of developing PE. Most of the cases were reported in ambulatory patients (80.3%), only one (0,4%) patient was under anticoagulation prophylaxis. Most hospitalized patients (80.4%) were receiving prophylaxis. More than a half of the PE (59.8%) were apparently asymptomatic and were diagnosed by radiological assessment for unrelated reasons. The association between deep venous thrombosis and PE was found in 12.8%. The probability of mortality at 30 days, based on the PESI's scale was 2.6% in class I, 6.6% in class II, 34.2% in class III, 31.6% in class IV and 25.0% in class V. There was an overall 30-day mortality of 13.2%. All patients were treated with low molecular weight heparin and oral anticoagulants were initiated approximately three months after.
Summary and Conclusions: In our study, PE was more frequently diagnosed in advanced stages of the disease, in patients undergoing chemotherapy treatment and with central venous catheter. The authors emphasize that most cases of PE were asymptomatic and occur mainly in ambulatory patients. This findings highlight the need to sensitize health´s professionals to recognize and assess the risk for PE in oncologic patients and further prevent it.

PB2044

HEMORHEOLOGIC DISTURBANCES IS VTE RISK FACTOR IN CHILDREN WITH ALL

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Background: Acute lymphoblastic leukaemia (ALL) is the commonest childhood malignancy with high risk of venous thromboembolism (VTE).
Aims: The aim was to investigate blood rheology properties in children with ALL during first remission and with not symptomatic organs failures.
Methods: The study's population was 48 children (age<16y.o.). Six patients had VTE during follow-up despite standard anti-VTE prevention. Whole blood viscosimetry, plasma viscosimetry, erythrocytes aggregability and deformability, B-type natriuretic peptide (BNP) level and plasma ions concentrations were assayed.
Results: All patients had normal plasma viscosity and unimpaired erythrocyte deformability. Whole blood viscosity was increased by shear rates 5-300 s-1 and mainly by shear rates 5-75 s-1. We found erythrocytes hyperaggregability in all patients. Elevated BNP level was found in 16 patients, and from them six VTE had been developed. These patients had the combination "most increased whole blood viscosity - erythrocytes hyperaggregability" but they had not symptomatic heart failure.
Summary and Conclusions: Totally increased blood viscosity with erythrocytes aggregability are factors leading to thrombosis. The slightly decrease of blood flow rate might be assumed when BNP-level have been raised. Both signs elevates the risk of VTE. Despite antithrombotic prevention directed to hypocoagulation the revealed hemorheologic disturbances could

be as a trigger to start of VTE in children with ALL. The obtained results suggest that the study should be continued.

PB2045

IMPACT OF DABIGATRAN ON PLATELET AGGREGATION, SECONDARY HAEMOSTASIS AND FIBRINOLYSIS, COMPARED TO ACENOCOUMAROL

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Background: Dabigatran etexilate (DE) is an oral direct thrombin inhibitor. Thrombin, which is inactivated by DE, has a central role in the whole haemostatic process by affecting all of its phases. It activates platelets; amplifies the haemostatic response by activating factors V, VIII and XI; converts fibrinogen to fibrin; stabilizes the fibrin clot through activation of FXIII; and further affects fibrinolysis by regulating an important factor in the clot stabilization, the thrombin activatable fibrinolysis inhibitor. Despite the crucial role of thrombin in almost all phases of coagulation, no research on the impact of DE on platelet function and fibrinolysis has been conducted, while there is disagreement about optimal methods to monitor its anticoagulant effect in certain clinical settings.
Aims: Our aim was to compare the impact of dabigatran and acenocoumarol on primary and secondary haemostasis and fibrinolysis, and to identify laboratory assays that can be used to assess the anticoagulant activity of DE.
Methods: We recruited 20 patients with non valvular atrial fibrillation (NV-AF) treated with DE (110 mg per os bid) and 20 patients on acenocoumarol, matched on age and sex, from Cardiology Department in a tertiary hospital in Athens, Greece. Exclusion criteria were the following: platelet count<100×109/l, active thrombosis or bleeding, simultaneous anticoagulant and antiplatelet therapy, INR out of the defined limits, renal, hepatic, thyroid dysfunction, malignancy and infectious disease. Conventional coagulation tests, endogenous thrombin potential (ETP), thromboelastometry (ROTEM), epinephrine-induced light transmission aggregometry (LTA), and hemoclot thrombin inhibitors (HTI) were performed. Non-parametric tests (the Fisher's exact test and the two-sample Wilcoxon rank-sum [Mann-Whitney] test) were used for the statistical evaluations. Correlations were assessed by the Spearman rank correlation coefficient and the respective p-value.
Results: The two groups were similar in terms of descriptive characteristics, comorbidities, haematological and biochemical parameters. ROTEM® analysis showed a greater impact of DE on procoagulant activity than acenocoumarol, but only the difference in clotting time reached statistical significance (p=0.004). The lysis index at 60 min was significantly lower in patients receiving dabigatran (p=0.011) indicating an increased fibrinolytic activity. In epinephrine-induced LTA, patients on dabigatran showed decreased aggregation compared to those on acenocoumarol, marginally insignificant (p=0.068). Regarding the correlation of dabigatran levels, as assessed by the HTI assay, with the haemostatic parameters, it is noteworthy the statistically significant associations between them and almost all parameters of ETP assay. Specifically, positive correlations between dabigatran levels and lag time (p=0.001) and time to peak (p=0.018) were detected, while area under the curve (AUC, p<0.001) and peak height (C max, p<0.001) were inversely correlated with HTI values.
Summary and Conclusions: Compared to acenocoumarol, dabigatran seems to affect more fibrinolysis, while a clear impact on platelet aggregation was not determined. Taking into consideration that a small sample size has been studied, this hypothesis deserves further research, since it could be of clinical relevance in certain clinical settings. On the other hand, ETP use in dabigatran monitoring seems to be helpful.

PB2046

PREVALENCE OF COMBINATION OF TRIPLE ALLELIC MUTATIONS ASSOCIATED WITH THROMBOPHILIA IN PATIENTS WITH VENOUS THROMBOEMBOLISM

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Background: Triple thrombophilic abnormalities have been rarely reported and most patients carried polymorphisms nowadays not included in the recommended laboratory investigation for thrombophilia, such as those in the MTHFR gene.
Aims: To assess the prevalence of triple allelic mutations associated with thrombophilia in patients with venous thromboembolism (VTE).
Methods: We selected from a cohort of 2,199 individuals with VTE who were referred to our Thrombosis Center those with triple thrombophilic abnormalities (*i.e.* deficiency of antithrombin [AT], protein C [PC], protein S [PS], factor V Leiden [FVL], prothrombin [PT] 20210A) and/or triple allelic mutations (*i.e.* triple heterozygosity [Het], homozygosity [Hom] plus Het).
Results: We identified 10 patients (M/F 5/5) (0,45%) (Table 1). Four patients had triple Het, and the remaining carried a combination of Hom and single Het. No patient in the cohort carried double Hom neither Hom for deficiency of natural anticoagulants, nor triple deficiency of natural anticoagulants. Het for deficiency of natural anticoagulants was present in 5 patients (AT=2, PC=1, PS=1, PC+PS=1), associated with FVL or PT20210A (Table). Four patients had triple abnormalities (various combinations of AT,PC,PS deficiency, and FVL or PT20210A) (0.18%), and 6 had double abnormalities (AT deficiency and Hom for FVL in one case, FVL and PT20210A in the remaining ones).
The first VTE was deep vein thrombosis (DVT) of one leg in 9 cases, in 2 of them with pulmonary embolism (PE), and superficial vein thrombosis (SVT) in 1 case. The median age of first VTE was 27 years (range 2-73), in 8 cases <45 years. The first event was provoked in all cases. Two of them had an additional thrombophilic acquired abnormality (hyperhomocysteinemia and lupus anticoagulant, respectively). Four patients had recurrent VTE events. Eight patients received lifelong treatment with vitamin K antagonists (VKA).

Table 1.

Summary and Conclusions: In patients with VTE triple allelic mutations associated with thrombophilia are uncommon but not exceedingly rare (0.45%), and diagnosis of a single thrombophilia abnormality should not discourage from an exhaustive laboratory investigation.
Abnormality can be a combination of Hom and Het or a combination of triple Het. The clinical onset occurs in young age in the large majority of cases. Surprisingly, such conditions seem not associated with unprovoked events, and recurrence occur in a minority of subjects.

PB2047

MICROPARTICLE (MPS) RATIO IS HIGHLY CORRELATED WITH ALL PARAMETERS OF THROMBIN GERENATION ASSAY (TGA) PARAMETERS IN MULTIPLE MYELOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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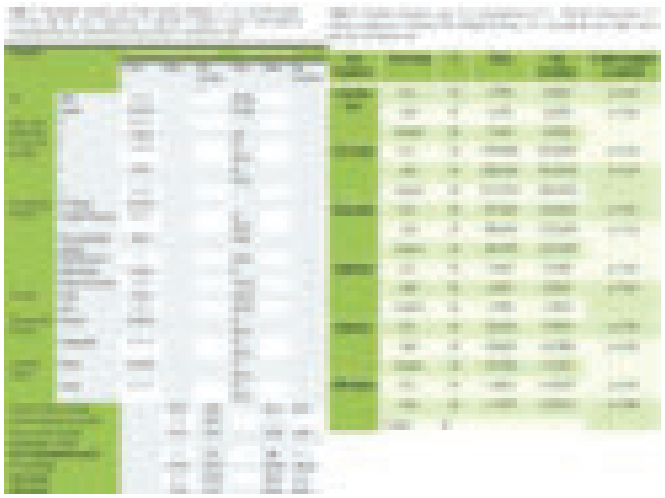
Background: Microparticles (MPs) are small membrane derivatives that generally released from either live cells or apoptosed cells. MPs have been studied with various types of diseases and has been shown that presence of MPs is accompanies with increased risk of thrombe embolic (TE) events.
Aims: In this study we aimed to detect presence of MPs , analyse Thrombin Generation Assay (TGA) parameters to improve understanding of pathophysiology in a patient population that includes Multiple Myeloma (MM) and Chronic Lymphocytic Leukemia (CLL) patients whose had a high risk for TE events compare to normal population.
Methods: We include 40 MM and 30 CLL patients who diagnosed and followed in Hematology Department and outpatient clinic of Atatürk University Medicine Faculty between January 2010 and July 2013. 26 Healthy individual also included to the study as control group. At the begining of the study samples for TGA and MPs tests were taken from the patients and control group after informed consents were given. Patients were followed prospectively during the study. Chemotherapy responses, disease related informations and demographic data were recorded. At the end of the study TGA and MPs test results were investigated statistically with collected data.
Results: In this study, patients' mean follow-up period was found 22.6 months for the MM, 38.8 months for CLL. The patient characteristics were given in

Table 1. At the end of the study; 13 TE events and 10 mortality observed in MM patients. 10 Thromboembolic events and 3 mortality was observed in CLL patients. Calculated MPs ratio of patients with CLL and MM were 1.46±1.03, 1.16±0.52, respectively (According to calculated reference value 0.99±0.29 of control group). During the study, The incidence of thromboembolic events during the study in patient group (CLL and PCM patients) was detected that statistically significantly has positive correlation with the stage of the disease (p=0.01), presence of co-morbid disease (p<0.001), mortality (p=0.002),age (p=0.011), Lag-time in TGA test (p=0.048). We also detected that the incidence of thromboembolic events has a statistically significant negative correlation with ETP and Peak time in TGA test (p=0.021 and 0.046 in orderly),quality of the therapy response (p=0.008). Decrease in ETP and peak values were statistically significantly accompanied by an increased incidence of thromboembolic morbidity. We also detected that all parameters of TGA test (Lag time, ETP, TtPeak, Peak value) were statistically significantly correlated with MPs ratio both in CLL (p<0.001, p=0.006, p<0.001, p=0.002 in orderly) and MM (p<0.001, p=0.006, p<0.001, p=0.002 in orderly)patient groups.TGA test parametersof CLL, MM and control groups were given in Table 2.

Table 1 and Table 2.



Summary and Conclusions: As a result, we detected that CLL and MM patients found significantly high incidence for TE. Stage of the disease, co-morbid diseases, age, chemotherapy response and some TGA test parameters were statistically significantly correlated with TE evente. We also detected that MPs ratio was statistically significanlty correlated with all parameters of TGA test. This close relationship of MPs with TGAin CLL and MM patient can be interpreted as a clue for increased acquired thrombophilia due to presence of MPs in these patient groups. TE events are significant morbidity and mortality cause for hematological malignancies and other cancers. We speculated that TGA and MPs evaluation to obtain an idea for Tissue Factor activity can be a valuable parameter for the determination of the high-risk patients to administer prophylaxis. Further multicentered prospective large scaled studies were required to support such an idea.

PB2048

CALIBRATED AUTOMATED THROMBOGRAM ALLOWS INDIVIDUALIZED APPROACH TO ANTITHROMBOTIC TREATMENT AND PROPHYLACTICS IN THROMBOPHILIA PATIENTS.

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Background: Proper thrombosis prophylaxis without increasing risk of hemorrhages is of great importance, so optimal duration of prolonged anticoagulant use is still of interest for thrombophilia patients, particularly those with lupus anticoagulant (LAC), factor V Leiden (FVL) and prothrombin G20210A mutations. The Calibrated Automated Thrombogram (CAT) is now considered to better correlate with patients phenotype compared to traditional coagulation tests and has wide clinical utility, including evaluation of prothrombotic risks .
Aims: Aim of our study was to assess the use of CAT in identifying patients who need prolong antithrombotic regimens.
Methods: Calibrated Automated Thrombogram (CAT) was done according to Hemker *et al.* with fluorogenic assay, at 5 pM TF and 4 µM phospholipids in platelet poor plasma (PPP) with PPP plasma+/-TM reagent (Thrombinoscope BV, Maastricht, The Netherlands). Thrombin generation curves were calculated using the Thrombinoscope software (Thrombinoscope BV, The Netherlands). Our selected group of 38 patients (M/F 15/23, mean age 38,0±9,0 yrs) with venous thromboembolism (VTE) manifestation under the age of 45 years involved 14 heterozygous for FVL, 4 LAC and 3 prothrombin G20210A mutation carriers respectively. Investigation was performed at least 6 months after the thrombotic episode. STATISTICA 6.1 was used.
Results: Endogenous thrombin potential (ETP) and peak thrombin (PT) were markedly reduced in the presence of TM. Normal ranges of inhibition were calculated in 28 age and sex matched controls. The percentage of inhibition below 21% for ETP and/or 14% for PT (*i.e.* activated protein C resistance - APRC) were found in 8 (57%) of patients with FVL and 4 (100%) patients with LAC. Importantly abnormal inhibition of PT was found to be more sensitive in determining of APCR, as it was in all these patients and abnormal inhibition of ETP only in 50% cases with FVL and 75% with LAC. Both in the absence and in the presence of TM significant correlation with LAC was found for lag time (R =0,45; p<0,05), ETP and PT (R =-0,45 and R =-0,44, respectively, p<0,05). One patient with prothrombin G20210A mutation and increased factor VIII activity also demonstrated APCR. Importantly not all patients with increased FVIII activity (>200%) demonstrated APCR, it was found in 50% of cases (3 from 6).). As for the parameters derived from the thrombin generation curves (lag time, ETP, PT, time to peak) both in presence and absence of TM, no one showed correlation with factor VIII activity.
Summary and Conclusions: APCR (both inherited and acquired) is associated with high risk of VTE and its recurrence. As FVL is the main cause of APCR, little attention is paid to the fact that not all the carriers of this mutation demonstrate APCR, at the same time acquired APCR is enough common. According to our data APCR could be effectively detected by CAT performed with PPP plasma+/- TM reagent. It is possible to find APCR and LAC even when patients are on vitamin K antagonists (VKA) treatment. We suggest important to use CAT before making decision on VKA discontinuation. Patients demonstrating APCR, would benefit from extended VKA treatment. Whether FVL patients without APCR phenotype do not need further prolongation of antithrombotic prophylactics remains to be established. Our data support the position that CAT allows individualized approach to antithrombotic treatment and prophylactics.

PB2049

DIFFERING COAGULATION PROFILES IN PATIENTS WITH MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE.

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Background: Armand Trousseau first noticed the link between cancer and thrombosis almost 150 years ago. The link between myeloma and thrombosis was first noted in the 1970s. It came to prominence with the advent of thalidomide and related agents which further increased this risk. The mechanism has not been well established so it has been difficult to measure an individual patients' risk. Monoclonal gammopathy of undetermined significance (MGUS) has also been associated with an increased risk of VTE, but to a lesser extent. Thromboelastography is a technique that measures the visicoelastic properties of whole blood under low shear stress. Trelinski et al recently demonstrated that patients with myeloma display changes in ROTEM tests at the time of diagnosis that may indicate a prothrombotic state. There is little data with regard to changes in thromboelastographic profiles in patients with myeloma or MGUS.
Aims: To investigate the differing coagulation profiles of patients of patients with myeloma, MGUS and normal controls by means of conventional coagulation tests and thromboelastography.
Methods: Having obtained informed consent, fresh whole blood was taken by direct venepuncture into tubes containing citrate 0.105M from patients with myeloma (prior to treatment), MGUS and normal age and sex-matched controls. It was stored at room temperature for 30 minutes prior to analysis. 500µl of whole blood was activated with 12.5µl of Kaolin and thromboelastography was performed by the same technician on 340µl of activated blood in combination with 20µl of calcium chloride using a TEG 5000 Thrombelastograph®analyzer according to the manufacturer's protocol. The remainder of the investigations were performed in the hospital laboratory. Statistical analysis was performed using the unpaired t-test for normally distributed variables and a Mann-Whitney U test for non-normally distributed variables. A *p*-value of <0.05 was considered statistically significant.
Results: Eight patients were recruited in each of the three groups. None of the patients were hospitalised. The mean age in each group was similar. Patients with myeloma had a significantly lower mean haemoglobin level than patients with MGUS or normal controls (10.7 *vs* 13.9 *vs* 14.9g/dl, p<0.001). Pateints with myeloma had a significantly more prolonged mean prothrombin time (PT) than normal controls (11.4 *vs* 10.6sec, p=0.024) but not patients with MGUS

(p=0.126). Patients with myeloma had significantly higher median D-Dimer levels than normal controls (0.98 *vs* 0.17mcg/ml, p=0.024), as did patients with MGUS (0.45 *vs* 0.17mcg/ml, p=0.015). Patients with myeloma had a significantly higher mean factor VIII level than normal controls (243.4 *vs* 131.6%, p=0.016) and there was a non-significant trend towards patients with MGUS having higher factor VIII levels than normal controls (208.75 *vs* 131.6%, p=0.084). There was no significant difference in TEG parameters (r time, k time, alpha angle, maximum amplitude) between the three groups.

Summary and Conclusions: In keeping with previous studies, patients with myeloma have prolonged PT times, elevated D-dimer levels and factor VIII levels when compared with normal controls. Moreover, patients with MGUS showed a trend towards hypercoagulability. No differences were seen in the thromboelastographic profiles of the three groups.

PB2050

WILMS TUMOUR AND VENO-OCCLUSIVE DISEASE. THE ROLE OF MICROPARTICLES AND PAI-1

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Background: Venous occlusive disease (VOD) of the liver has been reported to be associated with haematopoietic stem cells transplant. This condition has been associated with high mortality rate of up to 90% as multiorgane failure may occur [McDonald *et al.* 1993]. Several markers of thrombosis have been suggested to be involved in the VOD pathogenesis. However, this condition continues to have a poor prognosis and to be poorly understood. Recently an increment of plasminogen activator inibitor (PAI-1) has been identified as a possibile valid marker of VOD. Patients with solid tumours may also develop VOD. Interestingly, patients with Wilms Tumor (TW) develop VOD following chemotherapy treatment without any HSCT. Microparticles have been reported in several prothrombotic conditions [Piccin et al 2007], however studies on their association with PAI-1 are lacking. We report on VOD cases within a paediatric cohort of children affected by TW treated with chemotherapy.

Aims: The aim of this study was to investigate new biological marker involved with VOD.

Methods: Twelve consecutive patients (F=7; M=5) with a diagnosis of TW were treated with a combination of chemotherapy regimen based on vincristin, actinomicine-D and doxorubicine. Only five patients developed VOD after a median of 4 weeks from diagnosis (range 1-14 weeks). In all cases, poor platelets plasma was stored at -80C°. Three consecutive sampling at intervals of 3 weeks, were carried out during hospitalisation on each case. Standard coagulative parameters were studied (PT, PTT, Fibrinogen, D-Dimer). Microparticle studies were carried out using flow cytometry. PAI-1 antigen (PAI-1:Ag) was measured by ELISA (Tint-Elize PAI-1, Hyphen BioMed - Neuville-sur-Oise, France), while PAI-1 activity (PAI-1:act) was measured using chromogenic assay (TriniLIZE PAI-1 Activity, Trinity Biotech - Jamestown, NY, USA).

Results: The analysis of consecutive measurements over time revealed a statistically significant negative correlation for PT (-0.264; p<0.001) and for Fibrinogen (-0.023; p< 0.008). MP, PAI-1:Ag and PAI-1:act showed a positive trend over time, however statistical significance was not reached. A linear regression study showed an inverse relationship of PAI-1: Ag and MP coeff (Mp= -0.005, P= 0.521). Interestingly, a polynomial relationship between PAI-1:act and MP was seen [PAI-1:act = cost+ $(MP/1000)^{-2}$ + $(MP/1000)^{2}$; coeff MP^{-2}:23.0; p=0.002; MP^{2} :11.9; p=0.006; Model , p=0.006] (Figures 1 and 2).

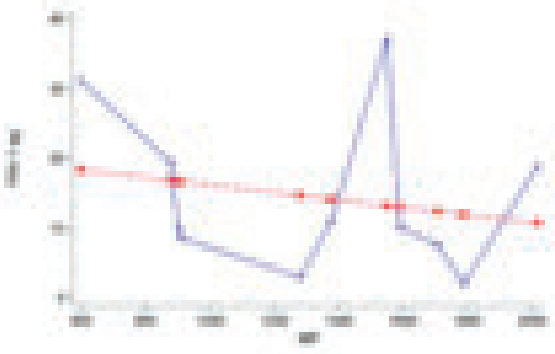

Figure 1. Inverse relationship of PAI-1: Ag and MP coeff (Mp=-0.005, P= 0.521.

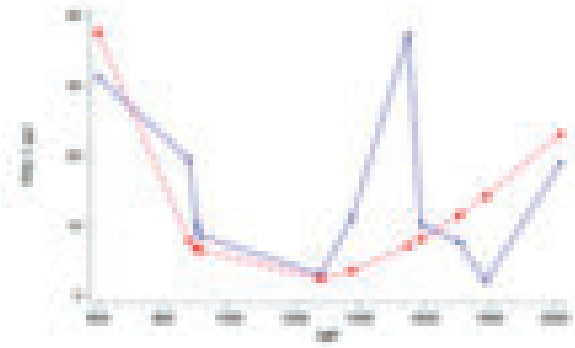

Figure 2. Polynomial relationship between PAI-1:act and MP was seen [PAI-1:act = cost+ $(MP/1000)^{-2}$ + $(MP/1000)^{2}$; coeff MP^{-2}:23.0; p=0.002; MP^{2} :11.9; p=0.006; Model , p=0.006.

Summary and Conclusions: In conclusion, this preliminary data shows that following chemotherapy for TW based on vincristin, actinomicine-D and doxorubicine, some coagulative changes are present and persist over time. Moreover, we demonstrated a polynomial relationship between PAI-1:act and MP in patients with WT after chemotherapy. Although our numbers are small, we believe that prospective studies on MP and PAI-1 may help to shed light on VOD pathophysiology.

PB2051

VENOUS THROMBOEMBOLISM: EPIDEMIOLOGICAL DATES AND RISK FACTORS IN OVER AGE 55

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Background: The venous thromboembolism (VTE) occurs in one-two of every thousand individuals per year and rises exponentially with age after 60. Certainly, around a third of patients with sintomatic VTE manifets pulmonary embolism (PE), whereas two-thirds manifest deep vein thormbosis (DVT) alone. Overall, 25% to 50% the patients with first-time VTE have an idiopatic condition, without a readily identifiable risk factor. Although use of oral contraceptives (OAC) have been associated with VTE in women, published data suggest no consistent differences in the incidence of VTE among men and women.

Aims: *This study aims to analyze* the etiopathogenic data patients that have VTE at age below 55 and check whether there were gender differences in relation to the location of the thrombotic event and the presence or absence of risk factors.

Methods: The analysis was performed on 300 consecutive patients (150 men and 150 women) with VTE at age below 55, seen in Doctor Negrin Hospital de GC from August 2007 to October 2013. Data were obtained from the software (Sintromac web 3.4) and a local base. Data were collected regarding a thrombotic event location, risk factors and results of the thrombophilia.

Results: In this observational study, the results was: 1. The mean age of presentation of the first thrombotic event was 38 years. 2. The location of the thrombotic event: DVT of the lower limbs (DVT LL) was seen in 215 patients (71.6%) of which 37 (17.2%) were embolized in lung; 28 patients showed pulmonary embolism (PE) without evidence of DVT so the overal prevalence of PE was 22,3%. We saw 24 (8%) DVT in upper limbs (DVT UL), 10 (3,3%) cerebral venous sinus thrombosis (CVST) and 15 (5%) abdominal thrombosis (AT). 3. No triggers of thrombosis were found in 182 patients at the prevalence of primary VTE was 60.6%. Among secondary VTE, major trigger of thrombosis were surgery, trauma and immobilization; in 6 patients the thrombotic event was paraneoplastic. A third of women taking oral contraceptives, half of which, presented a trigger usually associated with trauma/immobilization. 4. We found 54 cases to congenital thrombophilia (22.6%) of the 238 patients studied; one hundred and three patients (33%) were obese (BMI>30) and 59 (19%) presented two or more cardiovascular risk factors.

Summary and Conclusions: Results from the present study indicate that there are any differences among sexes except the higher frequency of LLL DVT in women and more CVST in women too, while the abdominal thrombosis is more common in men.

PB2052

VENOUS THROMBOEMBOLISM (VTE) IN PATIENTS WITH PANCREATIC CANCER

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Background: Venous thromboembolism (VTE) is a critical complication of malignant disease. Pancreatic cancer is one of the cancers most commonly associated VTE. In general, the VTE incidence rate of Asians is lower than that of Caucasians. In 2007, a Korean study reported that only four cases of VTE (5.3%) occurred in Seventy five patients with advanced pancreatic adenocarcinoma.
Aims: We evaluated VTE incidence in pancreatic cancer and characteristics of pancreatic cancer patients with VTE.
Methods: We retrospectively reviewed the medical records of patients with histopathologically proven pancreatic cancer from January 2006 to December 2012 at Soonchunhyang university hospital. We detected VTE through CT (chest CT, pulmonary embolism CT) and low extremity ultrasoundgraphy.
Results: Five hundred and fourteen patients with pancreatic adenocarcinoma were enrolled. (M: F, 300:214, localized: locally advanced: metastatic=31:230:253, mean age: 66.7 years). Ninety six of 514 patients (18.6%, symptomatic: aymptomatic=38:58, PE: DVT: PE+DVT: visceral thrombosis=20:19:19:38) were diagnosed as VTE. At the time of DVT diagnosis, cancer status of 50 patients cancer was progression, and that of 15 was stable. Thirty one patients were diagnosed with the pancreatic cancer and VTE, at the same time. They all had metastatic lesions. Fifty VTE patients were treated with antithrombotic therapy. Ninety three of 96 patients died, and three of them have probability that cause of death was VTE. The others died of pancreatic cancer progression. From pancreatic cancer diagnosis to VTE diagnosis, the period is 1.7month. (95%CI 1.1-2.3 month). Median overall survival (OS) was not significantly different between pancreatic cancer with VTE or without VTE. OS was significantly longer VTE patients after pancreatic cancer diagnosis than VTE patients with pancreatic cancer at the same time (10.73m *vs.* 1.7, p=0.00).
Summary and Conclusions: The incidence of VTE (18.6%) in Soonchunhyang university hospital with pancreatic cancer was not lower than that in western groups.

PB2053

PLATELET INDICES IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background: The involvement of platelets in the pathophysiology of coronary artery disease has long been recognized and well defined. Large platelets, which can easily be identified during routine hematological analysis, are associated with increased platelet activation and thrombosis, because they produce more prothrombotic factors and aggregate more easily. Mean platelet volume (MPV) reflects to the platelet size and the rate of platelet production in bone marrow, and it may be used as an indicator of platelet activation. PDW indicates the platelet distribution width and p-LCR indicates the percentage of platelets larger than 12 fL.
Aims: The aim of this study is to determine the possible impact of platelet indices, such as mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) on the incidence of acute myocardial infarction.
Methods: Over a period of nine months, from 12/05/2013 to 12/02/2014, platelet indices MPV, PDW and p-LCR of 77 patients with acute myocardial infarction were identified. Also, 77 patients without acute myocardial infarction were included in this study as a control group. The two groups were similar in regard to sex (66 males - 12 females) and age (29 - 93 years avg = 63 years). The blood samples were drawn and analyzed on the first morning after admission. Platelet indices were measured by SYSMEX XE-5000. A series of statistical analysis (descriptive statistics, compare means and correlations) have been used to demonstrate the relationship between patient with acute myocardial infarction and MPV, p-LCR, PDW indicators. The significance level was defined as $p<0.05$. All statistical analysis was performed using SPSS 20 and Mc Excel 2010.
Results: All PVI [mean platelet volume (MPV), platelet distribution width (PDW), and platelet large cell ratio (p□LCR)] were significantly raised in patients with AMI (mean MPV, 11.09 (SD, 1.02) fL, mean PDW, 13.95 (SD, 2.28) fL, mean p□LCR, 33.06% (SD, 8.32%) compared with those from the control group (mean MPV, 10.72 (SD, 0.99) fL, mean PDW, 12.93 (SD, 2.25) fL, mean p□LCR, 30.73% (SD, 8.09%). T test analysis was used in order to examine the statistical significance between patients with acute myocardial infarction and the indicators. All the findings revealed 0.000 ($p<0.05$) significance and a positive correlation between these indicators and acute myocardial infarction MPV ($r=0.15$, $p<0.05$), PDW ($r=0.004$, $p<0.05$), p-LCR ($r=0.021$, $p<0.05$).
Summary and Conclusions: This study has shown an elevation of MPV, PDW and p-LCR in patients with acute myocardial infarction and a statistically significant correlation between the platelet indices and AMI. Elevated values of the platelet indices suggest that the presence of large platelets, possibly juveniles, which are more active than the smaller ones, play a major role in coronary thrombus formation. Platelet indices are potentially useful biomarkers of platelet activity because they are easily accessible and widely used laboratory tests, as a part of a routine complete blood count. Further research is required in order to define the prognostic role of platelet indices in the pathogenesis of acute myocardial infarction.

PB2054

CEREBRAL VENOUS THROMBOSIS IN THE SETTING OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) AND APLASTIC ANEMIA (AA): A CASE REPORT

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Background: The most frequent and feared complication of paroxysmal nocturnal hemoglobinuria (PNH) is thrombosis. Because the mortality rate of cerebrovascular events in PNH patients remains high, prompt diagnosis and urgent intervention is required. The propagation of thrombosis and the occurrence of further thrombosis appears to be prevented by eculizumab.
Aims: Development of any thrombosis in a patient with PNH is now considered one of the primary indicators to commence eculizumab therapy, and this should be done without delay.
Results: We report a case of a 68-years old woman who was diagnosed with aplastic anemia (AA) in 2009 and was treated with rabbit anti-thymocyte globulin and cyclosporine A (CsA) with a good response for four years. In June 2013, for worsening of laboratory data, we performed bone marrow biopsy that showed a cellularity of 40% with a normal maturation of the three series. FC analysis revealed two discrete populations of cells one of which showed levels of CD55 and CD59 antigen expression compatible with the diagnosis of PNH (granulocyte 93,6%, monocyte 93,4%, red cell 8,6%). The eculizumab beginning was delayed for the difficulty to source tetravalent meningococcal vaccination. She was admitted to our hospital one month later for development of seizures. Laboratory data showed worsening of renal function (creatinine 1,6 mg/dl), marked elevation of LDH (1127 U/L) with haptoglobin consumption. An MRI scan documented thrombosis of a left parasagittal cortical vein. She was treated with therapeutic dose of low molecular heparin and then oral anticoagulation when platelet count was steadily above 50000/mm^3. She started seizures prophylaxis, that was continued until now. After meningococcal vaccination, she promptly started eculizumab. The MRI of 3 months later showed a good response to the treatment with a reduction of extension of thrombosis. Thrombophilia evaluation was negative. In September 2013 there was progressive worsening of laboratory data: hemoglobin 7,4 g/dl, white cell count 1570/mm^3, platelet count 55000/mm^3, despite normal markers of hemolysis. Bone marrow cellularity was decreased (20%), with a certain degree of dyserythropoiesis and megacaryocite with slight dysplastic changes. We started steroid and then oral CsA, suspended a month later for renal toxicity. Indeed we started Danazol, in association with manteinance dose of eculizumab, with a discrete response particularly on platelet count (Figure 1).

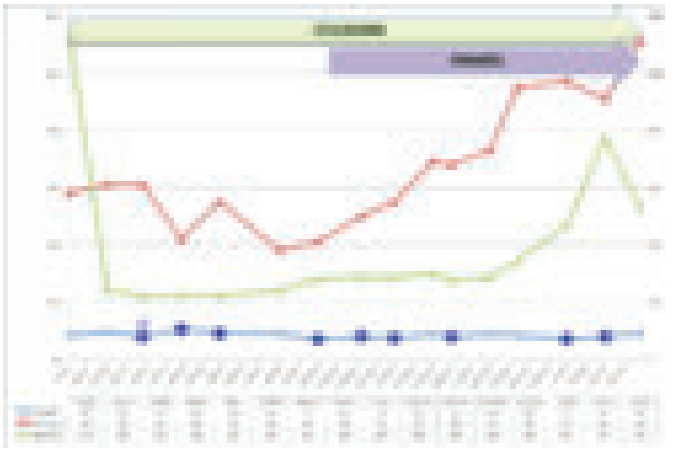

Figure 1.

Summary and Conclusions: Despite the availability of eculizumab, the management of paroxysmal nocturnal hemoglobinuria (PNH) remains complicated because of disease heterogeneity and close association with BM failure syndromes. The thrombophilia of PNH also appears to be ameliorated

by eculizumab, but the drug has no effect on the BM failure component of the disease. If BM failure dominates the clinical picture, treatment should focus on the underlying BM failure syndrome. A better understanding of the pathobiology that underlies the thrombophilia of PNH is needed, and this case report demonstrate the complex relationship between PNH and BM failure syndromes that determine clonal selection and clonal expansion and may ultimately lead to therapy that targets the disease at the level of the hematopoietic stem cell. In conclusion, thrombosis has been well-recognized as the leading cause of death in PNH and the impact of eculizumab on thrombosis largely explain the improved survival seen with eculizumab therapy.

PB2055

EVALUATION OF RISK FACTORS FOR DEVELOPMENT OF POST THROMBOTIC SYNDROME IN PATIENTS WITH DEEP VEIN THROMBOSIS

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Background: The post-thrombotic syndrome (PTS) is an important, under-appreciated, chronic consequence of deep vein thrombosis (DVT). It is characterized by symptoms as chronic, persistent pain, heaviness, swelling, cramps and itching in the affected limb. Various risk factors are attributed to the development of PTS. There is a paucity of data from India.
Aims: To evaluate the incidence and risk factors for development of PTS in Indian DVT patients.
Methods: All the DVT patients at least 6 months after the diagnosis, attending the thrombosis clinic in the department of hematology at AIIMS, New Delhi from April 2012 to December 2013 were analyzed. A total of 102 patients were screened for the signs and symptoms of PTS. The severity of PTS was graded according to Villalta Scale and all the patients with PTS were looked for various risk factors. Patients with oral anti-coagulants were regularly monitored with INR. D-dimer assay (semi-quantitative) and Doppler ultrasound were carried out at the time of enrollment and at 3, 6 months interval.
Results: Median age of study patients (male: female: 82:20) was 35 years (range 17 - 68 years). Duration of symptoms of DVT at the time of diagnosis was varied from 2 to 180 days with median of 14 days. Body Mass Index of patients was from 13 to 33 Kg/m^2 (median: 24 Kg/m^2). Ninety eight patients had proximal and rest four had distal DVT. Most common site of DVT was left lower limb (63 patients). Median duration of resolution of symptoms of DVT was 18 months (range: 5 – 60 months). Fifty six patients (55%) had signs and symptoms of PTS and out of these, 15 patients had mild, 18 patients had moderate and 23 patients had severe PTS. Heaviness (62.5%) and pain (56%) in lower limb were the most common symptoms experienced by PTS patients. Venous ulcer (41%) was present in all patients with severe PTS. In univariate analysis development of PTS was strongly associated with male sex (p=*0.002*), proximal DVT (p=*0.001*), lesser achievement of therapeutic INR (less than 50% of total visits) (p=*0.048*), prolonged symptoms duration of DVT at the time of diagnosis (p=*0.001*) and prolonged symptoms resolution of DVT (p=*0.001*). In multivariate analysis, male sex (odds ratio 0.067, 95% CI 0.004-1.286, p=0.001), proximal DVT (odds ratio 1.234, 95% CI 1.026-2.625,p=0.03), prolonged symptoms duration of DVT at the time of diagnosis (odds ratio 1.029, 95% CI 1.009-1.033, p=0.004) and prolonged symptoms resolution of DVT (odds ratio 1.289, 95% CI 1.150-1.427, p=0.001) showed strong correlation. However, achievement therapeutic INR has lost its significant correlation (odds ratio 0.081, 95% CI 0.001-4.601, p=0.223). D-Dimer remained elevated in all patients having chronic DVT and moderate and severe PTS but only in few cases of mild PTS and had high statistical significance (p=*0.001*).
Summary and Conclusions: Post thrombotic syndrome was seen 55% of DVT patients. The development of PTS was strongly associated with male sex, proximal DVT, prolonged symptoms duration of DVT and prolonged symptoms resolution of DVT. D-dimer showed useful screening test and showed strong correlation with DVT and PTS.

PB2056

ACUTE EFFECT OF SILDENAFIL ON FIBRINOGEN LEVELS IN ERECTILE DYSFUNCTION PATIENTS

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Background: Acute effect of sildenafil on fibrinogen levels in erectile dysfunction patients.
Aims: Aim of this study is to investigate the acute effect of sildenafil on fibrinogen levels in men with erectile dysfunction.
Methods: 20 erectile dysfunction patients without known history of cardiovascular disease participated in the study. The study comprised a randomized double-blind, crossover trial with sildenafil or placebo administration. The study was carried out on two separate arms: one with sildenafil, 100 mg and one with placebo. Thereafter, following a 1-week washout period, patients were switched to treatment period with the other drug. Fibrinogen levels were measured by immunonephelometry. The measurements of inflammatory markers were made by researchers unaware of the study hypothesis.
Results: There were no differences in baseline fibrinogen levels between sildenafil and placebo sessions. Response is defined as net Drug 1 effect minus Drug 2 effect at each time point. P value on the graph refers to repeated-measures analysis of variance significance between Drug 1 and Drug 2 session throughout the study (drug interaction). Acute administration of sildenafil produced a significant reduction of fibrinogen (P<0.05, maximal absolute response of −45mg/dl at 4 hours).
Summary and Conclusions: The present study shows for the first time that sildenafil has an acute favourable effect on fibrinogen levels in patients with erectile dysfunction. This finding may have important implications in erectile dysfunction patients who are considered to be at increased cardiovascular risk.

PB2057

INCIDENCE OF VENOUS THROMBOEMBOLISM (VTE) IN ACUTE MYELOID LEUKEMIA (AML): RISK ASSESSMENT BY PADUA PREDICTION SCORE (PPS)

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Background: The hematological malignancies are associated with a high risk of venous thromboembolism (VTE). Low molecular weight heparin (LMWH) is usually recommended for VTE prevention in cancer patients. However, no experience has been reported in patients with acute myeloid leukemia (AML), usually at risk of bleeding because thrombocytopenia. The Padua Prediction Score (PPS) is a simple model of assessment to identify the thrombotic risk in hospitalized patients.
Aims: To evaluate the risk of VTE in patients with acute myeloid leukemia (AML) undergone conventional chemotherapy by the PPS.
Methods: We performed a retrospective analysis of patients with acute myeloid leukemia (excluded promyelocytic leukemia and patients undergone to allogenic transplantation) between January 2007 and May 2013. All patients had a central venous catheter and received at least two cycles of conventional chemotherapy. None of the patients received thromboembolic prophylaxis. We assessed VTE risk during the hospitalization and 90 days the end of treatment after.
Results: 43 patients were considered. The median age was 55 years (range 32-74) and 26 (60%) were male. Eleven patients were at high risk and thirty-two were at low risk. We observed 4 venous thromboembolic events (9.2%); three in low risk and one in high-risk patients (table2) Moreover three patients had thrombosis with platelets counts less than 100x10^{9} /L.
Summary and Conclusions: In our study, PPS is not useful to identify patients affected by AML at high risk for VTE. Nevertheless in those patients VTE risk Is quite similar to solid tumor. In prospective it could be useful to evaluate a modified PPS where patients affected by AML are identified at high risk (3 points). Of course these preliminary data have to be on larger number of patients.

PB2058

HOW WE SHOULD TREAT THE PATIENTS WITH CRYPTOGENIC ISCHEMIC STROKE OR TRANSIENT ISCHEMIC ATTACKS AND ANTIPHOSPHOLIPID SYNDROME?

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Background: As a result of retrospective and observational studies it was thought that stroke associated with antiphospholipid syndrome (APS) carried a high risk of recurrence, so anticoagulation with vitamin K antagonists (VKA) should be considered, but there is no strong evidence that it is more effective than aspirin. Guidelines issued in 2011 by the American Heart Association and American Stroke Association recommended antiplatelet therapy for patients with cryptogenic ischemic stroke or transient ischemic attacks (TIA) and who test positive for antiphospholipid antibodies (aPL). In contrast, VKA are recommended for patients with ischemic stroke who meet all the criteria for APS. But how we should treat the patients with cryptogenic ischemic stroke or TIA who meet all the criteria for APS?
Aims: Analyze recurrence of stroke and risk factors for recurrence of a serie of patients with cryptogenic ischemic stroke or TIA as the first thrombotic manifestation of APS, who received low-dose aspirin as prophylactic treatment during five years of follow-up.

Methods: Small retrospective study following six consecutive patients (1 man and 5 women, range of age between 35 and 68 years) with APS treated with aspirin 100 mg daily for a median of 2 years (mean 3.9 years) after cryptogenic ischemic stroke (n:4) or TIA (n:2). Analysis included laboratory and clinical criteria of APS, together with established cardiovascular risk factors, inherited thrombophilias, malignancy, immobilization and surgery.
Results: There were no significant differences in the frequency or titers of different antiphospholipid antibodies, cardiovascular or thrombophilic risk factors, with the exception of the age. Only two young patients (<50 years) had a recurrent TIA (after 1 TIA and 1 cryptogenic ischemic stroke, respectively).
Summary and Conclusions: Our results suggest that young patients (<50 years) with cryptogenic ischaemic stroke or TIA and APS may be at high risk of recurrence and low-dose aspirin doesn't appear effective for preventing recurrent stroke in these patients.

PB2059

THE ROLE OF THROMBOELASTOGRAPHY IN EVALUATING THE EVOLVING HYPERCOAGULABILITY IN PATIENTS WITH MYELOMA ON TREATMENT: A PILOT STUDY

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Background: Patients with myeloma have an increased risk of venous thromboembolism (VTE). Increased rates of VTE, above the baseline, have been noted in patients on specific myeloma treatment regimens. This is likely due to enhanced expression of tissue factor, increased cellular adhesion molecules, Von Willebrand Factor and thrombin generation and a decreased capacity of endothelial cells to activate protein C. Immunomodulatory agents, in combination with steroids or anthracyclines, lead to the highest rates of VTE, especially in the absence of thromboprophylaxis. Thromboelastography assesses the viscoelastic properties of whole blood under low shear stress. Its main advantage over routine tests of haemostasis is the global assessment of the different components of clot formation such as coagulation factors and platelets. It has mainly been used in the operative setting, to assess bleeding patients. However, it has also been used to assess hypercoagulability in the obstetric and surgical oncology setting. A recent study (Trelinski et al 2014) demonstrated that patients with myeloma display changes in ROTEM tests at the time of diagnosis that may indicate a prothrombotic state.
Aims: To investigate whether thromboelastography was a useful tool in evaluating the evolving hypercoagulability state in patients with myeloma.
Methods: Patient s with newly diagnosed/relapsed myeloma who met criteria for treatment were recruited. Fresh whole blood was taken by direct venepuncture into tubes containing citrate 0.105M at diagnosis and prior to cycle 2 of treatment. Samples were stored at room temperature for 30 minutes prior to analysis. 500µl of whole blood was activated with 12.5µl of Kaolin and thromboelastography was performed by the same technician on 340µl of activated blood in combination with 20µl of calcium chloride using a TEG 5000 Thrombelastograph®analyzer according to the manufacturer's protocol. The remainder of the tests were performed in the accredited hospital laboratory. Patients were excluded if they had a history of VTE or were on an anticoagulant.
Results: 8 patients were recruited. The mean (SD) age at recruitment was 72.8 (11.1) years. 5/8 had IgG myeloma and 6/8 were women. Table 1 shows the laboratory values at baseline and after one cycle of chemotherapy. A paired samples t-test was performed to see if there were any differences between the groups. Both the FVIII level and the VWF:RiCoF were significantly different between the groups. Among the thromboelastography indices that were looked at, only the alpha angle was significantly different between the groups. While the maximum amplitude appeared higher in the chemotherapy group, this was not significant.
Summary and Conclusions: Similar to previous studies, there was a significant increase In FVIII and VWF after chemotherapy. The α- angle was also significantly elevated in the chemotherapy group suggesting that thromboelastography may have a role to play in assessing the coagulation status in patients with myeloma as an increased α-angle has been shown to be associated with hypercoagulability. Thromboelastography has the advantage of being a relatively quick and inexpensive means of assessing a patient's coagulation status when compared with testing for other markers of hypercoagulability such as FVIII and VWF. The main drawback is that it is heavily dependent on platelets so if a patient is thrombocytopenic secondary to their disease, it may not accurately assess their coagulation status. This is only a small pilot study and so it would need to be replicated on a larger scale to validate it.

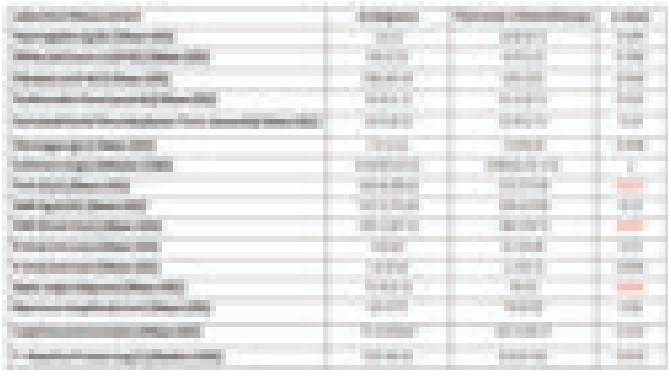

Table 1. Laboratory measurements at diagnosis and following one cycle of chemotherapy.

Quality of life, palliative care, ethics and health economics

PB2060

A CROSS SECTIONAL STUDY OF FACTORS EFFECTING BETA THALASSEMIA AMONG MULTI ETHNIC GROUPS IN PAKISTAN

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Background: Among hemoglobinopathies, thalassemia is constantly upturning the burden on economy of multicultural and multi-ethnic country like Pakistan. This autosomal recessive genetic disorder is caused by either reduced production or complete absence of beta-globin chains following production of immature erythroblasts due to dyserythropoiesis. Severe anemia, general fatigue, repeated or frequent blood transfusion following post transfusion hepatitis intensifies the severity of disease.
Aims: Present multicenter study undertaken in five cities of Pakistan aims to investigate the prevalence of beta thalassemia in different caste/ethnic groups using suitable approach.
Methods: Data for present study was collected from a period of 2011 to 2013. After consent, 450 patients of beta thalassemia excluding patients of any other blood disease were interviewed for different epidemiological parameters including their gender, age, transfusion history, family and personal history. Sero-negative blood samples were further preceded for RBC indices, quantification of hemoglobin including variant hemoglobin testing and other blood chemistry tests.
Results: Out of 450 patient samples 340 were seronegative. Beta thalassemia major (>95%) was most common followed by thalassemia intermedia (<5%) and very few structural variants (<1%) *e.g.* HbS and HbH. It was observed that in our population, females (55.29%) are more affected with beta thalassemia than males (48.82%). When compared to the normal age span, it was observed that only 2.64% of the patients crossed the second decade of their life. Early onset disease (before 6 months) was more common (24%) than the late onset *e.g.* above 24 months (10%). The high ratio of consanguinity (>80%) showed that it was in common practice among affected families. Further work on beta globin genotyping of these subjects is under progress.
Summary and Conclusions: Short life span and high number of HCV/ HbBAg status depicts that in a country like Pakistan, insufficient facilities, poor management and compromised socioeconomic status are deteriorating the disease status. More multicenter study covering cities from different regions of country are needed in developing preventive measurements at regional and national level.

PB2061

SAVING MONEY AND COMFORT BY GAINING CYTOSINE ARABINOSIDE MICROGRAMS

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Background: Cytarabine (4-Amino-1-(β-D-arabinofuranosyl)-2(1H)-pyrimidinone, Ara-C) a deoxycytidine analogue, is one of the most active drugs in the treatment of acute leukemias and is widely applied at a variety of other hematological malignancies. Although chemical and physical in-use stability of commercial Ara-C has been recommended for 48 hours at room temperature after dilution, from a microbiological point of view (data from drug informative sheet of Aracytin, available in Italy), the product should be used immediately. The cancer drugs preparation and dispensing in designated centralized chemotherapy (CHT) preparation pharmacy units (CPU) has been extensively studied to improve its quality and minimize personnel exposure to these drugs. We know relative little about the possibility to optimize and economize the use of CHT agents in CPU.
Aims: Here we explore the chemical and microbiological stability of a commercial Ara-C sample (Aracytin) in its pharmaceutical form after its reconstitution and storage at 4°C, in order to save drug.
Methods: Commercial Ara-C has been investigated under the common conditions of the clinical use to be degradable in water as in physiological solution. Ara-C samples reconstituted according to the commercial reported instructions and stored at 4°C, in controlled and validated aseptic conditions, were analyzed by high-field nuclear magnetic resonance spectroscopy (NMR). The results obtained were compared with those derived from identical samples bubbled with air at 4 °C, and 25 °C, at different time and concentrations, respectively. Microbiological assay, applying the cylinder-plate method, was performed. After an initial entry through the vial closure with a sterile syringe with needle, aliquots of reconstituted and unused solution were withdrawn after multiple aseptic entries trough the cap at 1 to 5 days and were assayed against solutions of reference standard.
Results: All the samples remained chemical unchanged for one month. Low temperature and scarce contact with air decrease the degradation process. We demonstrate the absence of any microbiological contamination, respecting the described aseptic condition, for at least 5 days.
Summary and Conclusions: We demonstrate chemical and microbiological stability of Ara-C solutions for at least 30 and 5 days respectively, allowing the use of the same vial for a prolonged time. It seems to be cheap and particularly useful for CPU and hospital, in order to economize without losing in efficacy and safety. We can also auspicate the use of the same vial, after a specific training about aseptic and sterile procedures to collect multiple doses, for outpatients who receive subcutaneously daily administration of low doses of Ara-C for more than a week. It could avoid to discard a great amount of drug and allow the outpatient to self administrate drug without daily return to the Hospital only for a simple and short-term injection. The opportunity to save Ara-C micrograms could positively impact both on health economic and patients quality of life.

PB2062

ASSESSMENT OF GERIATRIC SYNDROMES AND FUNCTIONAL STATUS IN ELDERLY HEMATOLOGIC CANCER PATIENTS IN TURKEY

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Background: Management of elderly patients with cancer is complex and requires a multidisciplinary approach. Utilizing Geriatric Screening tools for the identification of vulnerable older patients with hematologic cancer is needed.Geriatrics is an emerging and newly developed specialty in Turkey. This study is one of the first studies analyzing geriatric hematology cancer population by using geriatric assessment tools in Turkey.
Aims: The goal of this study was to characterize an elderly population with hematologic malignancies presented to hematology outpatient clinic and determine the prevalence of comorbidities, polypharmacy, functional dependencies and geriatric syndromes.
Methods: 48 patients with hematologic malignancies, age 51 years and older were assessed at hematology outpatient clinic over 3 months period. Numerous standard geriatric screening tests (ADL, IADL, MNA, Mini Cog and MMSE, TUG (timed up and go test), GDS (geriatrics depression scale) were administered to assess mood, functional status , nutritional and cognitive status. Demographic and medical data were obtained from patients' medical records.
Results: The mean age of the patients was 67.1 years (standard deviation, 10.6 years). There were 19 female (39.6%) and 29 male (60.4%). About 20.8% and 29.2% had limitations on one or more ADL and IADL domains respectively. Geriatric syndromes detected by geriatrician included cognitive impairment (minimal cognitive impairment (4.2%) and dementia (20.8%), depression (35.4%), risk of malnutrition (25%), and polypharmacy (54.2%).Out of 48 patients 83.3% had comorbidity score equals to 5 and more. TUG test as an indicator for fall risk found positive at 9 patients (18.8%).There were no statistically significant correlation between comorbidity and ADL , IADL scores. 18 patients were unaware of severity of their diagnosis but no correlation was found between awareness and mood.
Summary and Conclusions: In this descriptive study, many older hematologic cancer patients were found to have geriatric syndromes The study demonstrates that comprehensive geriatrics assessment provides insights into understanding the needs of elderly patients with hematologic cancer. Geriatrics is an emerging and newly developed specialty in Turkey. This study is one of the first studies analyzing geriatric hematology cancer population by using geriatric scales in Turkey. It revealed that numerous geriatric syndromes may be underdiagnosed in a regular visit. Larger and longer term well controlled clinical studies evaluating geriatric hematology cancer populations are needed in the future.

PB2063

THE BURDEN OF HEALTHCARE COST AMONG RELAPSED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS: (A SEER MEDICARE DATASET EXAMINATION)

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Background: DLBCL is the most common form of Non-Hodgkin Lymphoma in the US accounting for ~33% of newly diagnosed pts. For newly diagnosed pts treatment with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is the standard of care (Coiffier, NEJM 2002). While roughly 50% of first-line pts are cured with R-CHOP, older pts who relapse have a poorer prognosis and are often treated with a variety of therapies, but the downstream costs of this care are not well known.
Aims: This study examined initial and downstream health care costs among adult DLBCL pts who received additional lines of therapy (LOT) following R-CHOP.
Methods: Pts diagnosed with DLBCL and treated with R-CHOP first line between 2001 and 2009 were identified from the linked Surveillance, Epidemiology, and End Results (SEER) US Medicare database using ICD-O codes 9680-9689. Pts with at least two lines of therapy, neither of which were censored, were included in the study. The start of chemotherapy was considered the start of first line of therapy, and the regimen included all medications received in the first 36 days following therapy initiation. TheLOT ended with either the addition of a new chemotherapeutic agent or discontinuation of all agents. Second- and third-line therapy (LOT2 and LOT3) were identified using a similar algorithm. Receipt of stem cell transplant was not included when identifying LOT1 or LOT2. All health care costs from inpatient, outpatient, physician services, and medications were calculated during LOT1, during LOT2, and following LOT2. Mean monthly costs during LOT1 and LOT2 were calculated by dividing mean costs by length of LOT. Sensitivity analysis was conducted examining costs during LOT1 among pts with a non-censored LOT1.
Results: Among 6,617 pts diagnosed with DLBCL, 3,257 had a complete (non-censored) LOT1 of R-CHOP, and of those 352 had a complete LOT2. Mean age was 73 years, 47% were female, 86% were Caucasian. At diagnosis, 39% of pts had stage I/II, 53% had stage III/IV, and 7% no stage information. Mean (median) length of LOT1 was 113 days (104 days) with mean cost of $59,617 (median $37,539). Sensitivity analysis demonstrated that first-line costs (Mean: $62,055, Median: $37,168) were similar for pts with a first line of R-CHOP irrespective whether they subsequently received a 2nd line treatment or not. LOT2 started an average of 418 days after LOT1 (median 200 days; interquartile range 21 - 595 days) and lasted 56 days (median 43 days) with a mean total cost of $41,947 (median $23,276). LOT2 most commonly involved treatments with cyclophosphamide (55%), etoposide (43%), and vincristine (42%), but a number of different treatment strategies were used. For both LOT1 and LOT2, costs were comprised largely of outpatient services (60%), physician visits (33%), inpatient stays (6%), and pharmacy (0.5%,). Average monthly costs for LOT1 and LOT2 were $15,828 and $22,472, respectively. Median follow-up after LOT2 was 518 days (mean 753); initiation of a LOT3 was observed among 77 pts. Mean cost after LOT2 was $125,029 (median: $90,670).
Summary and Conclusions: Older DLBCL pts failing to respond adequately to first-line R-CHOP therapy incur substantial aggregate costs across their treatment history. Importantly, calculated average monthly costs were higher during a second line of therapy (*vs.* a first line) – representing almost a 42% increase in monthly costs when incur a first relapse.

PB2064

FEASIBILITY AND LONG TERM OUTCOME OF PERCUTANEOUS VERTEBROPLASTY IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Background: Vertebral fractures occur in over 70% multiple myeloma (MM) patients and can cause pain, disability and poor quality of life. This is particularly relevant for elderly patients, as pain and disability may add to other comorbidities. Vertebral fractures occur in over 70% multiple myeloma (MM) patients and can cause pain, disability and poor quality of life. This is particularly relevant for elderly patients, as pain and disability may add to other comorbidities.
Aims: The present study aimed at evaluating retrospectively the medium and long-term effects of percutaneous vertebroplasty when performed in elderly MM patients, in order to assess whether the procedure can result into subjective clinical improvement or lead to side effects not expected in younger patients populations.
Methods: Forty patients (24M/16F, median age 72 years, range 68-87 years) with newly diagnosed (n= 26) or relapsed-refractory (n = 14) MM and painful vertebral lesions underwent vertebroplasty prior to proceed to induction or salvage therapy. Eighty-four procedures were performed at D2-L5 levels, 26 patients were treated at ≥ 2 levels. The visual analog ten points scale (VAS) was used to evaluate pain prior to and upon completion of the procedure, on day +1 and monthly thereafter, till three months after the conclusion of the transplantation program. Pain response to vertebroplasty, irrespective of pretreatment values, was graded as follows: complete response VAS = 0; optimal response VAS = 1; good response VAS = 2-3, no response VAS ≥4
Results: Twenty-six patients (65%) obtained a complete or optimal pain control; this was not related to disease status, as at least optimal pain response was achieved in 69% newly diagnosed patients as compared to 57% relapsed-refractory ones. No difference in achievement of a complete/optimal pain response was observed in patients aged ≥ 75 yrs (70%) as compared to those<75 yrs (63%). Response to antimyeloma therapy, however, predicts for an optimal and more durable pain control, as patients achieving a very-good partial response or better had a significantly higher probability of pain control (p=0.04). No significant side effects were registered.
Summary and Conclusions: In conclusion, percutaneous vertebroplasty is useful in elderly MM patients with painful vertebral fractures as it allows a rapid and long-lasting pain control; the combination with an effective antimyeloma therapy may warrant better results

PB2065

AUTONOMIC DYSFUNCTION IS AUGMENTED DURING THERAPY, BUT CAN BE IMPROVED BY PHYSICAL EXERCISE IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY. RESULTS OF A PILOT TRIAL.

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Background: Autonomic dysfunction is common in patients with cancer and may have considerable negative effects on quality of life and mortality. Vagal nerve activity, indexed by heart rate variability (HRV), could have a role of prognosticator in cancer. Exercise enhances cardiac autonomic regulation of cancer patients. Because of the association of higher HRV-parameters and prolonged survival in cancer patients, improvement in autonomic control may be important goal of exercise.
Aims: Therefore, the aim of this prospective longitudinal study was to assess parameters of physical fitness and HRV in patients with hematological malignancy before, during chemotherapy and after supervised physical training.
Methods: Patients with hematological malignancies treated with systemic anticancer therapy were enrolled. HRV, physical fitness and muscle strength were measured in three time-points: before start o systemic therapy (15pts), after end of treatment (56pts with response) and after 12-week exercise program (30pts). HRV was tested by vagal activity (VA), sympatovagal balance (SVB) and global score (GS), physical fitness was measured with cycle ergometer (pVO_2max), muscle strength with hand grip test. Exercise training consisted of spinning (about 45-60%), resistance exercise (15-25%), remaining activities included balance exercise and stretching. Lesson duration was 50-90 minutes with intensity between 60-75% HRmax with frequency of three times per week.
Results: Totally 64 patients were included in this prospective study (43 NonHodgkin´s lymphoma, 14 Hodgkin lymphomas and 7 leukemias). Median age was 54 (19-77) years, and 36/64 pts were women. At baseline (before chemotherapy), physical fitness (pVO_2 max) was normal and HRV was only mildly reduced compared to common population. After treatment significant decrease in pVO_2max (p0.065) and in HRV were observed (SVB; p0.018). Exercise training lead to improvement of the global fitness measured by pVO_2max (p.0.0029), and influenced also HRV (VA p0.086; CS p 0.098); in 7 pair-matched patient subset HRV was significantly changed in all parameters (VA p.0.045, SVB p.0.01, CS p.0.01).
Summary and Conclusions: Exercise training is feasible and able to improve significantly physical fitness of patients with hematological malignancy. Training is also related to improvement in autonomic dysfunction and could potentially contribute to better survival. Further investigation is needed in this area.

PB2066

PROPHYLAXIS WITH LAMIVUDINE FOR THE PREVENTION OF REACTIVATION IN OCCULT HEPATITIS B (OBI) IN NHL : A COST-EFFECTIVE ANALYSIS

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Background: Occult HBV infection (OBI) is defined by the persistence of HBV in the liver without serum HBsAg and HBV-DNA. It represents a life threatening event during immunosuppressive chemotherapies. An OBI occurs in approximately 18% of HBcAb+ patients. International guidelines suggest surveillance for HBV markers in immunosuppressed patients, in particular

monoclonal antibodies. In our study, the prevalence of OBI reactivation in Non-Hodgkin Lymphoma (NHL), in 498 NHL patients in our centre of Southern Italy, was 10.42% in HBcAb+ HBsAb- patients. In this work, we want to perform a cost-effectiveness analysis regarding the use of Lamivudine for the prevention of reactivation in OBI in patients with Non-Hodgkin Lymphoma undergoing chemotherapy with or without Rituximab. In fact, considering guidelines and literature, universal prophylaxis should have been applied to all HBcAb positive HBsAg negative patient. A cost-benefit issue arises : is it more cost-effective to treat all the HBcAb positive HBsAg negative patients with Lamivudine to prevent the OBI reactivation occurrence in a small quote of them, or may it be more effective a "wait and see" protocol?

Aims: Our idea was to perform a cost-effectiveness analysis, comparing the costs of prophylaxis of an eventual HBV reactivation and the "monitoring" approach that was used in our patient based on the international guidelines.

Methods: We calculated the cost of prophylaxis with Lamivudine in a time interval of twelve months, which encompasses the time of a standard Rituximab-containing chemotherapeutic protocol and a minimum time of follow-up. It has to be noticed that, very often, NHL patients need more than one chemotherapy cycle to obtain NHL remission, and, sometimes, if they do not obtain a complete remission, undergo to long-term "maintenance" treatments with Rituximab. These patients (HBcAb positive) are at high risk of HBV reactivations, due to long times of immunosuppression.

Results: Nevertheless, even if our calculations underestimated the costs of prophylaxis, the "monitoring approach" resulted cost-effective. Moreover, even though in our series no serious events in terms of morbidity and/or mortality occurred, in other literature reports a monitoring approach did not guarantee patients survival. These detrimental results could be ascribed to the delayed start of Lamivudine treatment if the monitoring is not adequately strict (less than one evaluation/month). Also, it has been reported that performing only the transaminase monitoring should not be acceptable to prevent severe reactivations. Our monitoring approach resulted efficacious probably because of the monthly ALT assay was strictly observed (Table 1).

Table 1.

Summary and Conclusions: We report an advantage in the "monitoring" approach that was used in our patient in respect to universal prophylaxis. Due to the retrospective nature of our study, we cannot draw any firm conclusion on which should be the best approach for patients with isolated HBcAb positivity who should undergo chemotherapeutic regimens (universal prophylaxis *vs* monthly ALT monitoring). A randomized controlled trial might be needed to properly address this issue.

PB2067

THE SOUTHERN TRANSYLVANIA PATIENTS' OPINION ON THE RELATIONSHIP BETWEEN THROMBOPHILIA AND QUALITY OF LIFE

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Background: Thrombophilia is a public health problem which is increasingly being studied and whose complications are increasingly recognized in practice.

Aims: We aimed to study the opinion of hospitalized patients on the relationship between thrombophilia and quality of life.

Methods: A transversal study was performed on a sample of 246 consecutive hospitalized patients in medical departments of Emergency County Clinical Hospital Sibiu in January 2014 who responded to a questionnaire on the relationship between thrombophilia and quality of life. The results were analyzed and they have allowed conclusions that we hope to have implications for clinical practice.

Results: The mean age of surveyed patients was 53,32+/-15,35 years. Distribution by gender: women 56,10%, men 43,90%. Less than half of respondents (45.53%) have heard of thrombophilia, but we explained them that it is a hypercoagulable state that predisposes to thrombosis. The appearance of a lower limb deep venous thrombosis can be life threatening in the opinion of 60.16% of patients. Most patients (89.43%) would agree to about 10 days of injectable anticoagulant therapy if they had deep venous thrombosis, but only 62.68% of respondents would accept long-term therapy with acenocoumarol, under periodic control of INR (weekly in the first month, then monthly). Most people believe that the health ministry should include analyzes that allow diagnosis of thrombophilia in basic health services package in order to be free. The majority (86.18%) believe that loss of vision in one eye caused by central retinal vein thrombosis (one of the thrombophilia complications) reduces quality of life. A similar proportion of respondents (87.8%) believe that stroke increases maintenance costs related to thrombophilic patient. The vast majority of surveyed patients (91.06%) believe that a paralyzed and bedridden retired in Romania does not have sufficient income to support himself. Over 60% of those surveyed believe that a woman who is going to remain pregnant should be subject for thrombophilia screening because it can cause abortion. Fewer than half of them (47,97%) believe that the anticoagulant treatment carried out throughout all their life due to a thrombophilia complication reduces the quality of life of the patient. The majority (77.24%) advocates for popularizing thrombophilia. They opt for talks with your GP (58,78%) and media (20,94%) and less for letters addressed to the patients (12,16%), messages sent over the internet (5,41%), conferences, or facebook (by 1.35%). The majority (86.18%) agree that it is cheaper to prevent thrombophilia complications through early diagnosis and information than to treat them (after they have occurred).

Summary and Conclusions: Patients hospitalized in Southern Transylvania want to be informed about thrombophilia especially by their family doctor. Their complications reduce the quality of life, which is why it is desirable that they are prevented. They believe that to resolve the problems raised by thrombophilia the state should get involved financially.

PB2068

ASSESSMENT OF QUALITY OF LIFE IN THALASSEMIA PATIENTS IN A DEVELOPING COUNTRY

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Background: Thalassemia major is an inherited disorder requiring life- long blood transfusion and chelation therapy. It is also associated with various complications arising out of iron overload and transfusion transmitted infections. With increasing lifespan of these patients due to advances in the medical management, quality of life has become an important parameter reflecting the efficacy of health care system.

Aims: To assess the quality of life of thalassemia patients attending thalassemia day care centre in a tertiary care hospital in India.

Methods: 60 thalassemia patients between 10-18 years of age regularly attending Thalassemia Day Care Centre were included in the study. Quality of life was assessed by using WHOQOL-BREF scoring. The QOL was assessed on four parameters. The QOL was assessed on four parameters. Independent variables evaluated included clinical and sociodemographic characteristics.

Results: The mean overall score was found to be 119.37 with scores in physical, psychological, social and environmental domains being 135.7, 101.6, 59.23 and 134.89 respectively. Higher socioeconomic status was related to higher scores. Presence of co-morbidities like TTI'S & endocrinopathy was associated with lower overall score.

Summary and Conclusions: Adolescent thalassemics in our unit have reasonable quality of life. An association is found between co-morbidities and quality of life.

PB2069

EVALUATION OF MENTAL HEALTH AND PHYSICAL PAIN AMONG PATIENTS WITH BETA-THALASSEMIA MAJOR IN NORTH GREECE.

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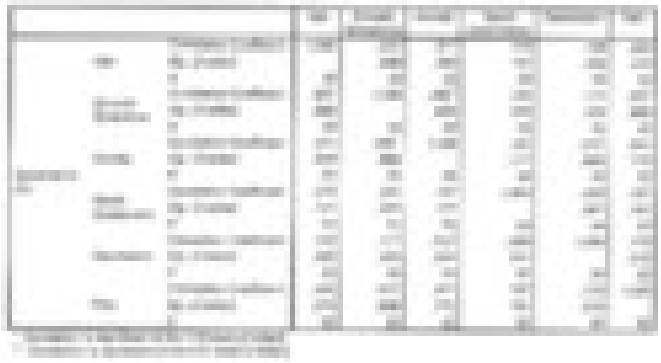
Background: Beta-thalassemia major (β-TM) is a chronic, genetic blood disorder. Patients are considered to be vulnerable to emotional and behaviour problems.

Aims: The aim of this study was to assess mental health and the somatic pain of patients with homozygous β-TM who are transfused systematically in our unit.

Methods: In this survey, 54 adults patients aged 37,5+/-18,5 years were studied. The female patients were 59,3%. The general health questionnaire by David Goldberg (GHQ-28) was used as mental health assessment model aiming to detect mental disorders. The model of Binary was used as scoring method of GHQ-28. Overall rating below 5 indicates no psychiatric problem,

while total score over or equal to 5 indicates the likelihood of diagnosis with any psychiatric disorder. The GHQ-28 allows separate evaluation of four subscales which are "somatic symptoms", "anxiety", social dysfunction" and "depression disorder". The Visual Analogue Scale of Pain (VAS) was used as model for pain evaluation. Data was analyzed with software SPSS 17.0 and Microsoft Office Excel 2010. The significance level was set at p=0.05.
Results: One out of four examined patients was presented with GHQ-28 score above or equal to 5 as having an increased chance to be diagnosed with any psychiatric disorder. Concerning the pain, the majority of the studied patients assessed at scores between 1 and 3 meaning that they are feeling mild pain. There is no statistical significant correlation between age and GHQ-28 score. There is a statistical significant correlation between age and somatic symptoms (p=0,026), anxiety and somatic symptoms (0,004) as well as anxiety and depression (p=0,022) (Table 1).

Table 1. Correlation between the examined parameters.



Summary and Conclusions: Thalassemic patients tend to be diagnosed with psychiatric disorders and it seems that they do not feel severe pain. More quantitive and comprehensive studies has to be conducted in order to estimate specific effective factors in psycho-social health.

PB2070

ECOG ROLE IN THE ASSESSMENT OF THE FUNCTIONAL STATUS OF ELDERLY PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Cancer is the leading cause of death in elderly patients aged 60 to 79 years. ECOG performance score is a common tool in everyday practice but an older cancer population requires more specific tools to access their condition as Comprenhensive Geriatric Assesment (CGA).
Aims: The aim was to assess whether ECOG score correlate with CGA assessment.
Methods: The 32 patients older than 65 years (with different ECOG score) diagnosed with hematologic malignancies hospitalized in Medical Center "Bezanijska kosa" underwent uniform CGA assessment including Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Mini Mental Status Examt (MMSE), Geriatric Depression Scale (GDS) and Mini Nutritional Assessment (MNA).
Results: Median age of 32 pts was 75 years (min 65, max 86). Eighteen (56%) patients were ECOG≤1, and 14 (34%) ECOG ≥2. According to CGA results, ADL dependence was found in 8 (25%) pts, IADL in 11 (34,4%) pts, minimal cognitive impairment was present in 8 (18.8%) pts; dementia in 13 (40.6%) pts; 11 (34,4%) pts were at risk of depression; 15 (46.8%) pts were at risk of malnutrition. ECOG correlated (Pearson coefficient) with ADL, IADL, MMSE, GDS and MNA with statistically significant difference with ADL (p=0.001), IADL (p=0.001), MMSE (p=0.038) and MNA (p=0.029).
Summary and Conclusions: A high proportion of elderly patients with hematological malignancy and poor ECOG score presents with abnormalities on a baseline CGA. ECOG correlate with CGA components and could be used in estimation of ADL, IADL, MMSE and MNA.

PB2071

ANALYSIS OF QUALITY-OF-LIFE AFTER RED BLOOD CELL TRANSFUSIONS IN HEMATOLOGICAL MALIGNANCIES PATIENTS WITH ANAEMIA

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Background: Anaemia frequently decreases survival rate and overall Quality-of- life (QoL) in patients with hematological malignancies (HM). Red blood cell (RBC) transfusions can quickly correct severe symptoms of anaemia, increase hemoglobin (Hb) and improve QoL.
Aims: To study QoL in HM patients with anaemia before and after RBC transfusions.
Methods: HM patients (n=69) with anaemia: myelodysplastic syndrome (n=12), acute myeloid leukemia (n=10), primary myelofybrosis (n=8), chronic myeloid leukemia BC (n=3), multiple myeloma (n=17), non-Hodgkin's lymphoma (n=5), chronic lymphocytic leukemia stadium C (n=12) and acute lymphoblastic leukemia (n=2). The median age of patients was 64.0 years (range 21-82). Initial Hb concentration was <8.0 g/dl and target Hb > 8.0-9.9 g/dl. QoL was assessed with the Functional Assessment of Cancer Therapy-Anemia questionnaire scale General (FACT-G) and Anaemia (FACT-An). Patients filled up the special forms of questionnaire before and after RBC transfusion.
Results: Mean baseline Hb concentration in our patients was 4.0-8.9 g/dl (Me=68 g/dl). During the hospitalization period were transfused 1-9 (mean 3.4±2.1) RBC Units. Hb concentration increased from baseline to 7.9-9.9 g/dl (Me=98; p<0.01). The Hb increasing was 0.88±0.54 g/dl per Unit. The most patients registered reducing symptoms of amaemia. After RBC transfusion in the most subscales of FACT-An we observed improving. In FACT-G in measuring domains of "Physical well-being" were ascertained of improving from 12.9±5.3 to 11.0±5.7 points (p<0.001), "Social/ family well-being" - from 13.4±4.4 to 13.6±4.3 (p=0.6), "Emotional well-being" - from 9.2±4.8 to 8.0±4.8 (p<0.05), and "Functional well-being" - from 9.6±4.7 to 11.0±4.5 (p<0.02). In FACT-An Anemia subscale we observed improve from 35.9±11.2 to 29.8±11.5 points (p=0.001). In detail study of Anemia subscale we found out statistically significant (p<0.05) improve at the next items: 1-6, 10-13 and 15 of the FACT-An questionnaire. 1) I feel fatigued; 2) I feel weak all over; 3) I feel listless ("washed out"); 4) I feel tired; 5) I have trouble starting things because I am tired; 6) I have trouble finishing things because I am tired; 7) I have energy; 8) I have trouble walking; 9) I am able to my usual activities; 10) I need sleep during the day; 11) I feel lightheaded; 12) I get headaches; 13) I have been short of breath; 14) I have pain in my chest 15) I am too tired to eat; 16) I am interested in sex; 17) I am motivated to do my usual activities; 18) I need help doing my usual activities; 19) I am frustrated by being too tired to do the things I want to do; 20) I have to limit my social activity because I am tired.
Summary and Conclusions: In this study was showed that RBC transfusions effective improve QoL in hematological malignancies patients with anaemia.

PB2072

ANALYSIS OF SOME ASPECTS OF QUALITY OF LIFE IN PATIENTS WITH ACUTE LEUKEMIA IN REPUBLIC OF ARMENIA

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Background: Since hematological cases can be treated and prolongation of life is achieved, many different spirituals and psychological concerns must be addressed to maintain smooth functioning and optimize quality of life (QoL).
Aims: Analysis of some aspects of quality of life in patients with acute leukemia in Republic of Armenia
Methods: This research study has been designed the first time to place into hematological practice in Armenia a modified QoL questionnaire and find out correlations between efficiency of treatment of AL and QoL.
QoL questionnaire include common and disease related specific problems: over 50 questions about psychological and functional conditions of the patients their relationship with others, self-esteem issues, etc. The questionnaires were first filled out within the 7 days after admittance and after two weeks.
Results: The study of questionnaires revealed that weakness impaired the patients' QoL in 80% (n=64), the bone pain – in 85% (n= 68), fever- in 75% (n=60), dyspepsia – in 60% (n=48), followed by the decreased ability to work – in 60% (n=48) and lower personal relations – in 62.5% (n=50) of cases. Patients with high-risk and AML have shown the poorer indicators of QoL then the patients with low-risk and ALL. Acknowledgment and understanding by the patients, that many of side effects and spiritual feelings are impermanent and predictable, may create sense of confidence. On the other hand, fear, anxiety and aloofness would complicate extensively the course of clinical management and expected outcomes in each single case. Our research data indicates that patients in general trust and are confident in their healthcare practitioners (physicians and nurses). The first step towards dealing with the routine chemotherapy treatment is communication. The second step is QoL information: perception that many of depressive feelings are predictable and not fatal, and many of side effects are impermanent, may become meaningful. Low personal relations, depression and anxiety, financial burden problems play negative and significantly impair patients' QoL.

Summary and Conclusions: The modified QoL questionnaire for the patients with AL is one of the well-validated instruments to place into everyday practice to obtain reliable scores and meaningful data on QoL and it should become an integral part in AL treatment decision-making.

PB2073

DEVELOPMENT OF PROGRESS TEST BASED ON EHA CURRICULUM AND EHA CV PASSPORT, USED FOR YEARLY EVALUATION OF HEMATOLOGY RESIDENCY IN SWEDEN

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Background: In Sweden, after 5.5 years of medical school and 1.5 years of general internship, you can pursue a residency in internal medicine and hematology, which takes an additional 6.5-7 years. This can take place at a University clinic, as well as at non-University hospitals.
Aims: The Swedish Hematology Association appointed a Work Unit (WU) of resident hematologists lead by two senior colleagues with a vast teaching experience, to develop a yearly progress test for evaluation of hematology residents in Sweden. The framework for the progress test was the EHA curriculum and EHA CV Passport, which divides the Hematology Specialty into eight large parts, with subunits to systematically cover the whole of Hematology.
Methods: Individuals or groups of Swedish experts in each field, *e.g.* the National Diagnostic Groups, were asked to produce a number of multiple choice (MCQ) questions. The questions were read, revised and sorted by the appointed WU, after which 75 MCQ questions were selected for the first progress test. These questions covered the eight major fields of the EHA CV Passport: benign clinical hematology (n=8), myeloid malignancies (n=11), lymphoid and plasma cell disorders (n=18), stem cell transplantation and special therapy (n=9), laboratory diagnosis (n=11), thrombosis and hemostasis (n=8), transfusion medicine (n=4 as this is a separate specialist education in Sweden, not sorting under Hematology), and general skills (n=5). The WU has prepared, revised and sorted questions for another three progression testes. The test was available via our homepage www.sfhem.se for one week in November 2013, whereafter the test was removed and the answers posted. Instructions were to complete the test within 2 hours, and when answers were available, sit down and discuss with the clinical mentor. Two months after the progress test, we sent out a questionnaire about the contents and success rate.
Results: Among 90 listed resident physicians in the Swedish Hematology Association registry, 48 work at university hospitals and 42 at non-university hospitals. Ten residents responded that they did not take the test, and until now 36/80 questionnaires have been completed. Correct answers varied from 36-60 points of the maximum 75 points. Years of active work in hematology varied from 1.5-5.5 years, where higher scores of correct progress test answers were associated with longer clinical experience. Comments from residents were that the test was well received, the questions adequate to assess skills, and that it will help point out weaknesses to work on. Furthermore, residents expressed eagerness to take the test again next year to see improvement.
Summary and Conclusions: There is a need for continuous evaluation of acquired skills during residency, and a progress test that reflects the EHA CV Passport may be good for yearly progress evaluation. Thus, we will continue this work with a forthcoming test in November 2014.

LATE BREAKING ABSTRACTS

Late breaking 1

LB2433

RESULTS OF A PHASE 3, MULTICENTER, RANDOMIZED, OPEN-LABEL STUDY OF AZACITIDINE (AZA) VS CONVENTIONAL CARE REGIMENS (CCR) IN OLDER PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML)

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Background: Older patients (pts) with AML have poor prognoses. Previous studies with a range of chemotherapy drugs or best supportive care (BSC) only have consistently shown median overall survival (OS) of 2-8 months (Oran 2012; Kantarjian 2012). Azacitidine (AZA) prolonged OS *vs* conventional care regimens (CCR) in older AML pts with low bone marrow (BM) blast percentage (20-30%) (Fenaux 2010).
Aims: To compare effects of AZA *vs* CCR on OS, response, and safety in a large cohort of older pts with newly diagnosed AML in an international phase 3 study.
Methods: This multicenter, randomized, open-label study (ClinicalTrials.gov NCT01074047) included pts aged ≥65 years (yrs) with newly diagnosed *de novo* or secondary AML (>30% BM blasts), ineligible for allogeneic stem cell transplant. Pts were to have ECOG PS 0-2; WBC ≤15x10^9/L; and intermediate- or poor-risk cytogenetics. Before randomization, pts were preselected to receive 1 of 3 CCRs per investigator choice of best treatment (Tx): intensive chemotherapy (IC; standard 7+3 regimen), low-dose Ara-C (LDAC; 20 mg SC BID x10d/28d cycle), or BSC only. Pts were then randomized to AZA (75 mg/m^2/d SC x7d/28d cycle) or CCR, and received their preselected Tx. The primary endpoint was OS. Additionally, a prespecified sensitivity analysis for OS censored pts at start of subsequent AML Tx. OS was estimated by Kaplan Meier methods and compared by log-rank test. Also measured were 1-yr survival and hematologic response per IWG AML criteria (Cheson 2003). Grade 3-4 hematologic adverse events (AEs) were assessed per CTCAE v4.0.
Results: In all, 488 pts (median age 75 yrs, 59% male) were randomized to AZA (n=241) or CCR (n=247 [BSC n=45, LDAC n=158, and IC n=44]). Baseline demographic and disease characteristics were similar between groups. In the AZA and CCR arms, 20% and 15%, respectively, had AML secondary to MDS, 35% and 34% poor-risk cytogenetics, median blast counts 70% and 74%, and 77% of both groups had ECOG PS 0-1. Median (range) Tx cycles were 6 (1-28) with AZA, 4 (1-25) with LDAC, 2 (1-3) with IC, and median exposure 65 days (6-535) with BSC. Median OS (95%CI) in the AZA arm was 10.4 (8.0-12.7) months (mos) *vs.* 6.5 (5.0-8.6) mos with CCR (unstratified HR=0.84 [95%CI 0.69, 1.02], p=0.0829; stratified HR=0.85 [95%CI 0.69, 1.03], p=0.1009) (Figure 1). The sensitivity analysis showed a significant AZA benefit: median OS was 12.1 (9.2–14.2) mos *vs* 6.9 (5.1–9.6) for CCR (stratified HR=0.76 [95%CI 0.60, 0.96], p=0.019) (Figure 2). One-yr survival was 47% with AZA *vs* 34% with CCR, for a significant difference of 12.3% (95%CI: 3.5%, 21.0%). Responses of CR and CRi, respectively, were seen in 20% and 8% of AZA pts and 22% and 3% of CCR pts. Grade 3-4 anemia, neutropenia, febrile neutropenia, and thrombocytopenia rates, respectively, were 16%, 26%, 28%, and 24% with AZA; 5%, 5%, 28%, 5% with BSC; 23%, 25%, 30%, 28% with LDAC; and 14%, 33%, 31%, 21% with IC. There was no difference in 30- or 60-day mortality between Tx groups.
Summary and Conclusions: Median OS with AZA was 10.4 mos, a 3.8 mos (58%) increase over CCR and 1-yr survival with AZA was 47%, a 36% increase *vs* CCR. These findings represent the largest OS and 1-yr survival benefits seen with a low-intensity therapy in elderly AML (Kantarjian 2012; Burnett 2007, 2013). Rates of grade 3-4 hematologic AEs were higher with AZA than BSC,

and similar to LDAC and IC, and consistent with previous experience. These data show that AZA is an effective and safe therapy for newly diagnosed elderly AML pts.

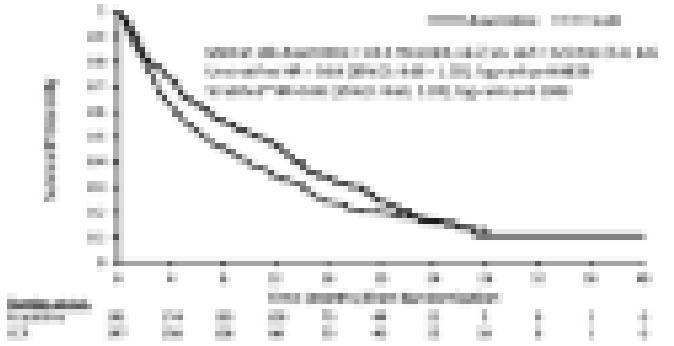
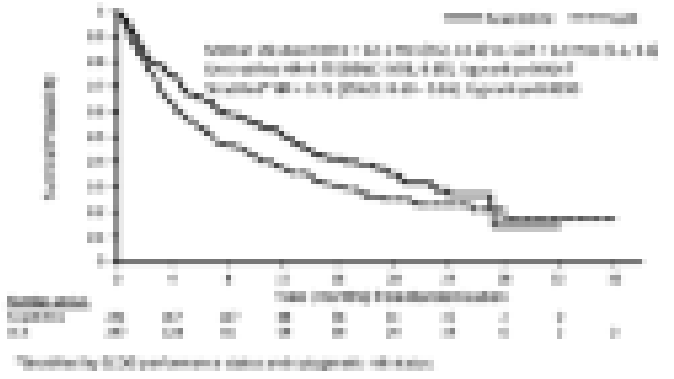


Figure 1. Overall survival: AZA *vs.* CCR (ITT population).



Fgure 2. Overall survival sensitivity analysis (Censored for subsequent AML therapy): AZA *vs.* CCR (ITT population).

LB2434

A PHASE I STUDY OF AG-221, A FIRST IN CLASS, POTENT INHIBITOR OF THE IDH2-MUTANT PROTEIN, IN PATIENTS WITH IDH2 MUTANT POSITIVE ADVANCED HEMATOLOGIC MALIGNANCIES

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New Information: AACR in 4/2014. The clinical data from this phase I study will be updated with new safety and clinical efficacy data for the EHA presentation. (Total N of at least 31 patients). Data on the first 22 patients enrolled was presented at AACR.

Background: Cancer metabolism represents an emerging field of novel drug discovery. Somatic point mutations in the metabolic enzymes isocitrate dehydrogenase 1/2 (IDH1/2) confer a novel gain-of-function in cancer cells resulting in accumulation and secretion of the onco-metabolite, R-2-hydroxyglutarate (2-HG). High levels of 2-HG have been shown to inhibit a-KG dependent dioxygenases including histone and DNA demethylases, regulating the epigenetic state of cells resulting in altered cellular differentiation. IDH2 mutations have been identified in a spectrum of solid tumors and hematologic malignancies. This first clinical study explores the safety and clinical activity of AG-221 in advanced hematologic malignancies, the first mutant IDH inhibitor in clinical trials. AG-221 is an oral, potent, reversible, and selective inhibitor of mutated IDH2 protein. In a primary human AML xenograft model, AG-221 treatment reduced 2-HG levels and demonstrated a survival benefit. Data from the ongoing dose escalation, phase 1 study of AG-221 in patients with advanced IDH2 mutant positive hematologic malignancies are reported here.

Aims: This first in man phase I study of oral AG-221 is designed to evaluate the safety, pharmacokinetics (PK), pharmacodynamics (PD) including 2-HG levels, and clinical activity. AG-221 is administered orally once (QD) or twice per day (BID) in continuous 28-day cycles. Sequential cohorts of patients are enrolled at higher dose levels, followed by planned expansion cohorts. Eligible patients include those with relapsed or refractory AML, myelodysplastic syndromes (MDS) and elderly untreated AML that harbor an IDH2 mutation. Blood and bone marrow is collected at multiple times for determination of the PK/PD effects of AG-221. Bone marrow examination for response are performed on Days 15, 29, 57, and every 56 days (2 cycles) thereafter.

Results: The study was activated in September 2013. As of the March 20th, 2014, 22 patients have been enrolled; 21 with AML and 1 with MDS. All patients were IDH2–mutant positive. AG-221 doses administered included 30 mg BID (n=7), 50 mg BID (n=5), 75 mg BID (n=5), and 100 mg QD (n=5). Two patients were added to the 30 mg BID cohort to replace PK un-evaluable patients. Sixteen of 22 patients remain on study drug treatment. Therapy has been well tolerated with no dose-limiting toxicities. The maximum tolerated dose has not been reached. Possible drug-related severe adverse events occurred in two patients: grade 2 hyperleukocytosis and grade 3 confusion. There were four deaths due to sepsis within 30 days of study drug termination, three on cohort 1 and one on cohort 4. Preliminary analysis of PK at 30 and 50 mg demonstrated excellent oral AG-221 exposure. Mean plasma half-life is greater than 40 hours. Evaluation of PD demonstrated sustained reduction in 2-HG plasma levels of up to 100% following AG-221 dosing in cohorts 1 and 2. Efficacy evaluation at the time of the data cutoff from patients enrolled in cohorts 1 and 2 are shown below in Table 1. Ten AML patients were treated: (N=5 at 30 mg BID, N=5 at 50 mg BID), 5 men and 5 women, with a median age (range) of 62.5 years (53-74). Eight patients had an R140Q mutation, two had a R172K mutation. Median number of prior regimens was 2 (1-4). One patient relapsed after an allogeneic bone marrow transplant. Six of 10 patients have had investigator assessed objective responses (International Working Group Criteria): 3 complete responses (CR), 2 complete responses with incomplete platelets recovery (CRp), 1 partial response (PR). Three were not evaluable for efficacy (NE). Marked differentiation of myeloblasts into mature forms, consistent with preclinical models, was associated with responses. All responding patients demonstrated neutrophil recovery. Only one patient experienced disease progression. One patient with a CR was removed from study to undergo allogeneic BMT. Five of the 6 responding patients remain on AG-221.

As of April 15th, 31 patients have been enrolled into the study into existing and new dose cohorts. Dose escalation continues and updated safety, efficacy, PK/PD, and molecular data will be presented.

Table 1. Summary of efficacy from cohorts 1 and 2.

Summary and Conclusions: AG-221, a novel, oral, potent IDH2 mutant inhibitor is well tolerated and as predicted by preclinical models, triggers the terminal differentiation of leukemic blast cells that ultimately leads to objective responses including complete remissions. These data provide early validation of mutant IDH2 as a therapeutic target in cancer.

LB2435

ORDER MATTERS: MUTATION ORDER INFLUENCES TUMOUR EVOLUTION AND STEM CELL POTENCY BY ALTERING TRANSCRIPTIONAL CONSEQUENCES OF THE SECOND MUTATION

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Background: Cancers result from accumulation of somatic mutations and their properties are thought to reflect the sum of these mutations. However, virtually nothing is known about the cellular and molecular consequences of altering the order of mutation acquisition.

Aims: To determine whether the order of mutation acquisition impacts tumour evolution and/or stem cell and progenitor cell biology in myeloproliferative neo-

plasm patients who carry both JAK2V617F and a TET2-inactivating mutation in the same clone.
Methods: We genotyped >7000 colonies from 24 patients with myeloproliferative neoplasms (MPNs) who harbored mutations in both *JAK2* and *TET2*. Stem and progenitor cells were isolated to study the impact of mutation order on mature and immature hematopoietic cells. Gene expression profiling was performed to determine whether mutation order impacted the transcriptional alterations caused by the second mutation.
Results: *JAK2* and *TET2* mutations each occurred first in 12/24 patients, with *TET2*-first and *JAK2*-first patients observed in all 3 MPN subtypes. Age of presentation, acquisition of JAK2V617F homozygosity and the balance of immature progenitors within a tumor were all influenced by the order in which *TET2* and *JAK2* mutations were acquired. In studies of single hematopoietic stem and progenitor cells (HSPCs), mutation order influenced the proliferative response to JAK2V617F and the generation of functional progenitors. The capacity of double-mutant HSPCs to generate colony-forming cells was markedly different in TET2-first compared to JAK2-first patients; and the HSPC compartment was dominated by *TET2* single-mutant cells in TET2-first patients but by JAK2/TET2 double-mutant cells in JAK2-first patients. Transcriptional profiling on individual colonies with different genotypes from 7 patients (4 TET2-first, 3 JAK2-first) was performed, thereby allowing direct comparison of genetically distinct cells within a patient, thus controlling for differences in age, sex, treatment, germline genetic background and other confounding variables. Mutation of *JAK2* or *TET2* was associated with altered patterns of gene expression that were strikingly dependent on the antecedent genotype (*e.g.*, most genes up-regulated or down-regulated when JAK2V617F was acquired on a TET2-wild-type background were not altered when JAK2V617F was acquired on a TET2-mutant background). Analysis of altered gene sets revealed up-regulation of translational machinery when JAK2V617F was acquired on a WT background and downregulation of cell cycle progression genes when JAK2V617F was acquired on a TET2-mutant background. Since JAK2 plays a key role in normal erythroid differentiation and TET2 modulates the epigenetic landscape, we investigated whether prior mutation of *TET2* influences the transcriptional response to JAK2V617F. We compared genes up-regulated when JAK2V617F was acquired by TET2-wildtype cells with those down-regulated when JAK2V617F was acquired by TET2-mutant cells. This approach identified 10 genes discordantly regulated by JAK2V617F depending on the *TET2* genotype. Remarkably, six of these have been implicated in DNA replication (MCM2, MCM4, MCM5) or regulation of mitosis (AURKB, FHOD1, TK1). These data demonstrate that acquisition of a prior *TET2* mutation dramatically alters the transcriptional consequences of JAK2V617F in a cell-intrinsic manner.
Summary and Conclusions: The order in which *JAK2* and *TET2* mutations are acquired influences clinical presentation, stem/progenitor cell biology and clonal evolution in MPN patients. In addition to providing a paradigm for other malignancies, these results have clinical implications for MPN patients including the prediction that mutation order will influence therapeutic efficacy.

LB2436

RUXOLITINIB PROVES SUPERIOR TO BEST AVAILABLE THERAPY IN A PROSPECTIVE, RANDOMIZED, PHASE 3 STUDY (RESPONSE) IN PATIENTS WITH POLYCYTHEMIA VERA RESISTANT TO OR INTOLERANT OF HYDROXYUREA

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Background: Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by erythrocytosis, burdensome symptoms, and an increased risk of vascular events. A key goal of therapy is to maintain hematocrit (HCT) control. Hydroxyurea (HU) is often used as a first-line cytoreductive treatment; however, not all patients (pts) achieve HCT control or tolerate therapy with HU.
Aims: RESPONSE is an open-label phase 3 study to evaluate the efficacy and safety of ruxolitinib (RUX), a JAK1/JAK2 inhibitor, compared with best available therapy (BAT) in PV pts resistant to or intolerant of HU.
Methods: HU-resistant/intolerant pts with splenomegaly (≥ 450 cm^3 by MRI) who required phlebotomy (PBT) for inadequate HCT control were randomized 1:1 to RUX 10 mg bid or BAT. The primary endpoint was the proportion of pts who achieved both HCT control without PBT from wk 8 to 32 (with no more than 1 PBT postrandomization and prior to wk 8) and a ≥ 35% reduction in spleen volume (SV) from baseline (BL) by MRI at wk 32. The key secondary endpoints included proportion of pts who maintained primary response at wk 48 and proportion of pts who achieved complete hematologic response (CHR) at wk 32. Other endpoints included duration of primary response, symptom improvement, and safety. The MPN-SAF diary was used to assess 14 disease-related symptoms on a scale of 0 (absent) to 10 (worst imaginable). In addition, symptom clusters related to the following were evaluated: cytokines, hyperviscosity, and splenomegaly. BAT-treated pts could cross over to RUX from wk 32. Primary analysis occurred when all pts reached wk 48 or discontinued.
Results: 110 and 112 pts were randomized to RUX and BAT, respectively. The median time since PV diagnosis was 8.2 and 9.3 y; 35% and 29% of pts had a history of thrombosis; and 31% and 42% had ≥ 3 PBTs within 24 wk of screening, respectively. The respective median BL SV in RUX and BAT pts was 1195 cm^3 and 1322 cm^3. The median duration of exposure to RUX and BAT was 81 and 34 wk, respectively: 17 (15%) of RUX and 108 (96%) of BAT pts discontinued randomized treatment (96/108 pts [89%] crossed over to RUX), of which 3.6% and 1.8% were due to adverse events (AEs). The primary endpoint was achieved in 21% of RUX *vs* 1% of BAT pts ($P < .0001$); 91% of RUX pts achieved durable response at wk 48. Overall, 77% of RUX pts met at least 1 component of the primary endpoint: 60% of RUX and 20% of BAT pts achieved HCT control without PBT from wk 8 to 32; 38% of RUX and 1% of BAT pts achieved ≥ 35% SV reduction at wk 32 (Figure 1A). CHR was achieved in 24% and 9% of RUX and BAT pts, respectively ($P = .003$). At wk 32, 49% *vs* 5% had ≥ 50% improvement in MPN-SAF 14-item total symptom diary score; a greater percentage of pts in the RUX arm also achieved ≥ 50% improvement in each symptom cluster (Figure 1B). AEs in the RUX and BAT groups during the first 32 wk of randomized treatment were evaluated. Grade 3/4 anemia or thrombocytopenia occurred in 1.8% and 5.5% of RUX pts, respectively, *vs* 0% and 3.6% of BAT pts. The most common grade 3/4 nonhematologic AEs that occurred in > 2 pts in either treatment group (RUX *vs* BAT) were dyspnea (2.7% *vs* 0%), pruritus (0.9% *vs* 3.6%), and fatigue (0% *vs* 2.7%); all of these events were grade 3. Thromboembolic events occurred in 1 RUX and 6 BAT pts.

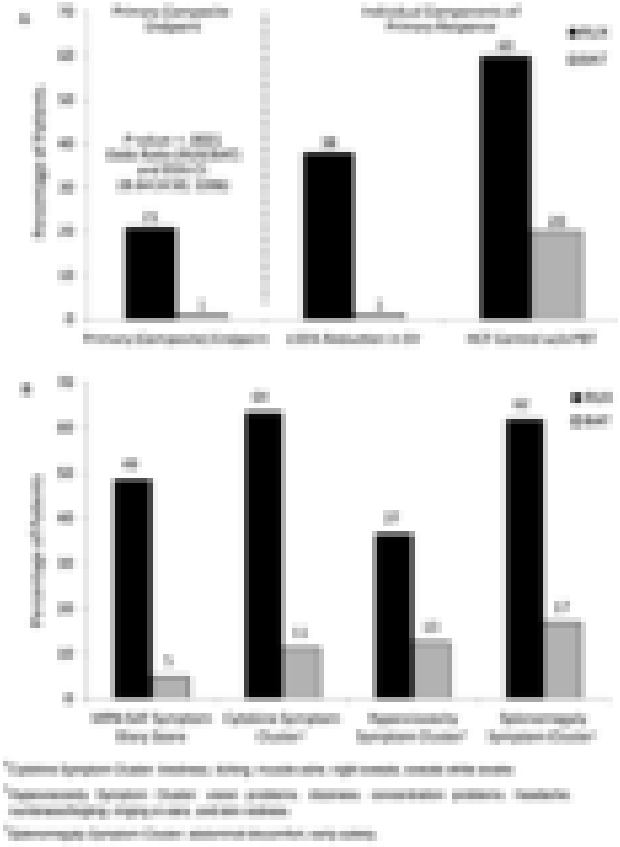

Figure 1. Percentage of patients achieving (A) the primary endpoint and components of the primary endpoint and (B) ≥50% improvement in MPN-SAF symptom scores.

Summary and Conclusions: This is the first phase 3 study of a JAK inhibitor demonstrating that in pts with PV resistant to or intolerant of HU, RUX treatment is well tolerated and effective in controlling HCT without PBT, decreasing SV, and improving PV-related symptoms.

Late breaking 2

LB2437

KIDS A-LONG: SAFETY, EFFICACY, AND PHARMACOKINETICS OF LONG-ACTING RECOMBINANT FACTOR VIII FC FUSION PROTEIN (RFVIIIFC) IN PREVIOUSLY-TREATED PAEDIATRIC SUBJECTS WITH HAEMOPHILIA A

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Background: Initiation of prophylactic factor VIII (FVIII) therapy at a young age is recommended for the treatment of severe haemophilia A to prevent bleeding and consequent joint disease. Current therapy requires frequent infusions of FVIII. Previous reports indicate that FVIII half-life is shorter in children than in adults, which could necessitate even more frequent dosing. Long-acting FVIII products may allow for prophylaxis with less frequent infusions. In a Phase 3 study of recombinant FVIII Fc fusion protein (rFVIIIFc) in adolescents and adults, rFVIIIFc demonstrated an increased half-life relative to rFVIII, and prophylactic rFVIIIFc infused once to twice a week yielded low annualized bleeding rates (ABR). Kids A-LONG was the first phase 3 study of a long-acting FVIII in previously-treated paediatric subjects.
Aims: The objective of Kids A-LONG was to evaluate the safety, efficacy, and pharmacokinetics (PK) of rFVIIIFc in previously-treated subjects less than 12 years of age with severe haemophilia A.
Methods: Kids A-LONG was a global, multi-centre, open-label, phase 3 study that enrolled male subjects <12 years old, with severe haemophilia A (<1 IU/dL FVIII activity), ≥50 prior exposure days (ED) to FVIII, and no history of FVIII inhibitors. All subjects were initially to be treated with twice-weekly prophylactic infusions of rFVIIIFc (25 IU/kg day 1, 50 IU/kg day 4). Dosing frequency could be increased (to as often as once every 2 days) and dose could be increased (to ≤80 IU/kg) as needed by the investigator. A subset of subjects (<6 yrs, n=25; 6 to <12 yrs, n = 35) underwent sequential PK evaluations with their prior FVIII therapy (50 IU/kg) followed by rFVIIIFc (50 IU/kg). The primary endpoint was development of inhibitors (neutralizing antibodies). Key efficacy endpoints were ABR and number of infusions required to control a bleed.
Results: 71 subjects from 23 centres (36 were <6 years of age [range, 1 – 5 yrs] and 35 were 6 to <12 years of age [range, 6 – 11 yrs]) were enrolled. Eighty-nine percent of subjects received a pre-study FVIII prophylaxis regimen, the majority (74.6%) of which required 3 or more infusions/week. The median time on study was 26 weeks, and 61 subjects had ≥50 EDs to study drug; 94.4% of subjects completed the study. No subject developed inhibitors to rFVIIIFc. Overall, the pattern of adverse events reported on rFVIIIFc treatment was typical of the population studied. No serious adverse events were assessed to be related to the product by the investigator. The arithmetic mean (95% CI) terminal half-life in subjects <6 years and 6 to <12 years was 12.67 hrs (11.23, 14.11) and 14.88 hrs (11.98, 17.77), respectively, based on the one-stage aPTT assay. The relative increase in half-life over prior FVIII therapy was consistent with the 1.5-fold increase in half-life seen in the A-LONG study of adults and adolescents. Median (IQR) ABR overall was 1.96 (0.00, 3.96) and for spontaneous bleeds was 0.00 (0.00, 0.00). Median average weekly dose and dosing interval were 88.1 IU/kg and 3.5 days, respectively. 93.0% of all bleeding episodes were controlled with 1-2 infusions (median dose per bleeding episode: 54.9 IU/kg).
Summary and Conclusions: In this study, rFVIIIFc prophylaxis was well-tolerated, resulted in low bleeding rates, and was efficacious for treating bleeding episodes. The increase in half-life compared to current FVIII and the safety profile were generally consistent with that observed in the Phase 3 study in adults and adolescents. rFVIIIFc offers the potential of prolonged dosing intervals and fewer infusions for paediatric patients with severe haemophilia A.

LB2438

MULTIPLEX GENOME EDITING OF TCRALPHA/CD52 GENES AS A PLATFORM FOR "OFF THE SHELF" ADOPTIVE T-CELL IMMUNOTHERAPIES

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Background: Adoptive immunotherapy using autologous T-cells endowed with chimeric antigen receptors (CARs) has emerged as a powerful approach to treating cancer. However, a limitation of this approach is that CAR T-cells must be generated on a bespoke basis.
Aims: To permit adoptive T cell therapy using allogeneic T cells, we have developed a platform for the large scale production of "off-the-shelf" CAR T-cells from unrelated 3rd party donor T-cells.
Results: We applied Transcription Activator-Like Effector Nucleases (TALEN) gene editing technology to overcome the key barriers to the adoptive transfer of 3rd party CAR T-cells. We eliminated the potential for T-cells bearing alloreactive TCR's to mediate GvHD by disrupting the TCRalpha constant (TRAC) gene, and we exploited the requirement for preparative lymphodepletion for engraftment of allogeneic CAR T-cells through disruption of the CD52 gene. Simultaneous editing of the TRAC and CD52 genes results in TCR/CD52-deficient T-cells that may follow alemtuzumab-mediated lymphodepletion/immunosuppression.
Summary and Conclusions: We provide proof of concept for the general applicability of this approach by manufacturing a TCR/CD52-deficient universal CD19 CAR T-cell (UCART19), and demonstrating that this product does not mediate alloreactivity. In addition, it can be selectively engrafted in the presence of alemtuzumab, while maintaining potent anti tumoral activity equivalent to standard CD19 CAR T-cells in an orthotopic CD19+ lymphoma mouse model. Our approach is being developed as a platform for large scale manufacturing of allogeneic, off-the-shelf, non alloreactive CD19 specific T Cells that offer the possibility to cure, in both salvage and consolidation therapy, high risk CD19+ B-cells leukemias.

LB2439

THE PD1/PD-L1 AXIS PROTECTS NORMAL KERATINOCYTES FROM CAR T-CELL ATTACK: RATIONALE FOR THE SKIN SAFETY OF CD44V6-TARGETED T CELLS

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Background: Off-tumor expression of the target antigen raises justified safety concerns about newly designed chimeric antigen receptors (CARs). We have recently developed a CAR targeting the tumor-promoting antigen CD44v6 and demonstrated potent antitumor effects against acute myeloid leukemia (AML) and multiple myeloma (MM) both *in vitro* and *in vivo* (Casucci et al, *Blood* 2013). Despite promising activity against epithelial tumors, the administration of the CD44v6-specific mAb used for deriving our CAR (bivatuzumab) showed reversible myelosuppression and severe skin toxicity when conjugated with the potent cytotoxic drug mertansine. Preclinically evaluating the potential off-tumor toxicities of CD44v6-targeted T cells is therefore crucial before they can be safely translated to the clinic.
Aims: To profile the off-tumor expression of CD44v6 and to verify the susceptibility of expressing cells to CAR-T cell killing.
Results: Quantitative RT-PCR analysis on a wide panel of cDNA from normal tissues revealed restricted CD44v6 expression on flat stratified epithelia, like the skin, albeit at considerably lower levels compared with primary leukemic blasts. We therefore addressed the issue of normal keratinocyte recognition in co-culture experiments. Strikingly, at the E:T ratios allowing the potent antitumor effects of CD44v6-targeted T cells, keratinocytes were not killed and there was no cytokine production. Differently from leukemic blasts, keratinocytes expressed significant lower levels of adhesion molecules (ICAM-1, LFA-3 and B7.2), but higher levels of the checkpoint molecule PD-L1. Blocking experiments with mAbs indicated that lower ICAM-1 expression is involved in keratinocyte resistance to direct CAR T-cell killing, while higher PD-L1 expression restrains secondary expansion of CAR T cells. Interestingly, comparative CFSE-dilution experiments revealed that normal keratinocytes are as immunosuppressive as mesenchymal stromal cells and that, similarly to them, can be licensed by interferon-gamma for even higher immunosuppressive activity. Of the different cells of the hematopoietic system analyzed, only circulating CD14+ monocytes expressed CD44v6 and were killed by CD44v6-targeted T cells. Moreover, by immunohistochemistry, we found no CD44v6 expression on bone-marrow monocytes, lymph-node macrophages, brain microglia, liver Kuppfer cells and dermal macrophages, suggesting a low risk for by-stander toxicity against these tissues. Accordingly with lack of CD44v6 expression on normal hematopoietic stem cells (HSCs) and progenitors, CD44v6-targeted T cells did not interfere with their clonogenic potential *in vitro* and, in co-culture experiments with whole bone marrow from MM patients, were able to selectively eliminate tumor cells, while sparing HSCs and progenitors. Finally, we tested the potential hematological toxicities of CD44v6-targeted T cells in NSG mice transgenic for human IL-3, SCF and GM-CSF (NSG-3GS). NSG-3GS mice transplanted with human CD34-selected cord blood cells showed enhanced myeloid reconstitution compared to NSG mice, including CD44v6+ monocytes. The infusion of CD44v6-targeted T cells in reconstituted NSG-3G mice resulted in the selective elimination of monocytes, but in the preservation of the HSC pool. For enabling rapid and conditional ablation of CD44v6-targeted T cells, we

have finally co-expressed the CD44v6 CAR with TK or the inducible caspase-9 and validated the suicide gene approach in hyper-acute xenogeneic GVHD surrogating maximal toxicity.
Summary and Conclusions: Our results suggest that the PD1/PD-L1 axis is involved in the protection of normal tissues from CAR T-cell attack and that, differently from immunotoxins, therapeutic doses of suicidal CD44v6-targeted T cells might associate with acceptable and/or reversible toxicities.

LB2440

FIRST INTERIM ANALYSIS OF A PAN-EUROPEAN STOP TRIAL USING STANDARDIZED MOLECULAR CRITERIA: RESULTS OF THE EURO-SKI TRIAL

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Background: The advent of tyrosine kinase inhibitors (TKI) has substantially improved survival of chronic myeloid leukemia (CML) with a high percentage of patients reaching deep molecular responses (MR; Hehlmann JCO 2014). There is a reasonable expectancy not only to further improve survival but to cure the disease. An important step to cure CML is to increase the rate of patients in durable deep MR (MR4 or deeper) after withdrawal of TKI. In several studies, it have been shown that in a substantial proportion of patients with deep MR, treatment can be safely and successfully stopped (Mahon 2010, Ross 2013).
Aims: The EURO-SKI study (European stop TKI study) was set up to define prognostic markers to increase the rate of patients in durable deep MR after stopping TKI. Further aims are the evaluation of harmonized methods of molecular monitoring, assessment of quality of life, and calculation of saved treatment costs per country.
Methods: Adult CML patients in chronic phase CML on TKI treatment in confirmed MR for at least one year (>4 log reduction on TKI therapy for >12 months confirmed by three consecutive PCR results) and under TKI treatment for at least 3 years were eligible. Final MR4 confirmation had to be performed in a standardized laboratory (n=6) according to the definition by Cross *et al.* (Leukemia 2012). Primary endpoint is the assessment of the duration of deep MR (defined by loss of MMR) after stopping TKI. Patients with previous or planned allogeneic stem cell transplantation or after a prior TKI failure were excluded. According to protocol, an interim analysis was planned after 200 patients with eligible molecular results at month 6 were available to test the null hypothesis that relapse-free survival at 6 months is less or equal 40%.
Results: From June 2012 to July 2013, 254 patients in chronic phase from 8 countries were registered. 54 were excluded (consent withdrawal n=1, protocol violation n=1, not eligible n=34, restart of TKI without relapse n=4, atypical or unknown transcript n=6, missing data n=8). Of the eligible 200 patients, 41.5% were female. Median age at diagnosis was 53.3 years (range 13.8 to 85.5). In assessable patients with spleen size recording 8.7%, 18.2 % and 0% were high-risk according to EUTOS, Sokal and EURO-Score, respectively. 103 patients were pretreated prior to TKI therapy, mostly with hydroxyurea and/or interferon. First-line TKI was imatinib in 97%, dasatinib in 1.5% and nilotinib in 1.5% of the patients. Twenty-four patients switched to second-line TKI due to intolerance, 16 to dasatinib, 2 to imatinib, and 6 to nilotinib. Time from diagnosis of CML to first day of stopping TKI varied from 34.4 months to 232.9 months, median time was 95.4 months. Median duration of TKI treatment was 94.8 months (range 34.2 to 150.6 months) and median duration of MR4 before stopping TKI was 65.1 months (range 11.1 to 140.7 months). For all patients MR4 could be confirmed through a MR4-standardized laboratory. Nineteen patients with detectable transcripts were in MR 4 (9.5%), 37 patients in MR4.5 (18.5%) and 18 in MR5 (9.0 %), respectively. 123 patients had undetectable transcripts, of them 49 patients were in MR4 (24.5%), 48 in MR4.5 (24%) and 25 in MR5 (12.5%); exact classification of 3 patients with confirmed MR4 is pending.
A pre-planned interim hypothesis testing was performed. Since 123 of the 200 patients (61.5%, 95% confidence interval: [54.4%; 68.3%]) remained without relapse the first 6 months, the null hypothesis could be discarded ($p < 0.0001$).
Summary and Conclusions: As compared to the "A-STIM" study (Rousselot *et al.* JCO 2014) this first European Leukemianet stop trial in CML confirms that loss of MMR can be used as a criterion for restarting therapy. In addition, it improves the chance to stay in treatment-free remission in the setting of standardized molecular testing. The EURO-SKI trial will further elucidate prognostic factors which allow a better selection of patients to improve the rate of durable deep MR after withdrawal of TKI.

Late breaking posters

LB2441

TREATMENT OF IRON DEFICIENCY ANAEMIA OF LATE PREGNANCY WITH A SINGLE INTRAVENOUS IRON POLYMALTOSE OR FERRIC CARBOXYMALTOSE *VERSUS* ORAL IRON SULPHATE: A PROSPECTIVE RANDOMIZED CONTROLLED STUDY (TIDAL)

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Background: Conferring the World Health Organisation (WHO) statistics, iron deficiency (ID) is the most common nutritional deficiency disorder in the world, affecting at least two billion people, with pregnant women at particularly high risk. ID affects more women than any other health disorder in the world, constituting a public health crisis in many developing countries. Recent studies in the developed world showed that ID is present in up to 20% of pregnant women (PMID: 20546462). Therefore, it is important to diagnose ID during pregnancy and hence to offer the appropriate management avoiding the significant side effects of ID.
Aims: We conducted a comparative, prospective, open labelled randomized-controlled trial to compare the efficacy, safety, tolerability and compliance of standard daily oral iron sulfate 325 mg (elemental iron 105 mg) *versus*1g of intravenous iron polymaltose or carboxymaltose.
Methods: We recruited 205 pregnant women between September 2013 and April 2014 with iron deficiency at our tertiary referral Hospital. Median age was 28 years (range, 18-47) with a median and mean gestational age of 27 weeks. Median baseline serum ferritin level was 9 mcg/L (mean, 10 mcg/L) with serum transferrin saturation below 20%.
Results: Hb and iron stores after 4 weeks of treatment showed that the intravenous iron group was superior to the oral iron arm as measured by the increase in Hb level (mean of 9.5 g⁄L *vs.* 3.2 g⁄L; P < 0.02); the increase in mean serum ferritin level was 196 mcg⁄L in the IV iron group *vs.* 62 mcg⁄L in the oral iron arm; (P < 0.001). The percentage of pregnant women with ferritin levels below 30 mcg⁄L was 14% in the IV iron group *vs.* 42% in the oral iron group after 4 weeks of treatment (P < 0.01). There was no significant difference noticed in patients who received iron carboxymaltose *versus* polymaltose in terms of Hb increment. However, ferric carboxymaltose showed greater ferritin increments from baseline with a mean ferritin increase after 4 weeks of treatment to 198 mcg/L in the ferric carboxymaltose arm *versus* a mean of 119 mcg/L in the iron polymaltose arm. Furthermore, a single dose of intravenous iron carboxymaltose was better tolerated than iron polymaltose in terms of delayed side effects after treatment with bone aches and nausea, however without significant side effects in both groups.
Summary and Conclusions: Our data indicate that intravenous iron carboxymaltose application during pregnancy is safe and leads to improved efficacy and improvement of iron stores compared to oral iron in pregnancy-related ID. Integration of iron studies as a screening tool in addition to regular full blood count in the routine antenatal visit allows early diagnosis and management of ID in pregnanacy.

LB2442

UNIQUE DEPENDENCE OF MALIGNANT, BUT NOT NORMAL HEMATOPOIESIS, ON CD44V6-MEDIATED SURVIVAL SIGNALS WITHIN THE BONE MARROW

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New Information: A preliminary report presented at ASH 2013. This abstract includes comprehensive and conclusive results, including HSCs silencing, *in vivo* proliferation and cell death of CD44v6-silenced tumor cells and primary AML and MM susceptibility to CD44v6 mAb.
Background: Targeting the unique interactions within the tumor microenvironment is an exciting new frontier in cancer therapy. Tumors of hematopoietic origin are addicted to specific survival signals provided within the bone marrow (BM). Unfortunately, the majority of these signals are shared with normal hematopoietic stem cells (HSCs), rendering their therapeutic targeting a challenging task. Monoclonal antibodies (mAbs) to the hyaluronate receptor CD44 inhibit leukemia initiation in immunocompromised mice and are currently investigated in humans. In principle, targeting the tumor-restricted CD44 isoform variant 6 (CD44v6) would have clear advantages over targeting the ubiquitously expressed standard molecule (CD44s). However, before proceding, it is needed to validate CD44v6 as a clinically relevant tumor antigen.
Aims: To demonstrate a non-redundant role for CD44v6 in hematopoietic tumor-cell biology and to preclinically estimate the safety and the therapeutic efficacy of targeted strategies.
Results: By RT-qPCR and FACS analysis on a wide range of hematopoietic tumor cell lines and primary cells, we established CD44v6 over-expression in a relevant fraction of acute myeloid leukemia (AML) samples (15/25, 60%) and in the majority of multiple myeloma (MM) patients (13/15, 80%). We previously showed that CD44v6 silencing by means of a shRNA completely inhibited primary AML and MM cell lines engraftment in NSG mice (P<0.01, Casucci *et al.,* 2013). Conversely, CD44v6-silenced HSCs engrafted and efficiently repopulated both myeloid and lymphoid compartments in NSG mice triple transgenic for human c-Kit ligand, GM-CSF and IL-3 (NSG-3GS). NSG-3GS mice drive human CD34+ toward a higher reconstitution of the myeloid compartment, allowing the study of CD44v6+ monocytes. These results suggest that CD44v6 has a non-redundant role in tumor biology. To assess its specific functions in tumor growth, we interfered with CD44v6 by either a mAb or by shRNA and observed no inhibition in tumor-cell homing to the BM. In line with these findings, CD44v6-silenced cells lost their *in vivo* tumorigenicity even when directly injected in the BM (P<0.05). Interestingly, this phenomenon is not associated with a reduced *in vivo* proliferation, but with a dramatic increase in spontaneous apoptosis, especially in the BM (P<0.05). Hypothesizing that CD44v6-silenced tumor cells had lost responsiveness to specific microenvironmental signals mediating survival, we set-up a co-culture system with BM-derived mesenchymal stromal cells (MSCs). MSCs, or their supernatants, protected AML and MM cells from spontaneous (P<0.01) and drug-induced apoptosis (P<0.01). Although present, known CD44v6 co-factors such as VEGF and HGF, were not involved in the phenomenon, as demonstrated by inhibition experiments with bevacizumab and crizotinib, respectively. Contrariwise, protection from apoptosis by MSCs associated with significant CD44v6 up-regulation (P<0.01) and with tumor cell-autonomous production of the CD44v6 ligand osteopontin, suggesting an autocrine loop. Preventing CD44v6 up-regulation by shRNA interference restored AML and MM cell lines sensitivity to apoptosis without affecting their ability to adhere to MSCs. Consistently, interference with CD44v6 by a mAb derived from VFF-18 clone, enhanced AML and MM primary cells susceptibility to drug-induced cell death (P<0.01 and P<0.05).
Summary and Conclusions: CD44v6 expression by hematopoietic tumors, but not by normal HSCs, is required for sensing survival signals within the BM microenvironment. Targeting CD44v6 in combination with chemotherapy is therefore a promising therapeutic approach for AML and MM.

LB2443

THE NOTCH PATHWAY IS RECURRENTLY MUTATED IN DIFFUSE LARGE B CELL LYMPHOMA ASSOCIATED WITH HEPATITIS C VIRUS INFECTION

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Background: Hepatitis C virus (HCV) has been found to be associated with B-cell non-Hodgkin's lymphomas, mostly marginal zone B-cell lymphomas (MZL) and diffuse large B cell lymphoma (DLBCL). Deregulation of signaling pathways involved in normal marginal zone development (NOTCH pathway, NF-κB, and B-cell receptor signaling) has been demonstrated in splenic MZL (SMZL).
Aims: To assess deregulation of NOTCH, NF-κB and B-cell receptor signaling in HCV-related DLBCL in comparison with HCV- DLBCL cases, the relationship of molecular lesion with clinical and histological features and the impact of signaling deregulation on the outcome of HCV-related DLBCL.
Methods: We studied mutations of NOTCH, NF-κB and B-cell receptor signaling in 46 pts with HCV-related DLBCL and in 64 pts with HCV- DLBCL.
Results: All cases were HCV-RNA positive. Extranodal disease was present in 29 pts and 17 pts had splenic involvement (primary splenic in 15 pts). In 12/46 (26%) cases a minor area of the diagnostic biopsy was composed of small to medium sized monocytoid B-cells, suggesting a progression from a MZL that was not previously recognized. Overall, the NOTCH pathway was recurrently

mutated in 26% (12/46) of HCV+ DLBCL: *NOTCH2* was mutated in 9/46 (19.6%), *NOTCH1* in 2/46 (4.4%), and SPEN in 1/46 (2.2%). *NOTCH2* mutations were represented in all instances by truncating events and clustered within a hotspot region in exon 34, including a recurrent p.R2400* nonsense mutation in 4 cases. *NOTCH1* was affected by a recurrent two-bp deletion (p.P2515fs*4) that truncates the PEST domain, similarly to *NOTCH2* lesions. The prevalence and type of NF-kB pathway mutations overlapped with the ones of DLBCL of non-GC phenotype arising in the HCV- pts. Among NF-kB genes, *TNFAIP3* was mutated in 5 (26.3%) (all of non-GC origin), *MYD88* was mutated in 1 patient (5.3%) while *IKBKB*, *BIRC3* and *TRAF3* were wild type in all cases. Differently from DLBCL of the HCV- population, in HCV+ DLBCL the BCR pathway (*CARD11*, *CD79A* and *CD79B*) was never affected by genetic lesions. Among pathologic features, NOTCH pathway mutations were enriched in cases harbouring a small to medium cells component, histologically resembling MZL, coexisted with the large cell component proper of DLBCL (6/12, 50% in cases with low-grade component *vs* 6/34, 17.7% in cases without low grade component; p=0.05). *NOTCH2* and *NOTCH1* mutations were investigated in a cohort of 64 HCV- DLBCL. *NOTCH2* was rarely mutated in 1/64 (1.6%) in HCV- DLBCL (comparison with HCV-positive cases resulted statistically significant, p = 0.002) while *NOTCH1* was never affected (0/64). After a median F-UP of 3.7 yrs for entire series, 5-yr OS was 53% (95% CI: 37%>67%). In univariate analysis, a worse OS resulted associated with age > 60 yrs (p=0.02), mutation of *NOTCH2* (p=0.03), NOTCH pathway disruption (p=0.008) and mutation of *NOTCH1* or *NOTCH2* (p=0.03). 5-yr OS was 29% (95% CI: 5%>59%) for pts carrying *NOTCH1* or *NOTCH2* mutation and 60% (95%CI: 41%>75%) for pts without these mutations. 5-y OS was 22% (95% CI: 1%>59%) for pts carrying *NOTCH2* mutation and 60% (95%CI: 41%>74%) for pts without *NOTCH2* mutation. 5-yr OS was 27% (95% CI: 5%>56%) for pts carrying NOTCH pathway mutation and 62% (95%CI: 42%>77%) for pts without these mutations. In multivariate analysis, NOTCH pathway disruption retained its prognostic effect also adjusting for IPI score (HR=7.7; 95%CI: 1.3-47.3; p=0.028), while IPI score was not statistically significant. When adjusting also for age (as continuous variable), NOTCH pathway was still significant (HR=9.0, 95%CI: 1.3-60.1; p=0.024) as well as age (HR=1.1; 95%CI: 1.0-1.2; p=0.043) (Figure 1).

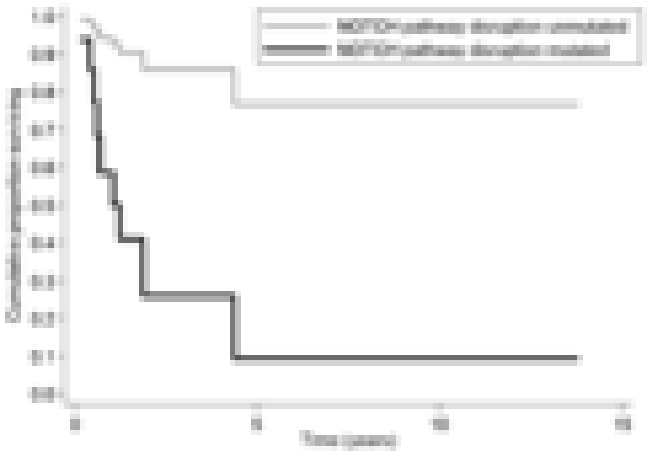

Figure 1.

Summary and Conclusions: In conclusion, a subset of patients with HCV-positive DLBCL displays a molecular signature of splenic MZL and has a worse clinical outcome.

LB2444

ACCELERATION OF BCR-ABL+ LEUKEMIA INDUCED BY DELETION OF JAK2

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Background: The role of Jak2 in leukemia and lymphoma formation is beyond doubt. As a consequence several clinical studies on Jak2 inhibitors are still ongoing. Hematopoietic side effects observed upon treatment with Jak2 inhibitors indicated a crucial requirement for JAK2 in normal hematopoiesis.
Aims: To investigate the role of Jak2 in hematopoietic stem cells and for slowly evolving Bcr-Abl ^{p210} driven chronic myeloid leukemia we used *Jak2$^{f/f}$Mx1Cre* mice. Bone marrow derived from *Jak2$^{+/+}$* and *Jak2$^{f/f}$Mx1Cre^{+}* mice was transduced with a retrovirus encoding Bcr-Abl^{p210} and transplanted into non-irradiated NSG recipient mice. Jak2´s role for the hematopoietic stem cell compartment was investigated by performing serial as well as competitive transplants. To analyse whether differences in homing contribute to the inability of *Jak2* HSCs to repopulate we also performed competitive transplant experiments where we mixed *Jak2$^{+/+}$* Ly5.1 and *Jak2$^{f/f}$Mx1Cre^{+}* Ly5.2 BM cells in a 1:1 ratio and reconstituted lethally irradiated Ly5.1 recipient animals. Deletion of Jak2 was initiated after transplantation.
Results: BM transplantation experiments of non-irradiated NSG mice were performed using 1x10^6 Bcr-Abl^{p210} transduced BM cells. Under these conditions, disease evolves slowly and the absence of *Jak2* accelerated disease development with increased white blood cell counts (WBCs) and severe splenomegaly. Disease acceleration was attributed to the fact that Bcr-Abl transformed leukemic stem cells have overcome the necessity for Jak2-dependent signalling whereas non transformed HSCs depend on the presence of Jak2. In line, *Jak2* deletion was associated with a drastic reduction of HSCs (hematopoietic stem cells) and HSCs fail to repopulate lethally irradiated recipients upon Jak2 deletion in serial transplant settings. In a competitive transplant a detailed analysis over a period of 17 weeks revealed a pronounced and continuous decrease of *Jak2* Ly5.2^{+} cells in the peripheral blood, whereas numbers of *Jak2$^{+/+}$* Ly5.2^{+} cells remained constant. The drop in *Jak2* Ly5.2^{+} cells was pronounced for B220^{+} cells and even more prominent for CD11b^{+}Gr1^{+} cells, which were no longer detectable after 7 weeks. After 17 weeks almost complete absence of hematopoietic stem cells and progenitors in *Jak2* recipients was confirmed.
Summary and Conclusions: Jak2 is critical for HSC maintenance, survival and function. This dependence does not extend to Bcr-Abl^{p210} transformed leukemic stem cells. Thus, in the absence of Jak2 Bcr-Abl^{+} leukemic stem cells outcompete regular HSCs and accelerate Bcr-Abl^{+} leukemia.

LB2445

KINETICS OF ORGAN RESPONSE AND SURVIVAL FOLLOWING NORMALIZATION OF THE SERUM FREE LIGHT CHAIN RATIO IN AL AMYLOIDOSIS

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Background: Despite successful treatment of the clonal plasma cell implicated in its pathogenesis, patients (pts) with AL amyloidosis (AL) have significant morbidity related to amyloid mediated organ dysfunction. While normalization of the serum free light chain measurements (normal ratio of involved and uninvolved free light chains (nFLCr)) is the goal of therapies and centerpiece of hematologic response criteria, achieving meaningful organ response (OR) remains the ultimate goal of treatment. Expectations for OR following successful therapy leading to nFLCr in AL remain poorly described.
Aims: To evaluate the clinical implications of the kinetics of OR following successful nFLCr in AL.
Methods: Pts with confirmed AL, treated and followed at Mayo Clinic Rochester, who obtained nFLCr were retrospectively studied for evidence of OR. Demographic and clinical information including hematologic and organ related labs were obtained from medical records. Organ involvement and response criteria were defined by established standards. The research was performed with IRB approval. Survival curves were constructed per Kaplan-Meier estimates and compared using the log-rank test. Landmark analysis comparing groups of organ responders and non-responders was performed at one-year from initiation of therapy and from nFLCr. Cox-proportional hazard analysis was performed to assess variables influencing both time to OR and overall survival (OS). Effect likelihood ratio tests, hazard rates and 95% confidence intervals were calculated. Statistical analysis was performed using JMP v.10.
Results: Three-hundred-thirteen pts with AL obtaining nFLCr after therapy initiation formed the study cohort. Median follow up from diagnosis was 46.7 months (4.2-180.0). Renal, heart, liver and nervous system involvement were seen in 75%, 54%, 14%, and 12% of pts respectively. Primary treatments included auto-BMT (54%), melphalan-dexamethasone (28%), and proteasome inhibitor inclusive regimens (13%). Median time to nFLCr from start of therapy was 3.8 months (interquartile range 3.2-7.7). Seventy-two percent of pts obtaining nFLCr achieved OR in at least one organ. The median time to first OR from initiation of therapy was 6.7 months (interquartile range 3.4-12.2) and was 0.4 months (interquartile range [-1.2]-[+7.9]) from documented nFLCr. Median OS for the entire cohort was not reached, while it was 58.5 months for those who failed to obtain any OR. Landmark analysis of pts surviving at least 12 months from the start of treatment and nFLCr, showed superior OS in early organ responders (log-rank test p=0.038 and p=0.009 respectively) Figure 1. Having high levels of the involved FLC (iFLC) at diagnosis was predictive of having an OR following nFLCr (HR 1.45 (1.09-1.92) p=0.012). Other factors predicting OR include the presence of monosomy 13 on plasma cell FISH panel (HR 1.46 (1.01-2.08) p =0.044). In pts with renal involvement, having a lambda iFLC was predictive of not obtaining an OR despite nFLCr (HR 0.61 (0.44-0.86) p=0.005).

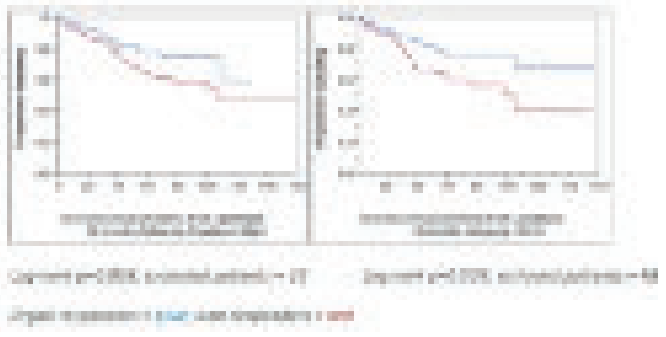

Figure 1. Landmarked survival curves comparing overall survival between organ responders and non-responders at landmarked time.

Summary and Conclusions: AL pts obtaining early OR following nFLCr have superior OS compared to those who obtain nFLCr but do not have early OR. High iFLC at diagnosis and monosomy 13 predict early OR. Interestingly, for pts with renal amyloid involvement, the presence of iFLC lambda predicts against obtaining renal response despite achieving nFLCr. These results provide new and significant clinical insight for the prognostication and management of successfully treated AL.

LB2446

IRON-INDUCED EPIGENETIC ABNORMALITY OF MOUSE BONE MARROW THROUGH ABERRANT ACTIVATION OF ACONITASE AND ISOCITRATE DEHYDROGENASE WITHOUT GENE MUTATION: ANALYSIS BY HIGH THROUGHPUT SEQUENCER AND GC-MS.

M Yamamoto[1,*], H Tanaka[2], Y Toki[1], M Hatayama[1], S Ito[1], J Inamura[1], M Shindo[1], K Sasaki[2], K Ikuta[1], T Ohtake[1], M Fujiya[1], Y Torimoto[3], Y Kohgo[1]
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Background: Iron overload in MDS contribute to a poor prognosis of the patients due to organ insufficiency and/or conversion to acute leukemia, both of which could be improved by iron chelation therapy. Iron is utilized in many biological processes such as oxygen transfer, energy metabolism and gene expression; we therefore hypothesized that iron overload itself may induce epigenetic change by systemic alterations of cellular metabolism, which may affect the production of 2-hydroxyglutarate (2HG), the crucial metabolite of isocitrate dehydrogenase (IDH) gene-mutated leukemia.
Aims: In order to clarify the role of iron on this issue, we evaluated the whole changes of gene expressions relating energy and glucose metabolism in the iron-overloaded mouse bone marrow cells using RNA sequencing and digital PCR analysis.
Methods: For iron overload model mice, iron dextran (10 mg/head/day) was intraperitoneally injected into C57Bl/6 mice for 5 days. After injection of iron dextran, deferoxamine (DFO) was also administered into the some mice. The mice were sacrificed, and peripheral blood and bone marrow cells were taken. RNA samples were then extracted from the bone marrow cells, and comprehensive gene expression analysis was performed using the high throughput sequencer Ion Proton (Life Technologies). The gene expression levels were further validated by the digital PCR system QuantStudio 3D (Life Technologies). Intracellular levels of 2-HG were enumerated by GC-MS analysis and DNA methylation levels were analyzed by ELISA.
Results: From the RNA sequencing and digital PCR analysis, the increased expression of metabolic enzymes, which are involved in glycogenolysis (Agl, Pgm1) and glucose metabolism (Aco1, Idh1) were observed in the bone marrow cells of iron overloaded mice. Furthermore, by GC-MS and ELISA, the levels of 2-HG and DNA methylation were also increased in these mice. These observations were canceled by DFO treatment (Figure 1).
Summary and Conclusions: As ACO1 is an iron-dependent enzyme, which converts citrate into isocitrate, and supplies isocitrate to IDH1/2, which converts isocitrate into a-ketoglutarate in the TCA cycle. We hypothesized that aberrant activation of the ACO1-IDH pathway by iron overload might induce 2-HG production and further cause DNA methylation even in the absence of IDH mutation. These whole RNA sequence analyses clearly indicate that the up-regulation of the ACO1-IDH pathway caused by iron overload trigger the production of 2-HG and finally induce DNA methylation that may develop leukemia even in the absence of IDH mutation.

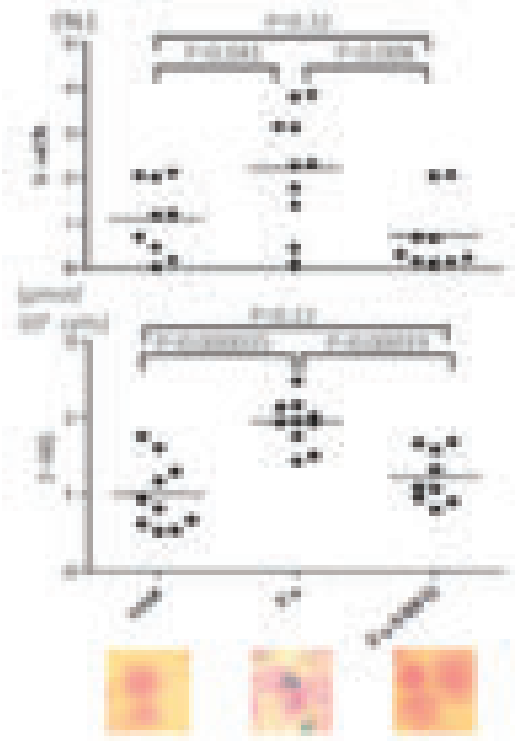

Figure 1.

LB2447

Abstract withdrawn

LB2448

RESPONSE TO LISINOPRIL IN PATIENTS WITH SICKLE CELL ANEMIA AND PROTEINURIA

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Background: Patients with Sickle cell anemia (SCA) are surviving longer and this has led to more chronic complications of SCA. Kidney is particularly susceptible to sickling of red cells and hypoxic damage because of the hypertonic and acidic environment of renal medulla. Proteinuria is an early manifestation of sickle cell related renal disease and considered to be a risk factor for developing renal impairment in future. Previous small studies have shown the benefit of ACE-inhibitors but this role remains to be defined.
Aims: The aim of this study was to assess the role of lisinopril, an angiotensin-converting enzyme inhibitor, in reducing proteinuria in patients with sickle cell anemia.
Methods: Patients older than 15 years with known sickle cell disease (HbSS or HbS-β^0) participated in the study. Patients with renal impairment and sickle cell trait were excluded. Twenty four hour urine collection was carried out to quantify proteinuria and urine collection was done in steady state without an acute illness during the last 2 weeks. All patients gave informed consent and the study was approved by the institutional review board.
Results: A total of 35 patients were recruited with a mean age of 28.5 ± 6.98 (range 20-47, 54% males). The mean Hb was 9.26 g/dl, mean HbF 11.2%, mean LDH 369 IU/ml, and mean serum creatinine 44 µmol/l. Median Pre-treatment 24 hour urine-protein was 0.3006 grams while median 24 hour urine-protein post-treatment was 0.150 grams (p=0.01). After a median follow-up of 38 months, 24 hour urine-protein decreased in 27 (77%) patients and normalized in 18 (52%) patients. Protein increased in 2 (6%) patients and remained stable (no change) in 6 (17%) patients. There was no significant difference in the pre and post systolic and diastolic blood pressures. Average dose of lisinopril was 5 mg. Nineteen patients are still on lisinopril. The reason(s) to stop lisinopril include: normalization of protein (9), non-compliance (3), side effects (2), and pregnancy (1). Adverse events include cough (5), dizziness (1), and diarrhea (1). None of the patients in the study developed deterioration of renal function.
Summary and Conclusions: Lisinopril is effective in reducing proteinuria in

patients with SCA without significant drop in the blood pressure. Only few patients developed adverse events which were mild & tolerable. It is unclear for how long to continue lisinopril and whether it can be stopped in patients who normalize urine protein. Larger studies with longer follow-up are needed to address these questions.

LB2449

ICARS: ENGINEERED SAFETY FOR T CELL IMMUNOTHERAPY

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Background: Despite being potentially curative, T cell immunotherapies have been hampered by toxic side effects in clinical use. "On-target but off-tumor" fatalities have occurred in immunotherapy trials due to toxic cross-reactivity with heart, lung, and brain tissues. Similarly, donor lymphocyte infusion in allogeneic bone marrow transplantation can lead to lethal acute or chronic graft-*versus*-host disease. Available approaches to limit T cell-mediated toxicities are inadequate and abrogate the beneficial effects of the therapies.
Aims: Using novel synthetic receptors, we sought to engineer dynamic cell intrinsic control to allow T cells to regulate their own functions and limit unwanted toxicities. Thus, trying to educate T cells on what to kill and what not to kill.
Methods: We engineered human T cells to express inhibitory chimeric antigen receptors (iCARs), which combined unique surface antigen recognition and intracellular inhibitory signaling based on CTLA-4, PD-1, LAG-3, 2B4, or BTLA immune-inhibitory receptors.
Results: Using iCARs in human T cells, we were able to control cytokine secretion, cytotoxicity, and proliferation driven either by endogenous T cell receptors or by clinically relevant chimeric antigen receptors. *In vivo,* iCARs could restrict tumor target T cells to eliminate on-target tumor cells but not off-target cells expressing the iCAR antigen. Additionally, iCAR-expressing T cells protected human cells expressing the iCAR antigen despite potent TCR-mediated allogenicity, in a novel *in vivo* induced pluripotent stem cell allogeneic response model. We have defined that iCARs function through reversible stoichiometric signaling allowing for sequential antigen encounters and potent function.
Summary and Conclusions: iCARs provide novel *engineered safety* to prevent toxicities in autologous and allogeneic T cell therapies, while preserving therapeutic efficacy through dynamic self-regulation.

LB2450

EFFECT OF FTY720 ON THE SET-PP2A COMPLEX IN ACUTE MYELOID LEUKEMIA. SET BINDING DRUGS HAVE ANTAGONISTIC ACTIVITY

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Background: PP2A is a phosphatase that functions as a tumor suppressor in several cancers. Our group showed that PP2A inactivation is a recurrent event in acute myeloid leukemia (AML), and that overexpression of SET, an endogenous inhibitor of PP2A, is a poor prognostic factor in this disease. Furthermore, restoration of tumor-suppressor activity by PP2A-activating drugs has anti-leukemic effects in AML cells. Interestingly, the anticancer activity of several PP2A activating drugs, such as FTY720 and OP449, depends on interaction/sequestration of SET.
Aims: Our aim was to clarify the domains involved in SET and PP2A catalytic subunit (PP2Ac) binding in AML and to elucidate the molecular mechanisms of PP2A activation by FTY720, in order to establish the basis of new PP2A activating drugs in AML.
Results: Immunoprecipitation of PP2Ac in untreated cells confirmed that SET interacts with PP2Ac, though treatment with FTY720 effectively disrupted this association. Next, we treated HL-60 cells with biotin-labeled FTY720, and immunoprecipitated the complexes formed in cell lysates with streptavidin-labeled dynabeads. We detected endogenous SET, but not PP2Ac, indicating that FTY720 is interacting with SET and activates PP2A in AML by preventing the PP2Ac/SET complex formation, in agreement with the recent observations obtained in lung cancer and in chronic myeloid leukemia cells (CML). We next analyzed the effect of FTY720 on the SET/PP2Ac complex by affinity chromatography analysis. Our results indicate that FTY720 binds SET within the last 100 amino acids of the C-terminal fragment of SET (from 177 to 277), in agreement with Saddoughi *et al.* (2013). This interaction produces a destabilization of the inhibitory SET/PP2Ac complex, with a consequent re-activation of PP2A activity and a reduction of cell viability. Additionally, we found that SET is predominantly cytoplasmic; however, after treatment with FTY720 there was a marked increase of nuclear localized SET, together with an intensification of PP2Ac into the cytoplasm. Our group and others have demonstrated that both FTY720 and OP449 can inhibit cell growth and re-activates PP2A activity in CML and AML cells. FTY720 is a relatively nontoxic drug currently used in patients with relapsing multiple sclerosis (MS). However, the dose used as an anticancer drug is much higher than the dose given to MS patients. Therefore, we evaluated the effect of combining FTY720 with OP449 to establish whether OP449 could help to reduce the dose of FTY720. Our results showed that FTY720 and OP449 have antagonistic effects, suggesting the need for testing new combinations of OP449 with second generation FTY720-derivatives for the treatment of AML.
Summary and Conclusions: SET forms an inhibitory complex with PP2Ac in AML, impairing the activity of PP2A, and the whole structure of SET is involved in the binding. We confirm that FTY720 reduces the inhibition of PP2A by SET, and shed light on the mode of action of this drug. Furthermore, we demonstrate for the first time that FTY720 induces an accumulation of SET into the nucleus, allowing PP2A to recover its activity in the cytoplasmic compartment. Finally, we show that FTY720 and OP449 have antagonistic effects, encouraging the hypothesis of an alternative combination of OP449 with derivatives of FTY720 could be a better strategy for SET inactivation in AML.

LB2451

PRETREATMENT D-2-HYDROXYGLUTARATE SERUM LEVELS PREDICT OUTCOME IN IDH1-MUTATED ACUTE MYELOID LEUKEMIA

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Background: Mutations in isocitrate dehydrogenases (IDH) 1 and 2 frequently occur in acute myeloid leukemia (AML) and result in the production of the oncometabolite D-2-hydroxyglutarate (D2HG), which is elevated in the serum of most patients with IDH-mutated (IDHmut) AML. Measurement of serum D2HG has been shown to be useful in identifying patients with IDH mutations, and can serve as a marker of tumor burden and response to treatment. Interestingly, D2HG has been shown to have leukemogenic properties *in vitro*, independent of mutated IDH. Whereas the impact of IDH mutations on outcomes has been investigated in large studies, the relevance of pretreatment serum D2HG levels is a matter still open to debate.
Aims: This studied investigated the impact of pretreatment D2HG serum levels on outcomes in the to date largest cohort of uniformly treated patients with IDHmut AML.
Methods: Pretreatment D2HG serum levels were measured in 86 IDHmut and 70 IDHwt AML patients enrolled in the prospective, randomized, multicenter AML2003 trial of the German Study Alliance Leukemia (SAL). D2HG was measured using gas chromatography/mass spectrometry (GC/MS) as well as a fluorometric assay (Balss et al, Acta Neuropathol. 2012 Dec;124(6):883-91).
Results: Measurement of D2HG with GC/MS and the fluorometric assay in 93 samples showed a strong correlation between the two methods (R=0.83). Further analyses were carried out using the fluorometric method. Median D2HG serum levels were significantly higher in patients with IDHmut than IDHwt AML (41.6 µmol/l (range 1.9 - 595 µmol/l) and 5.0 µmol/l (range 0.1 - 13.7 µmol/l), respectively, p<0.0001). D2HG correlated with leukocyte count, serum LDH and bone marrow blast percentage (R=0.67, R=0.5, R=0.39, respectively). In a multivariate Cox regression model, D2HG had a negative impact on event-free survival (EFS; HR 1.33; p=0.04), but not overall-survival (OS; HR 1.1; p=0.47), independently of the established risk factors age, ELN risk group, WBC, and LDH. A subgroup analysis showed that this impact on EFS solely resulted from a striking effect of D2HG levels on EFS in patients with mutations in IDH1 (HR 2.42; p=0.0029) , with no effect in patients with mutations in IDH2 (HR 1.0; p=0.99).
Summary and Conclusions: Recent studies investigating D2HG in AML have been performed with only small numbers of uniformly treated patients, or measured total 2HG instead of the actual oncometabolite D2HG. Our study comprises the to date largest number of uniformly treated patients with IDHmut AML. Pretreatment D2HG serum levels negatively impacted on EFS in IDH1mut AML, independent of established risk factors, and did so as a continuous variable, thus consistent with the concept of a dose-dependent effect of the metabolite. The effect on OS was not statistically significant, likely due to the frequent use of allogeneic SCT in the AML2003 trial, which could have rescued the negative effect of D2HG. No significant impact of D2HG on outcome was found in patients with IDH2mut AML. This variable effect of D2HG levels, apparently dependent on the isoenzyme mutated, *i.e.* IDH1 *versus* IDH2, or possibly the localization of the D2HG-producing mutant isoenzyme warrants further investigation.

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